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Evaluation of some pro-inflammatory and anti-inflammatory cytokiens in serum level of children infected with *Enterobuis vermicularis*

A Thesis

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University of Babylon, in Partial Fulfillment of the Requirements
for the Degree of Master in Science / Medical Microbiology

By

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ وَقُل رَّبِّ زِدْنِي عِلْمًا ۝ ﴾

صدق الله العلي العظيم

سورة طه (جزء من الآية ١١٤)

Certification

I certify that this thesis entitled "***Evaluation of some pro-inflammatory and anti-inflammatory cytokiens in children infected with entrobuis vermicularis***" was prepared by **Aseel Sekar Naji AL-Bermani** under my supervision at the college of Medicine **,** University of Babylon, as a partial requirement for the degree of Master in **Medical Microbiology.**

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Dedication

To

The Prophet Muhammad, may God's prayers and peace be upon him

Imam Mahdi, may God hasten his reappearance

My lovely parents, who motivated me….

My faithful husband, who supported and encouraged me....

My sister and my brothers for their help….

My children, who gave me the hope…..

Aseel

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First of all, I thank Allah who gave me support to fulfill these part thesis., from the depths of my heart and thanks to our honorable prophet Muhammad,may GOD bless him and grant him peace.

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Finally for those who I missed to mention their names, apologize thanks and appreciation to them all

Aseel Al-Bermani

Summary:

This study was conducted during the period from September 2021 to March 2022, a group of eighty-seven and thirty healthy children were selected, their ages ranged between (2-13 years), and included both sexes (37 males and 50 females). Samples were collected from Al-Noor Children's Hospital, Babel Children's Hospital, the second Hilla sector, and the villages and countryside in the city of Hilla, a special questionnaire form was prepared for each child participating in the study and was filled out by interviewing their mothers. Blood samples were taken from all study subjects for epidemiological and immunological study.

The current result showed that all clinically infected patients 87 (100%) of them had positive diagnostic results, while laboratory examination and 53 (60%) had positive diagnostic results. Also the result showed 79 (90.8 %) of infected clinically patients have treatment history in compared to patients with lab. examination 53 (100 %) have treatment history, and the difference was significant (p = 0.023), also showed only 1 (1.1 %) of infected clinically patients have history of enuresis in compared to 3 (5.7%) patients with lab. examination have history of enuresis, but the difference was non-significant (p = 0.120), and 83 (95.4%) of the clinically affected patients had a positive family history compared to the tested patients. Screening showing that all 53 patients (100%) had a positive family history, but the difference between clinically affected and tested patients was not vast.

According to residency, infected clinically patients group included 15 (17.2 %) cases from urban areas and 72 (82.8 %) cases from rural areas, while patients with lab. examination group included 12 (22.6 %) cases

form urban areas and 41 (77.4%) cases from rural areas and there was no significant difference in the frequency distribution of patients and control subjects according to residency (P = 0.432)

Infected clinically patients included 37 males and 50 females (42.5% and 57.5 %) respectively, whereas, patients with lab. examination included 21 males and 32 females (39.6% and 60.4 %) respectively and there was no significant difference in the frequency distribution of infected clinically patients and patients with lab. examination according to gender (P = 0.735), and the distribution of infection was higher in the age groups > 10 years of age, but the difference was not vast of another age groups.

The relationship between the prevalence of *E. vermicularis* and sex in the current study showed that the risk of *E. vermicularis* infection was higher but not significant($p \geq 0.05$) among female children.

The immunological biomarker (IL-1 beta , IL-2 and IL-10) results showed the patients with *E. vermicularis* concentration were slightly higher than in comparison to the concentration levels of control groups, , but the difference was non-significant (p = 0.782, p = 0.459 and p = 0.264) respectively.

The present result indicate age group less than 6 years have high levels of IL-1 beta , IL-2 and IL-10 in compared with other age groups, but the difference was non-significant (p=0.278) (p=0.15) (p=0.399). sequentially. Also the results show patients with male gender have high levels of IL-1 beta , IL-2 and IL-10 in compared with patients with female genders, but the difference also was non-significant (p=0.268) (p=0.082), (p=0.034) sequentially .

The correlation between serum immunological marker (IL-1 beta and IL-2 and IL-10) in patients with *Enterobius vermicularis* infection

showed a highly significant level. And the correlation between IL-10 levels and age were negatively; *i.e.*, as children's age increased, IL-10 levels tended to decrease. A significant negatively correlation between age and immunological marker ¸(IL-1β ¸ IL-2 and IL-10) ¸while significant positively correlation among the interleukins together

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List of abbreviations

AAMs	Alternatively activated macrophages
AAMs	Alkali-activated materials
ADCC	Antibody-dependent cellular cytotoxicity
AICD	Activation-induced cell death
AIM2	Absent in melanoma 2
APCs	Antigen presednting cells
CD4	T – helper cell cluster differentiation
CD8+	Cluster differentiation
CNS	Central nervous system
DC	Dendritic cells
dsDNA	Double-stranded DNA
ECP	Eosinophil cationic protein
GIT	Gastrointestinal tract
IFN-γ	Interferon-gamma
IgE	Immunoglobulin Epsilon
IL	Interleukins
IL – 10	Interleukin – 10
IL-1	Interleukin – one

IL-12	Interleukin – 12
IL-13	Interleukin -13
IL-17	Interleukin-17
IL-17E	Interleukin –17E
IL-18	Interleukins-18
IL1RL1	IL-1 receptor-like 1
IL-1α	Interleukin – one alpha
IL-1β	Interleukin one beta
IL-2	Interleukin – 2
IL-25	Interleukin –25
IL-33	Interleukin –33
IL-4	Interleukin -4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-9	Interleukin – 9
ILC2s	Type 2 innate lymphoid cells
ILCs	Innate lymphoid cells
IPI	International Prognostic Index
LPS	Lipopolysaccharide
MHC	Major histocoampatibility complex
Nk cells	Natural killer cell
NLRP3	Nod like resceptor
nTreg	Natural regulatory cells

PSC	Preschool-aged children
TGF-B1	Transforming growth factor-β1
TGF-β	Transforming growth factor-β
Th1	T-helper cells 1
Th17	Interleukins-17
Th2	T-helper cells 2
TLR3	Toll-like receptor 3
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
Treg	T regulatory cell
TSLP	Thymic stromal lymphopoietin

1.Introduction:

Enterobius (syn. *Oxyuris*) *vermicularis* is a human-pathogenic intestinal parasite belonging to the nematodes .Synonyms include "threadworm" and "seatworm." Symptomatic pinworm infection is referred to as enterobiasis (Deplazes *et al.*¸2013)

Infection most commonly occurs in children, but any individual is susceptible to *E. vermicularis* infection. People from tropical climates

and school-aged children are the most vulnerable. Infection is caused by ingestion of the pinworm eggs. (Yoon *et al*., 2000) 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 very related with high population density,socio-economic factors and the habit of fingers sucking (Tomanakan *etal*., 2020).

After ingestion, the embryonated eggs hatched in small intestine, and the larvae are released. The adult pinworms mainly attach themselves in the caecum and the appendix (Dunphy *et al*., 2017). Re-infection occurs easily in enterobiasis and the prevalence of this infection is mainly depend on public health and personal hygiene (Moosazadeh *et al*., 2017).

Due to its contagious nature, enterobiasis tends to occur more among large family members and institutions such as nurseries and kindergartens, especially with Although highly prevalent¸this infection is often overlooked as most infections remain asymptomatic or crowded conditions(Gunawardena *etal*.¸2013)

Infection with *E. vermicularis* commonly affect the gastrointestinal tract (GIT), and the ectopic infections is rare specially in the liver (Zakharia *et al*., 2019). Female genital tract and peritoneum are the common sites of ectopic infestation, and rarely they may also be seen in tissues like kidney, male urinary tract, conjunctiva, spleen, and lung (Nair and Balan, 2018). In the case of sever infestation, the worms may be visibly expelled with stool, adult worms also can be visualized by proctoscopy or colonoscopy (Wendt *et al*., 2019).

The disease is highly correlated with a lack of hygiene, contaminated bed linen and clothes may also play a role in transmission. Patients are often asymptomatic; however, when female worms deposit large numbers of eggs in the perianal region, pruritus is a typical

symptom, especially at night complication such as extra-intestinal infection ¸may occur but are uncommon (Djakovic *et al*.¸2006)

Occasionally, pinworms penetrate into the submucosa resulting to a fatal diseases. They may also, penetrate extraintestinal sites like vulva, vagina, uterus, fallopian tubes, ovary, peritoneum, lung, liver, breast, spleen (Ng *et al.*, 2011**)** male urinary tract (Zahariou *et al.*, 2007**)**

The immune response caused by parasitic infection is complex and multiple. Helminths induce strongly Th2-skewed responses associated with cytokines such as IL-4, IL-5, and IL-13, with mastocytosis, eosinophilia, and antibody class-switching to produce IgE (Allen and Maizels, 2011).

It has been reported that the *E. vermicularis* can stimulate the formation of regulatory cytokines as IL-10 which provides both host and parasite viability (Wilson MSI *etal* .¸2005)

Interleukin -1 is expressed in two forms, IL-1α and IL-1β, that share many biological properties, although the genes are distinct from each other and the sequence homology between them is low. Both forms play a number of roles in the immune system, including the activation of monocytes and macrophages the stimulation of proliferation and differentiation of B cells (in synergy with IL-4 and IL-6), and the potentiation of antibody production IL-1 markedly prolongs the lifespan and stimulates the effector function of neutrophils and macrophages (Mantovani *etal.*¸2011).

Interleukin-2 (IL-2) is a cytokine that controls the activitiers of white blood cells (leukocytes, especially lymphocytes) that are respoonsible for immunity. It constitutes part of the body's natural response to microbial infection (Miller *et al*., 2009).

Intreleuken-10 also acts on a wide range of cell type with one of its main roles being the downregulation of major histocoampatibility complex (MHC) and costimulatory molecules on antigen presednting cells (APCs), thereby preventing their full activation capacity and limiting T cell responses (Fiorentino *et al*.,1991)

1.1 Aim of this study:

In the present study, we have tried to illustrate the relationship between pro-inflammatory and anti-inflammatory cytokines in children infected with *E. vermicularis*¸this aim was achived by the following objectives:

1-Diagnosis of *E. vermicularis* by cellophane tape technique

2-Demographical study including (age, sex, residence, number of family and suffering from enuresis or not)

3-Study any relationship between infection with *E. vermiculars* and nocturnal enuresis

4-Determination of some pro-inflammatory cytokines (IL-2, IL-1β,) levels in patients and control

5-Determination of anti-inflammatory cytokines (IL-10) levels in patients with *E.vermicularis* and control group

1.2.Literatures Review

1.2.1. Historical Background of *Enterobius vermicularis*

Enterobius vermicularis (pinworms) is one of the most common human parasitic helminthes that may cause enterobiasis, which is

common among primary school children in many countries (Moosazadeh *et al*., 2017) *Enterobiasis* is an intestinal nematode infection caused by *Enterobius vermicularis*, commonly known as pinworms. *E. vermicularis* infection is an important public health problem among school children, especially in tropical and subtropical countries (Chen *et al* .,2018; Dudlová, *et al* .,2018)

E. vermicularis is called a pinworm due to its long pointed tail that resembles a straight pin in the adult worm (Ridley, 2012). Infection rates very up to 40%, depending on age and race (Bowman, 2009) *E. vermicularis* was first described in 1758 by Karl Linnaeus, who called it *Oxyuris vermicularis* (Gillespie and Pearson, 2001), the disease was named as oxyriasis for years. It is thought to be the oldest parasite defined and was identified in ancient Egyptian mummified human remains in addition to DNA samples from ancient human coprolite remnants from North and South America (Satoskar *et al.,* 2009).

The earliest known instance of pinworms is evidenced by pinworm eggs found in coprolite, carbon dated to 7837 B.C. at western Utah. Pinworm infection is not classified as a neglected tropical disease unlike many other parasitic worm infections .In Egypt the first evidence of pinworm infection dates back to Roman- occupied (30 BC – AD 395) Egypt (Horne, 2006).

Paleoparasitological analyses were performed in Iran (2500 and 1500 years BP) revealed the presence of human *E. vermicularis* (Nezamabadi *etal.*, 2013). In a second archeological site in Tehran city the samples shown the presence of one *E. vermicularis* egg and this was evaluated to be 7000 years old, this findings may represent the oldest evidence of human pinworm infection in Asia (Paknazhad *et al*., 2016).

Also eggs of *E. vermicularis* were recently identified in coprolites from one pre-Columbian mummy. This is the first evidence of ancient intestinal parasites in Bolivian mummies, being dated from 1150 to 1450 C.E. (Valverde *et al*., 2020).

The samples from North America archaeological sites revealed the presence of *E. vermicularis* eggs that identified from prehistoric populations of American Indians (Araujo *et al*., 2008). In Brazil the microscopic examination for coprolite from archeological sites in mid-western region of Brazil detected human *E. vermicularis* eggs and this is the first finding of pinworm in paleoparasitological analysis of coprolite in this region (Lino *et al*., 2018).

Most of *Enterobiasis* the infections are asymptomatic¸ Common *Enterobiasis* symptoms include itching, irritation of the perianal region, and vaginal pruritus in females (BurkhartandBurkhart,2005)

Enterobiasis has a wide range of geographic distribution and is prevalent in developing and even in developed nations. *Enterobiasis* is often described as a childhood infection caused by *E. vermicularis* (human pinworm), also known as oxyuriasis (Roberts and Janovy Junior *etal.*¸2009)

Pinworm eggs from archaeological sites have been recovered from many sites, primarily in the Americas(Reinhard *et al*.,2016). By comparing prevalence and intensity of infection from mummies and coprolites, these studies defined the New World paleoepidemiology and prehistoric biogeography of pinworm infection. Importantly, pinworm prevalence is affected by the development of complex societies and urbanization (Camacho and Reinhard,2019).

1.2.2. Classification of *Enterobius vermicularis*

Pinworms are a parasite group occurring in most families and genera of the order Primates including human. Enterobiasis caused by *Enterobius vermicularis* is the most frequently worldwide gastrointestinal diseases reported among preschool children and younger (Valverde *et al*., 2020).

Kingdom : Animalia

Phylum : Nematoda

Class : Rhabditea

Order : Oxyurata

Family : Oxyuridae

Genus *: Enterobius*

Species *:E. vermicularis*

Classification by Linnaeus (AL-Jaf. , 2018)

1.2.3. Morphology *Enterobius vermicularis*

Enterobius vermicularis is a worm that can enter the mouth of the body through food, air, soil that will nest in the large intestine . The adult pinworm (*Enterobius vermicularis*) is small, colored white. The female worm , is much larger than the male worm . In the anterior area around

the neck, the cuticle of the worm is widened. The characteristic extension of this worm is called the cervical alae (Trasia, 2021).

The cecum of the large intestine is the major site for pinworms to live and the gravid female migrates at night to lay up to 15,000 eggs. Ingested eggs hatch in the duodenum, and larvae mature during their migration to the large intestine. In the absence of host autoinfection, infestation usually lasts only four to six weeks (Kucik *etal* .,2004;Burkhart *etal* .,2005**)**

Female *E. vermicularis*, measuring 8 to 13 mm long by 0.4 mm wide, are characterized by the presence of wing like expansion (alae) of the body wall at the anterior end, distension of the body due to the large number of eggs in the uteri, and a pointed tail. Males, smaller in size, are 2 to 5 mm long and possess a curved tail **(**Bogitsh *et al*., 2012)**.**

The egg measures 50 to 54 micrometer in length by 20 to 27 micrometer in width and has a characteristic shape, flattened on one side. It is almost colourless, with a bean-shaped double contour shell a fully formed embryo **(**Cook and Zumla, 2009**).**

The eggs have five membranes: one inner, lipoidal layer, three middle layers known as membrana lucida, and one outer, albuminous membrane which coats the egg. This membrane makes the eggs sticky and therefore itchy to the host, which is important in the life cycle (Brusca and Brusca, 2003). Eggs have a thick shell and are convex on side and are flattened on the other (D-shape) ,they become infected within a few hours and the viability of egg 2-3 weeks (Taher 2017).

The larvae may be visible inside the egg due to the colorless shell of the embryonated eggs Like all nematodes, *E. vermicularis* has a thick outer protective covering (cuticle) (Gutiérrez, 2000; Ridley, 2012)**.**

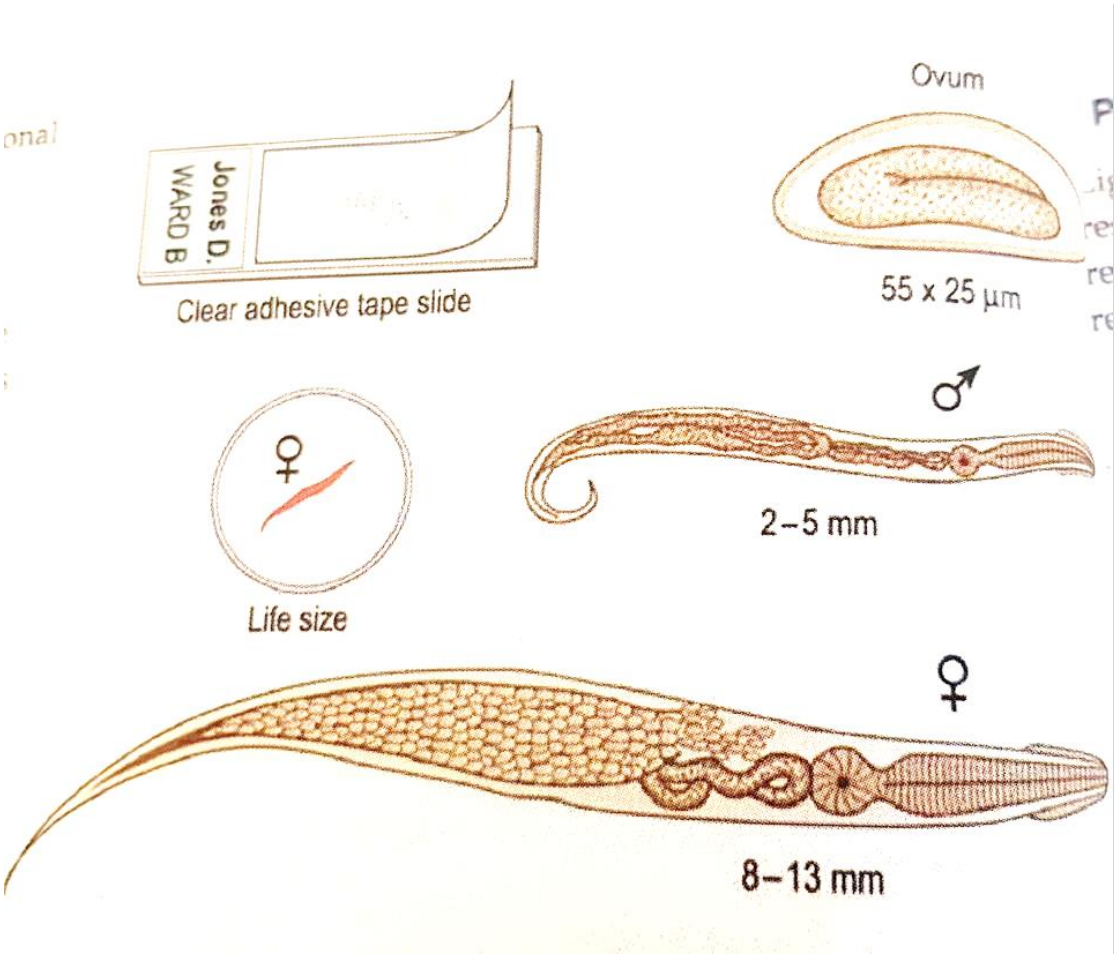

Figure (1-1) Morphology of *E. vermicularis* (Chiodini *et al*., 2003).

1.2.4. The life-cycle of *Enterobius vermicularis*

Human infection was directly associated with the ingestion of infective eggs through oral routes or from contaminated clothes and bed linens. Additionally, transmission through the respiratory tract has also been speculated by inhaling dust contaminated with eggs(Chang *etal*.¸2009)

life cycle of pinworm is uncomplicated, utilizing the human host gastrointestinal tract to become adults, and the gravid female worm migrates to the anus to lay fertilized eggs at night. In some cases, the ovulating female pinworm may also move to the external genitalia to produce fertilized egg(Meletis *etal*.¸2019)

E. vermicularis life cycle is simple, direct, and it is occur in the gastrointestinal lumen, and the infection take place via fecal-oral route following ingestion of embryonated eggs that hatche in small intestine develop into adult worms and reside in the caecum, appendix, colon, and rectum (Sharma *et al*., 2018).

Coupling between adult worms take place in the colon, and gravid female migrate to the perianal area especially during the sleep of host where they lay eggs *(*Dudlova. *et al.,* 2018). The air contact act as a stimulator to the gravid females to lay eggs (Dahal and Maharjan, 2015). The eggs which contain larva become infective within as little as 4-6 hr following oviposition (Wendt *et al.,* 2019). Males die after copulation, while egg-bearing females travel to the perianal and perineal regions, where they lay up to 11,000 eggs. The female usually return to the colon or die after laying eggs (Bogitsh *et al.,* 2013).

Eggs may also be deposited on clothing and bed linen, and are subsequently either ingested or inhaled (Park *et al*., 2005).

eggs transmission from person to person, directly via the anus-to-mouth route and finger contamination or indirectly by contaminated objects, e.g., toys, classroom tables, chairs, or the ground (Burkhart *etal.*¸2005; kim *etal* .¸2013) Since personal hygiene and exposure are important transmission factors, preschool-aged children (PSC) who live in crowded environments such as kindergartens are the most common group susceptible to pinworm infection therefore, children may acquire an infection through ingestion of eggs-contaminated foods or inhalation of infective eggs in the dust or retrograde migration of hatched larvae from the anus to the intestine (Burkhart *etal* .¸2005).

The eggs embryonate and become infective within six hours. Re-infection can occur if larvae hatch on the skin and crawl back into the colon The nocturnal activity of the worm may have been selected for to

provide the parasite with low levels of host activity so her presence is not noticed and to ensure that when the host wakes up, they are able to contaminate their hands with the infectious egg. The transmission cycle continues when an individual ingests the eggs, and re-infection of the same host is common (Kim *etal* .¸2010).

Although embryonation of the egg can only occur if the ambient temperature is above 23°C, the eggs can remain viable for weeks if conditions are moist and cool However, these conditions are rarely sustained long enough to prolong egg survival, and time outside of the host weakens the infective potential of the egg. After 1-2 days outside of the host, the egg expenences a sharp decline in infectivity The low virulence and correspondingly low durability of *E. vermicularis* is in line with the prediction that durability in the external environment is positively correlated with virulence Environmental and Behavioral Risk Factors Due to the life cycle characteristics of *E. vermicularis,* environmental variables such as rainfall or soil content do not strongly impact parasite development or survival Host behavior plays a much bigger role in the transmission dynamics of pinworm. Close contact among potential and infected hosts is necessary for transmission to occur (This indicates that host mobility is much more important than sit-and-wait (Kim *et al* .¸2010).

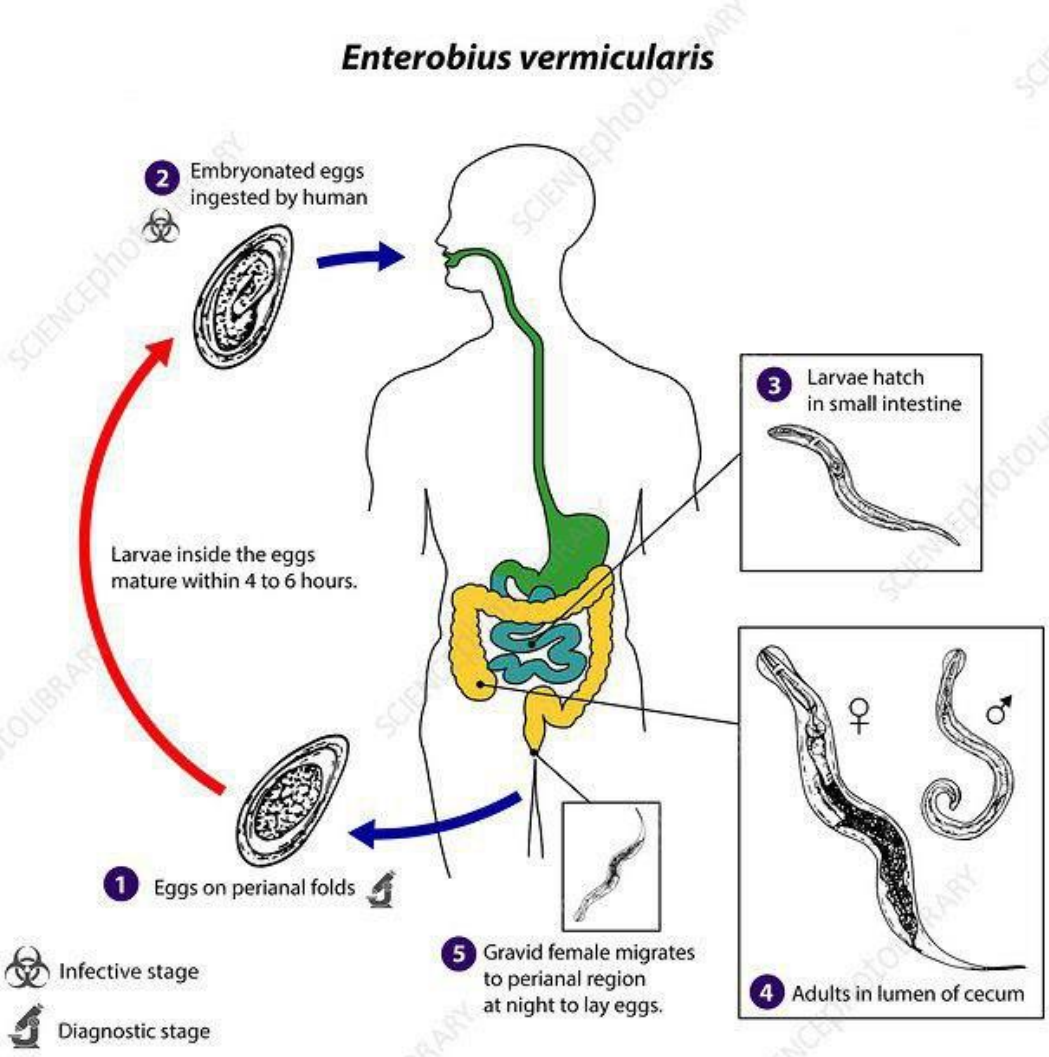

Figure (1.2) life cycle of *E . vermicularis* (Wendt *etal* .¸2019)

1.2.5. Transmission of *Enterobius vermicularis*

A common way for infection after the hand have contact with the perianal area and transfers the infection to the mouth (Sato *et al.*, 2008 ; Mehlhorn,2016). The infection also can be transmitted from person to person by contaminated hands, especially in crowded conditions, *E. vermicularis* can be transmitted by fecal-oral rout, self-reinfection is furthermore children with habit of sucking fingers or toys showed high rates ofinfection (Cranston *et al.*, 2015 ; Wang *et al.*, 2017). Fingernails can cause infection when children have a biting habit specially when fingernails is long and could transport mature eggs to the mouth (Abdullah and Al- Shirifi, 2005).

Biting pencils, not cleaning hands before meals, and playing on the earth contribute to higher risk factor among young children (Li *et al.*¸2015).

Infection can spread also when people handle contaminated fomites such as pajamas, bed linen, and underwear (Snow,2006). *E. vermicularis* air born eggs may be swallowed and cause infection (Taher, 2017).

Fruits and vegetables, particularly those eaten raw and without peeling, have been demonstrated to be vehicle for transmission of *E. vermicularis* eggs and cause infection, using of sewage on agriculturall and and using this waste water for agricultural purposes well lead to cause fruit and leafed vegetable contamination with *E. vermicularis* ova (Al-Mozan and Dakhil, 2019). Lettuce, cabbage, and strawberry were found to be fairly more heavily contaminated than the other crops because it would have large contact areas with the soil surface and root crops also would behave large contact areas with the soil surface and root crops also would be continuously exposed to the contaminants (Erdoğrul and Şener, 2005).

River water that penetrates populated areas and agricultural lands and has human waste is contaminated with *E. vermicularis* eggs, will provide a source of infection and cause transmission of these eggs to the human (Alkhalidy, 2019).

Cockroaches are proven carriers of the *E. vermicularis* eggs which were isolated from the external body of German cockroach, because the cockroaches feed on the contaminated fecal material which might be carrying those eggs and sticking with cockroaches bodies which works as a mechanical vectors through various body parts (Al-aredhi, 2014).

Pinworm ova have been recovered from 90% of dust samples in the houses of infested children, also from the walls of dining rooms in the schools, fur of domestic pets, swimming pools, and from soap bars (Gale, 2002)

1.2.6. Epidemiology

1.2.6.1.*Enterobiasis* in world

E. vermicularis infection is an important public health problem among schoolchildren, especially in tropical and subtropical countries(Chen *et al* .,2018; Dudlová *et al*.,2018) , with an estimate of over 1 billion infections (Lohiya *et al*., 2000). *E. vermicularis* infects 300 million people worldwide. Symptomatic and asymptomatic infections can occur in children and adult, there are also very rarely ectopic lesions in female genital tract and in peritoneum(Presterl *et al*., 2019). This infection is more common in the temperate than in the tropic(David ,2006).

E. vermicularis is the representative contact-borne contagious helminth in the Republic of Korea.It is especially prevalent among children in

crowded and unsanitary conditions (Song *et al*.,2003). the egg positive rate (EPR) of *E. vermicularis* in preschool children was reported to be 18.1% in western and southern coastal islands (Park *et al*.,2005) and 7.9% in Cheongju-si (Kang *et al*., 2006).

Enterobiosis has a wide range of geographic distribution and is prevalent in developing and even in developed nations. Intestinal enterobiosis affects more than 400 million people throughout the world. It rarely affects tropical people because its eggs cannot survive in hot dry conditions for long time (Afrakhteh *et al*., 2016)

Worldwide epidemiological studies showed the pinworm infection among schoolchildren in Asia, e.g., 54.9% in China (Li *etal.*¸2015) 8.8% in Thailand (Tomanakan *etal.*¸2020) 47.2% in Myanmar(Chai *etal.*¸2015) and 4.4% in South Korea (Kim andYu.¸2014)and 29.1% in Argentina(pezzani *etal*.¸2004); and Europe, e.g., 17.4% in Germany (Friesen *etal* .¸2019), 3.4% in Slovakia Republic (Dudlova *etal* .¸2018)and 19.3% in Osh Oblast, Kyrgyzstan(Steinmann *etal* .¸2010.)

In the United States, pinworm infestation is the most common helminth infection, with over 40 million people being infected (Mkhize *etal* .¸2012)and 29.1% in Argentina(Pezzani *etal.*¸2004)

Studies in several countries around the world have shown a high prevalence of *E. vermicularis* in different age groups: for example, 30–80% in North America, 18% in China and 17% in Tanzania(Chen *etal.*¸2013; Salim *etal.*¸2014). Other studies indicate that a prevalence of more than 20% is common in many parts of the world (Chen *etal*.¸2018)

Though most parasitic infections have been eradicated in developed nations, *E. vermicularis* infection is still often reported in many developed nations. In Pakistan the prevalence of *Enterobiosis* has sharply increased from 0.2% in 1964 to 14.1% in 2017 (Khan *et al.*, 2017).

Studies from different parts of Pakistan in the last two decades report prevalence rates of *enterobiosis*, as 2.61% Shaikh *et al.* (2003); 1.3% Chaudhry *et al*. (2004); 16.3% Maqbool *et al.* (2007); 10.3% (Nisa *et al*., 2011); 12% (Khan *et al*., 2012); 8% (Khan *et al.*, 2015); 9.73% (Khan *et al.*, 2017) and 9.52% (Khan *et al.*, 2018) and 6.48 (Khan *et al*., 2018)

In Palestine and according to the Ministry of Health annual reports, the average prevalence of the infection reaches very high levels (Hamarsheh and Amro 2020).

1.2.6.2.*Enterobiasis* in Iraq

In Iraq, an epidemiological study was carried out in Erbil province, showed that the incidence of *E.vermicularis* was 29.8% among primary school children (Hama and Rahemo, 2014),that the prevalence of *Entrobiasis* infection was (32%) by Vaseline-paraffin swab method in Babylon and Karbala governorates (AL-Masudy *etal* .،2004). The results of demonstrated the infection with pinworms was more high in population of marshland (70.75%) when compared with previous studies (31.2%), (32%), (37.12%), (49.05%) carried out in Kirkuk, karbala, Najaf and Babylon cities (kadir *etal* .،1999; AL-Masudy .،2004; AL-Quraishi.،2006; AL-Esawi .،2010).

Another study in Sulaimania province in Kalar town among children revealed that the prevalence of infection were 24.9%out of 1008 preschool and school children (Kadir and Amin, 2017).

A high prevalence of *E. vermicularis* was recorded in Wasit Province, and 62% of cases gave positive results by direct microscopic examination, and 52% of cases gave positive results by Scotch tape (Rahi and Morad, 2017).

In Al-Qadisiyah city the prevalence with *E. vermicularis* was 34% (Alwaily *et al*., 2019). The prevalence of *E. vermicularis* was detected as 6.2% among appendicitis patients in Samarra city (Al-Ammash *et al*., 2019).

epidemiological study Al-Masudy, refer In an epidemiological study in Basrah marshes villages, the prevalence of infection with *Enterobius vermicularis* was (50.32 % and 44.6%) in Abu-Malah and Harer village respectively (Jarallah *etal* .¸2012).

In Thi-Qar province south of Iraq prevalence infected 53(7.80%)out of 681 children (KHAZAAL *etal* .¸2020) .In Baghdad orphanage was 84.31% (Al-Qadhi *etal* .¸2011).In Al-Najaf city prevalence was 37.8% (Al-Qadhi *etal* .¸2011).In AL-Diwaniyah city total infection rate was 73.77% (Dohan *etal* .¸2022).

1.2.7.Pathogensis of *Enterobius vermicularis*

gastrointestinal nematode *E. vermicularis* also appears to be the oldest parasitic helminth to infect ancient populations(Paknazhad *etal.*¸2016).

pinworm infection may be symptomless in most patients, some of them may suffer perianal pruritus, insomnia, restlessness, and irritability,

particularly children .It should be stressed that pinworms may cause serious morbidity such as appendicitis and eosinophilic enterocolitis, and sometimes ectopic infections can result in pelvic inflammatory disease or urinary tract infections in females(Altun *et al*., 2017; Tsai *et al*.,2018).

Some evidence exists that *E. vermicularis* infestation may be a cause of secondaryenuresis(AlQadhi*etal*.,2011)

If autoinfection does not occur, pinworm infection is self-limiting due to the short life span of adult worms (Mehlhorn *etal* .,2012);(Centers for Disease control and prevention 2018)The main symptom of infection is pronounced (peri-)anal pruritus, which occurs primarily at nighttime while the affected individual sleeps. This can lead to disturbed sleep, childhood enuresis (in up to 53% of cases) and impaired concentration during the day. In some cases, childhood developmental disorders have been linked to enterobiasis (Out-Bassey *etal* .,2005).

Scratching in the perianal region can cause ulceration (excoriation) that shows a tendency toward bacterial superinfection. Anal dermatitis, perianal folliculitis, or ischiorectal abscess may develop. Very rarely, pinworms also migrate to the vaginal area, where they can cause vulvovaginitis or be indirectly responsible for urinary tract infections due to adherent enterobacteria such as *Escherichia coli* (Eder *etal* .,2018).

The role of *E. vermicularis* in relation to the pathogenesis of some cases of acute appendicitis has been the subject of controversy for many years, despite the fact that no causality has been reliably demonstrated (Fleming CA *etal* .,2015; Vleeschouweers *etal* .,2013)

Extraintestinal infection patterns in the vagina, urinary bladder, peritoneum, kidneys, liver, and eye have been described in isolated cases(Babady *etal* .¸2011; Kiliç S *etal* .¸2014).

Enterobiasis can sometimes also overlap with the clinical picture of chronic inflammatory bowel disease. Invasive systemic infections do not occur even in severely immunosuppressed patients. Alongside the intense itch, the disease is also characterized by marked psychosocial strain. that known to be related to such kind of morbidity . (AL-saffar *etal* .¸2015)

These diseases pose public health problems of high, not only medical, but also social and economic importance .These diseases display high prevalence in poor communities and especially in school and nursery children as a general population, since such conditions produce severe health problems and are associated with growth deficiency, anaemia, vitamins deficiency, and diminished general health status (Papier *etal* .¸2014).

The parasitosis causes some complaints such as restlessness, insomnia, perianal itching especially occurring at nights, nocturnal enuresis, teeth grinding at night, nasal itching, mouth drooling to the pillow at nights, involuntary movements. The seconder infection may occur in the itchy areas. Less often pseudo-meningitis findings can be seen depending on meningeal irritation as a result of allergic reactions developing against the excretas of the parasite. Particularly in girls, depending on adults entering to the vagina the vulvar itching and vulvovaginitis may occur, depending on adults entering to the urethra the urethritis may occur. (Garcia-Zamalloa *etal* .¸2012)

All *E. vermicularis* stages grow in the gastrointestinal tract, hence the host does not suffer from any systemic reaction until the worm burden become high, or there is ectopic infection, and as a result many cases are

asymptomatic. The worm triggers a mild, local inflammatory response, also when eosinophilic colitis has been described, circulating eosinophilia does not develop (Despommier *et al*., 2017).

In rare cases, *E. vermicularis* can lead to inflammation that will appear as ulceration in colon which may mimic tubercular ulcers, this ulceration because of the worms attach themselves to the mucosa using their heads and this necessary for pinworm to be invasive, there are two primary types of lesions that associated with tubular ulceration, ulcerative and ulcerohypertrophic. It is believed that *E. vermicularis* cannot penetrate the intestinal mucosa unless there is some insult to the mucosal barrier (Mukherjee *et al*., 2015).

Pinworms can mimic other serious diseases like colon carcinoma, Crons' disease (Johansson *et al*., 2013; Zouari and Mhiri, 2019).

The gravid female worm can move and wander through the female genital tract through the vagina and uterus causing vaginitis, cervicitis, endometritis, myometritis, and oophoritis which require surgical intervention (Ngui *et al*., 2014).

Central nervous system (CNS) infection can be caused Central nervous system (CNS) infection can be caused by *E. vermicularis* and this infection could have persisted for long period, attributed to immunological response due to malnutrition or continues reinfection as in bad hygiene or low socioeconomic status (Kandi *et al*.,2019).

E.vermicularis can increase the risk of developing psychiatric disorders in patients with pinworm infections (Chao *et al*., 2019). Also there is association between enterobiasis and nocturnal enuresis among children specially in crowded institutes like orphanages, kindergartens (Hussein, 2015).

Enterobiasis is one of many parasitic infections that cause malnutrition among children which is serious public health problem specially in developing countries (Obaid, 2015)

This infection can also cause significantly decrease in hemoglobin concentrations among infected children causing anemia with other low parameters of anemia including packed cell volume (PCV) and red blood cells (RBCs) (Hama and Rahemo, 2014).

1.2.8.Treatment and prevention

Only the two benzimidazole derivates, mebendazole and albendazole, are both adulticidal and ovicidal and are therefore considered to be the most effective drugs. (Berner *etal* .,2018)

In the case of persistent infection of the urogenital system, treatment with the systemically effective substance albendazole (or ivermectin) can be considered in specialized centers (Kashyap *etal* .,2014) The literature reports eradication rates of 94%–100% for albendazole and 53%–85% for ivermectin Intestinal lavage, "garlic cures," Karlovy Vary salts, and appendectomy (in the absence of appendicitis) are all obsolete (Wen LY *etal*.,2008)

Or Piperazine, Pyrantel pamoate 11 mg/kg body weight as a single dose. And Albendazole 400 mg as a single dose more than 2 years. Children (1-2 years) Albendazole 200 mg as a single dose (Sangeeta *et al.,* 2009; Cook and Zumla, 2009).

The whole family should be treated and treatment repeated two to four weeks later to eradicate any worms hatched since first treatment (Strobel *et al.*, 2006).

Complete eradication of pinworm infection from a population is highly unlikely. Personal hygiene is the most effective deterrent.

Fingernails should be cut short, and hand should be washed thoroughly after toilet use and before food is prepared or eaten. Since infection is most prevalent in urban areas where relatively large population intermingle, education of parents has proven most effective Parents should be informed that it is a self-limiting non-fatal infection, widespread among children and that no social stigma should be attached to it. There is no evidence that dogs can transmit the infection. Infected children as well as other members of the household should be treated promptly. Bedclothes, towels, and washcloth from infected homes should be carefully laundered in hot water and aired in sunlight (Bogitsh *et al.*, 2012).

1.2.9 Immune Response

1.2.9.1 Impact of Host Immune Response to Helminthic Infection

Helminthic infections are the leading cause of chronic infections in humans, which are characterized by the ability to survive for many years within the host cell (Van Riet *etal.*¸2007) .Some of those may not be pathological unless exposed by aggravating factors. The question is how those helminths can survive for a prolonged time within the immunocompetent host cells harmoniously? What substances of the helminthic products are involved in immunomodulation? This is because of the development of sophisticated survival strategies. Modulation and manipulation of our immune system are supposed to be the main mechanism/strategy for survival. (Helmby *etal.*¸2009)

The immune responses in enterobiasis infected children include higher concentrations of eosinophil cationic protein (ECP), eosinophils, and immunoglobulin E (IgE) in infested than in healthy children,

demonstrating a type-2 immune response activation for the period of infection. Eosinophilia may be a characteristic of pinworm infections in pediatric patients, even in the lack of classic symptoms. Physicians who inspect patients with gastrointestinal disorder and unexplained eosinophilia should consider enterobiasis as part of their differential diagnosis(Patsantara *et al*., 2016 ; Kara and Volkan, 2018) .

Most helminthic infections stimulate maturation of Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) along with the deterring of Th1 cytokine response (IL-12 and interferon-gamma (IFN-γ)(Else *et a*l .،1994) In response to this, the activity of IgE, mast cell, and eosinophil will be initiated. The stimulated IgE diminishes the worm's fitness as well as its fecundity via antibody-dependent cellular cytotoxicity (ADCC). Mast cell degranulation and release of protease helps the degradation of the junction of the epithelial cell layer and participates in the weep and sweep process. Most of the intestinal helminthic infections are responsible to induce the maturation of Th2 cytokines (producing IL-4 and IL-13), which promotes worm expulsion from the gastrointestinal tract (GIT). The signaling cascade of IL-13 and IL-4 is through the activation of the IL-4Rα-STAT6 in the intestinal mucosal epithelium that enhances goblet cell differentiation and mucus production (Artis *etal* .،2008 ; Babu s *etal* .،2019)

Similar to Th2 cells, alarmins and parasite-derived ES products can also stimulate innate lymphoid cells (ILC2s) and secrete Th2-related cytokines, including IL-5, IL-9, and IL-13. Consequently, it plays a key role in the expulsion of the parasite from the intestinal lumen by promoting goblet cell mucus secretion and smooth muscle contraction that increases its permeability for flushing of the parasite in the lumen of

the intestine. These modes of host cell immune response against the helminthic infection in the intestinal epithelium are called the “weep and sweep” process. Moreover, Th2 related cytokines produced by ILC2 contribute to airway hyper-reactivity and acute allergic immune response(Lambrechat *etal* .¸2012)

In addition, the tuft cell is a brush cell of epithelial layers of many hollow organ systems like the intestine and respiratory system. these cells are a potent source of alarmin cytokines (IL-25) and eicosanoids associated with allergic immunity, and the neurotransmitter acetylcholine required to coordinate type 2 immunity during helminthic infection (Fallon *etal* .¸2006; Von Moltke *etal* .¸2016)

Moreover, IL-33 and IL-18 as members of the IL-1 superfamily act as alerting immune molecules to the injured epithelial cell and generate a strong type 2 immune response. In addition, after it binds with the IL-1 receptor-like 1 (IL1RL1), also known as the ST2 receptor, found on the membrane of Th2 cells and ILC2s, IL-33 plays a role in inducing proliferation and repairing of the epithelium results in healing of the mucosa of the gut(Neill *etal.*¸2010).

Besides this, when a patient is previously exposed to helminths, T regulatory cell (Treg) generates immunoregulatory cytokines (TGF-β and IL-10) to decrease T and dendritic cell responses. Modified Th2 activitates the generation of TGF-ß, IL-10, and antigen-specific IgG4 that has a major effect by inhibiting IgE-associated allergic reactivity for helminth antigens. Hence, permanent antigenic infection with the parasite is related to a Th2 modified activation, which may decrease allergies (Medeiros *etal* .¸2003).

It has been reported that helminth infections may induce an evolutionary benefit by preserving host immune tolerance via modulation of immune pathway. Chronic helminth infection induces the activations of Th2 cells and T regulatory cells which shift the activation of adipose tissue macrophage from classical activation into an alternative activation. Alternatively activated macrophages (AAMs) secret anti-inflammatory cytokines such as IL-10, 1L-4, 1L-5, and IL-13 which per se inhibit pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. At first helminth infection provokes epithelial cells to secret alarming cytokines (IL-25, IL-33) which activate specific innate lymphoid cells (nuocytes) to produce anti-inflammatory cytokines in particular IL-13, IL-5 which initiate Th2 cells activations.(Neill *etal* .,2010).

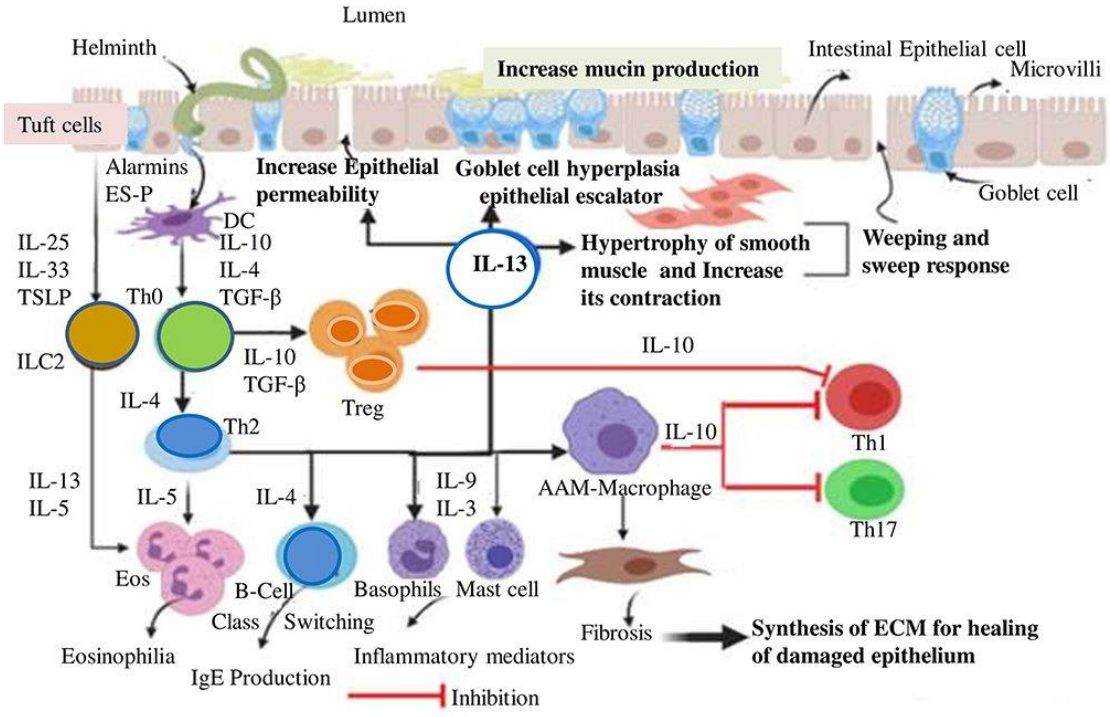

figure (1-3) Immune response to Helminth infection (Ayelign *etal* .,2020)

1.2.9.2 Interleukin :

Interleukins (IL) are a type of cytokine first thought to be expressed by leukocytes alone but have later been found to be produced by many other body cells. They play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. They also have pro-inflammatory and anti-inflammatory properties. The primary function of interleukins is, therefore, to modulate growth, differentiation, and activation during inflammatory and immune responses. Interleukins consist of a large group of proteins that can elicit many reactions in cells and tissues by binding to high-affinity receptors in cell surfaces. They have both paracrine and autocrine function. Interleukins are also used in animal studies to investigate aspect related to clinical medicine.(Akdis .,2011)

Interleukin-1 (IL-1)

The IL-1R family consists of 10 type-1 transmembrane proteins with a similar structure, which includes three Ig-like domains (D1, D2, and D3) responsible for ligand binding in an extracellular portion, a transmembrane domain, and an intracellular portion with the Toll-IL-1-receptor (TIR) domain, important for the initiation of signaling (Boraschi *etal* .,2018; Dinarello *etal* .,2019) .

It should be noted that pro-inflammatory cytokines from the IL-1 family, such as IL-1 β or IL-18, are produced in the form of inactive precursors, and their full activation requires the action of inflammasomes (NLRP3 and AIM2) and caspase 1 or the proteolytic activation of the cytokine in the extracellular environment (Dinarello *etal* .,2019)

Interleukin 1 (IL-1) the broad cytokine family, located on chromosome 2q12 made up of 17 kda (IL-1α IL1β) mature

proteinsWhich activate innate immune defenses to protect the body against infectious and non-infectious diseases It also plays a substantial role in tissue repair, cell growth and chronic inflammatory diseases Family of IL-1 composed of 11 member proteins (IL-1F1 to IL-1F11) (Dinarello *etal* .,2010).

IL-1β s mainly produced by DCs monocytes tissue macrophages, but it is also produced by B cells and Nk cells, endothelial cells, lymphocytes, smooth muscles and fibroblasts. IL-1β involves in the both the acute and chronic inflammation It is one of the most potent cytokines influencing almost every organ by activating a wide spectrum of cytokines and chemokine (Bird *etal* .,2002 ; Miller *etal* .,2006 ; Gabay *etal* ., 2010).

High circulating IL-1β levels contribute development immune response by stimulation of production of different in the host response to disease development (Lagathu *etal* ., 2006).

Macrophages, large granular lymphocytes, B cells, endothelium, fibroblasts, and astrocytes secrete IL-1. T cells, B cells, macrophages, endothelium and tissue cells are the principal targets. IL-1 causes lymphocyte activation, macrophage stimulation, increased leukocyte/endothelial adhesion, fever due to hypothalamus stimulation, and release of acute phase proteins by the liver. It may also cause apoptosis in many cell types and cachexia.(zhu *etal* .,2017)

Interleukin-2 (IL-2)

Interleukin-2, known as a major T-lymphocyte growth factor, promotes proliferation and maturation of activated T cells as well as

controls B cell proliferation and natural killer (NK) cell cytolytic activity IL-2 is the first short-chain type I cytokine, for which the receptor structure has been discovered (Malek *et al* .,2010; Damoiseaux etal .,2020)

T cells produce IL-2. The principal targets are T cells. Its primary effects are T-cell proliferation and differentiation, increased cytokine synthesis, potentiating Fas-mediated apoptosis, and promoting regulatory T cell development. It causes proliferation and activation of NK cells and B-cell proliferation and antibody synthesis. Also, it stimulates the activation of cytotoxic lymphocytes and macrophages(Bachmann *etal* .,2007)

Interleukin-2 (IL-2) is a cytokine that controls the activitiers of white blood cells (leukocytes, especially lymphocytes) that are respoonsible for immunity. It constitutes part of the body's natural response to microbial infection (Miller *et al*., 2009).

pathways activated by IL-2 occur via three major signal transduction systems, the JAK-STAT pathway, the PI3K/AKT pathway, and the MAPK pathway, leading to the transcription of target genes that contribute to IL-2-dependent biological activity (Damoiseaux *etal* .,2020)

On a functional level, growth-promoting effects of IL-2 on different lymphocytes, which have been widely explored in in-vitro studies, lie in striking contrast to lymphoproliferation and autoimmunity that characterizes in-vivo genetic deletion of IL-2 or its signalling chains (Suzuki *et al.*, 1995; Willerford *et al.*, 1995).

Espite the fact that interleukin-2 (IL-2) and its receptors represent one of the most extensively studied cytokine signalling systems,

unexpected findings emerging from therapeutic manipulations of this system in-*vivo* cannot be explained by simple conceptual models. Instead, mathematical modelling is likely required to elucidate the varied effects that IL-2 exerts on the immune system(Boyman and Sprent, 2012; Malek andCastro, 2010)

Interleukin-10 (IL-10)

Interleukin 10(IL-10) is a pleiotropic cytokine that is produced by different cell types, including myeloid ceglls (dendritic cells, macrophages, eosinophils, neutrophils, and mast cells) and lymphoid cells (NK, B cells, and T cells) with broad anti-inflammatgory activity. Macrophages and myeloid dendritic cells pathways such as TLR3 and TLR4, by stimulation with dsDNA and LPS, respecktively (Boonstra *et al.*, 2006).

Th2 cells produce IL-10. Its principal targets are Th1 cells. It causes inhibition of IL-2 and interferon gamma. It decreases the antigen presentation, and MHC class II expression of dendritic cells, co-stimulatory molecules on macrophages and it also downregulates pathogenic Th17 cell responses. It inhibits IL-12 production by macrophages.(Belghith M *etal* .,2018 ;Couper *etal* .,2008)

Structurally, IL-10 belongs to the class II cytokine family, which, and interferons (IFN-α, -β, and -γ). Tregs also can exert their actions by se10 (IL-10) and transforming (Ouyang and O'Garra, 2019)

IL-10 is a potent cytokine with multiple pleiotropic effects on immunoregulation It is produced predominantly by leukocytes including T and B lymphocytes, monocytes, macrophages, and DC, as well as by some epithelial cells. The anti-inflammatory activity of IL-10 includes,

inter alia (i.a)., downregulating the expression of MHC class II molecules and co-stimulatory molecules on monocytes and macrophages, as well as reducing the production of pro-inflammatory cytokines and chemokines .Dysregulation of IL-10 production is associated with increased immunopathology in response to infection as well as an increased risk of developing various autoimmune disorders(Couper *etal* .,2008; Lyer *etal* .,2012)

IL-10 is a cytokine of Th2-type activation, produced from AAMs, it involves in the suppression of pro-inflammatory cytokines mainly IL-6 and TNF-α . IL-10 is an antiinflammatory cytokine. During infection it inhibits the activity of Th1 cells, NK cells, and macrophages. Regulatory cytokine interleukin-10 (IL-10) has been proposed as a key molecule involved in the attenuation of helminth infection and chronic inflammation (Tabri *etal* .,2016) .

1.2.10 . Laboratory diagnosis of *Enterobius vermicularis*

Diagnosis of pinworm is made by identifying the worm or its eggs by direct visualization of female adult worms near anus or on sheets or underclothing at night , about 2-3 hours after patient falls asleep , by microscopic identification of worm eggs by using the (scotch tape test). The adhesive side of clear transparent not translucent cellophane tape is pressed to the skin around the anus at the first morning , before washing or get bathing . The tape is then directly fixed to a microscope slide and examined for eggs under low power (Mathieu, 2011).

Serological techniques are of no diagnostic significance , likewise neither blood eosinophilia nor raised immunoglobulin E (IgE) levels are

usually predictable due to the low invasiveness of the pinworms (Wendt *et al*., 2019).

Vaginal smear can be also used to diagnose *E. vermicularis* in rare cases of female genital tract involvement with this parasite, the present of the ova in cervicovaginal smears has been reported in certain cases of vaginitis (Kidambi and Lee, 2018; Shetty *et al*., 2012).

The diagnosis can be made during endoscopy and the pinworm can be visualized during a screening colonoscopy of asymptomatic patients (Kolli *et al*., 2019). Colonoscopy test can be used to examine patients that suffering from eosinophilic enteritis as a result to *E. vermicularis* infection, this test can show a purulent discharge from the rectum until the terminal ileum and ulcerations (Despommier *et al*., 2017).

The gold standard for diagnosing *Enterobius vermicularis* infection is the Scotch tape test. However, detection of *E. vermicularis*-specific DNA in stool might offer a more functional approach to confirmed the diagnosis (Rune *et al*., 2014). Polymerase Chain Reaction (PCR) technique was useful to confirm the DNA of *Enterobius vermicularis* (Dawood *et al* .,2016).

Chapter Two Materials and Methods

2. Materials and methods

2.1. Materials

2.1.1. Equipments and Instruments

Equipments and Instruments that used in the present study with their manufacturer and Origin was shown in table (2-1).

Table (2-1): Equipments and Instruments.

No.	Instrumnts and Equipments	Company	Origin
1.	Automatic Micropipette	Slamed	Germany
2.	Beaker	Slamed	Germany
3.	Centrifuge	Hettich	Germany
4.	Cool box	Cool box	India
5.	Cotton	MAY	Turkey
6.	Cuvette in optical glass	Citoclase	China
7.	Cylinder	Lab	Germany
8.	Deep freeze	Argelik	Turkey
9.	Disposable pipette tips	Biobasic	Canda
10.	ELISA reader	Biocheck	USA
11.	ELISA washer	Biocheck	USA
12.	Eppendorf- tubes (1.5)	Afco	Jordan
13.	Filter paper	Afco	Jordan
14.	Flat-head Vortex mixer	Elabascience	USA
15.	Gel tubes	Afco	Jordan
16.	Gloves	Sempermed	Thailand

17.	Graph paper	CALBIOTECH	Italia
18.	Incubator	Memmert	Germany
19.	Micro ELISA Plate	Elabascience	USA
20.	Microplate Reader		
21.	Plan tubes	Afco	Jordan
22.	Plate shaker	Elabascience	USA
23.	Precision pipettes	CALBIOTECH	Italia
24.	Rack	Afco	Jordan
25.	Refrigerator	Hettich	Europe
26.	Stick	Citoclase	China
27.	Syringes (5cc)	Medijecte	China
28.	Timer	Citoclase	China
29.	Volumetric flask 100ml	Lab	Germany
30.	Vortex mixer	Slamed	Germany
31.	Water path	Lab	Germany

2.1.2. Laboratory Kits

The kits that used in the present study with their companies and countries of origin are listed in table (2-2).

Table (2-2) : laboratory kits.

Number	Kits	Company	Origin
1	Human Interleukin 10 ELISA kit	BT	China
2	Human Interleukin 2 ELISA kit	BT	China
3	Human Interleukin 1Beta ELISA kit	BT	China

2.2. Methods

2.2.1. Ethical approval and study design:case control study .

The necessary ethical approval was obtained by verbal consent from mothers of patients. This study was approved by the committee of publication ethics at college of medicine , Babylon university , Iraq،this study was a case – control study .

Study duration and samples 2.2.2.

During the period of time from September 2021 to end of March 2022 , a total of eighty seven children participated and thirty control and their ages ranged between (2-13)years old , included both sex (37males and 50females), the chosen areas for sampling included AL- Noor hospital for children, Emergency Babylon hospital for maternity and children , the second AL Hilla sector , and villages and countryside in Hilla city, Iraq. Aspecial questionnaire form was prepared for each participant child in the study and filled out by interviewing their mothers.

.2.2.2.1. Cellophane tape sample collection

The samples were collected by using cellophane tape technique by pressing the sticky side of the tape several times on the anal and perianal region of the children and then sticking the tape on the labeled glass slide and putting it in a sterile clean nylon envelope and then enclosing it tightly . This method was carried out with the help of the children's mothers at night or in the early morning before defecation , using the toilet or taking bath. The collected samples were examined under light microscope(AL-Jaf .،2018)

2.2.2.2.Blood sample collection .

Three milliliter of venus blood was collected from each child infected with *E. vermicularis* and healthy (control group).The blood sample was placing in gel tube, the gel tube was left standing for 15-20 minutes at room temperature to clot, then the tubes were centrifuged at 3000 rpm for 10 minutes to collect the serum.The serum obtained was added in eppendorf tubes (200µl) into many alquites for different tests to avoid repeated freezing and thawing of the samples which is not recommended because this may affect the quality of the results. All sera were stored at-20^{0}C until being analyzed for immunological . After completing the sampling , all samples were simultaneously extracted from the freezer and tested

2.2.2.3.Inclusion and Exclusion criteria

The inclusion criteria was all patients were clinically infected with *E. vermicularis* while exclusion criteria included patients with other-intestinal protozoal infections, and chronic diseases as well as immunocompromised patients**.**

2.2.3. Immunological Tests

Several tests were included in this study especially immunological tests involved IL-1B¸IL2 and IL-10¸collected blood sample of patients infected with *E. vermicularis* and healthy controls ¸about (87 *E. vermicularis* and 30 sample as control)

2.2.3.1. Human Interleukin 1Beta (IL-1B) ELISA Test

A. Assay Principle:

This Kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IL-1B antibody. IL-1B present in the sample is added. Substrate solution is then added and color develops in proportion to the amount of human IL-1B. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B. Reagent Provided:

The components of reagent were used in the present study was shown in table (2-3)

Table (2-3): Components of reagent of IL-1 Beta test

1. Standard Solution (2400ng/L)	0.5mlx1
2. Pre-cated ELISA Plate	12*8 well strips x1
3. Standard Diluent	3ml x1
4. Streptvidin-HRP	6ml x1
5. Stop Solution	6ml x1
6. Substrate Solution A	6ml x1
7. Substae Solution B	6ml x1
8. Was Bufer Concentrate (25x)	20ml x1
9. Biotnylated human IL-2 Antibody	1ml x1
10. User Instruction	1
11. Plte Sealer	2 pics
12. Zpper bag	1 pic

C. Asay Procedure:

1. all reagents,was prepared standard solutions and samples as instructed. Bring all reagnts to room temperature before use. The assay is performed at room tmperature.

2. the number of strips required for the assay. Insert the strips in t frames for use.

3. Afiften ml was added to standard well

4. A fourty µlwas added sample to sample wells and then add 10µl anti-IL-10 antibody to sample wells control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37ºC.

5. The sealer was removed and washed the plate 5 times with wash buffer. Soak wells with at least 0.35 all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material

6.Then Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. plate covered with a new sealer for 10 minutes at 37ºC in the dark.

7.After that add 50 µl Stop Solution to each well, the blue color will change into yellow immediately.

8.finally Determine the optical density each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution

D.Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based

curve-fitting software and the best fit line can be determined by regression analysis.

2.2.3.2. Human Interleukin 2 (IL-2) ELISA Test

This sandwich method kit to the exact quantitative specific of human interleukin 2 in serum

A. Assay Principle:

This Kit is an Enzyme-Linked Immunosorbent Assay (ELISA).
The plate has been pre-coated with human I-2 antibody. IL-2 present in the sample is added. Substrate solution is then added and color develops in proportion to the amount of human I2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B. Reagent Provided:

present study was shown in table (2-4). The components of reagent were used in the

Table (2-4): Components of reagent of IL-2 test

Comonents	Quantity

1. Standard Solution (2400ng/L)	0.5mlx1
2. Pre-coted ELISA Plate	12*8 well strips x1
3. Standard Diluent	3ml x1
4. Streptvidin-HRP	6ml x1
5. Stop Solution	6ml x1
6. Substrate Solution A	6ml x1
7. Substae Solution B	6ml x1
8 Bufer Concentrate (25x)	20ml x1
9. Biotnylated human IL-2 Antibody	1ml x1
10. User Instruction	1
11. Plte Sealer	2 pics
12. Zpper bag	1 pic

C .Assay procedure:

1. All reagents,was prepared standard solutions and samples as instructed. Bring all reagnts to room temperature before use. The assay is performed at room tmperature.

2. The number of strips required for the assay. Insert the strips in t frames for use..

3. Afiften ml was added to standard well

4. A fourtyµlwas added sample to sample wells and then add 10µl anti-IL-10 antibody to sample wells control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37ºC.

5. The sealer was removed and washed the plate 5 times with wash buffer. Soak wells with at least 0.35 all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material

6.Then Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. plate covered with a new sealer for 10 minutes at 37ºC in the dark.

7.After that add 50 µl Stop Solution to each well, the blue color will change into yellow immediately.

8.finally Determine the optical density each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution

D.Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting

2.2.3.3.Human Interleukin 10(IL- 10) Elisa kit

This sandwich method kit is to the exact quantitative specific of human interleukin 10 in serum

A. Assay Principle:

This Kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human anti IL-10 antibody. IL-10 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human congucated IL-10 Antibody added to immune complex and then added the substrated . the reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B. Reagent Provided:

The components that were used in the present study was shown in table (2-5).

Table (2-5): Components of reagent of IL-10 test

Components	Quantity
1. Standard Soluton (1600pg/ml)	0.5mlx1

2. Pre-coated ELISA Plate	12*8 well strips x1
3. Standard Dilunt	3ml x1
4. Streptavidin-RP	6ml x1
5. Stop Solution	6ml x1
6. Substrate Slution A	6ml x1
7. Substrate olution B	6ml x1
8. Wash Bufer Concentrate (25x)	20ml x1
9. Biotinyled human IL-10 Antibody	1ml x1
10. User Instruction	1
11. Plate ealer	2 pics
12. Zippr bag	1 pic

C.Assay Procedure:

1. All reagents,was prepared standard solutions and samples as instructed. Bring all reagnts to room temperature before use. The assay is performed at room tmperature.
2. The number of strips required for the assay. Insert the strips in t frames for use..
3. Afiften ml was added to standard well
4. A fourtyµlwas added sample to sample wells and then add 10µl anti-IL-10 antibody to sample wells control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37ºC.
5. The sealer was removed and washed the plate 5 times with wash buffer. Soak wells with at least 0.35 all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material
6. Then Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. plate covered with a new sealer for 10 minutes at 37ºC in the dark.

7. After that add 50 µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Finally Determine the optical density each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

D.Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting

2.3.Data analysis:

Statistical analysis was carried out using SPSS version 27.
Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. ANOVA test was used to compare means between four groups. Pearson Chi-square test was used to find the association between categorical variables. A p-value of ≤ 0.05and≤ 0.001 was considered as significant (Al-Ukaelii and Al-Shaeb, 1998).

Chapter Three Results and Discussion

3. Results

3.1. Distribution of patients with *E. vermicularis* according to diagnosis methods.

The frequency distribution of patients with *E. vermicularis* according to diagnostic method has been carried out and the results were demonstrated in table (3-1). The present result showed all infected clinically patients 87 (100.0%) have positive diagnostic results, while the patients with laboratory examination was 53 (39.08%) have positive diagnostic results.

and healthy Table (3.1):Distribution of patients with *E. vermicularis* control according to diagnosis method.

Diagnosis	Patients clinically diagnosis		Patients with lab. 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	

0.05 ≤*n*: number of cases; ¥: Chi-square test; S: significant at *P*

The present results showed that scotch tape was the best technique for detection of *E. vermicularis* eggs, because the tape picks up any egg present in the perineal area. The result is in agreement with most of the researchers in which they observed low rate of infection when they used tests other than scotch tape. Among them in Sulaimania (Kadir and Amin,2011) and in Erbil(Al-Daoody,2020) . This low rate is related to that during oviposition the female worm migrate out onto the perianal area to deposit her eggs, which commonly occur at night, so the eggs are rarely seen in feces . This study was performed using the Scotch tape technique, a well know, widely used, easy, safe and reliable technique(Jeandron *etal* .¸ 2010)

The infection caused by *E. vermicularis* is relatively innocuous. Nevertheless, eggs deposition may cause perineal, perianal, and even vaginal irritation, and infected persons may try to relieve the irritation of the constant itching, possibly leading to potentially debilitating sleep disturbance, impaired concentration, emotional instability, or enuresis ,Furthermore, these uncomfortable symptoms can result in weight loss, urinary tract infections, and even acute or chronic appendicitis which can lead to death without appropriate surgical treatment. Therefore, children who exhibit perianal pruritus and nocturnal restlessness should be suspected of having pinworm infection(Altun *et al*., 2017).

Anal pruritus was the most common symptom detected, being significantly associated with *E. vermicularis* infection, whose eggs need about 6 hours for hatching in the anus . Anal pruritus plus finger sucking, a child behaviour also found to be significantly associated with enterobiasis, could increase the rate of anal-oral contamination(Meletis *et al.,* 2019).

3.2. Distribution of patients with *E. vermicularis* according to sex .

The frequency distribution of patients with *E. vermicularis* according to sex has been carried out and the results were demonstrated in table (3-2). Infected clinically patients included 37 males and 50 females (42.5% and 57.5 %) respectively, whereas, patients with lab. examination included 21 males and 32 females (39.6% and 60.4 %) respectively and there was no significant difference in the frequency distribution of infected clinically patients and patients with lab. examination according to gender (P = 0.735)

Table (3.2):Distribution of patients with *E. vermicularis* according to gender

Gender	Patients clinically Infected		Patients with lab. examination		χ^2	*P*
	N	%	*N*	%		
Male	37	42.5	21	39.6	0.115	0.735 ¥ NS
Female	50	57.5	32	60.4		
Total	87	100.0	53	100.0		

***n*: number of cases; ¥: Chi-square test; NS: non-significant at *P*≥0.05**

The relationship between the prevalence of *E. vermicularis* and sex in the current study showed that the risk of *E. vermicularis* infection was higher but not significant(p≥0.05) among female children using the adhesive tape method. These data support the findings of other studies that have reported a higher prevalence of *E. vermicularis* among female children such as Al-Daoody(2020) in Mosul City and Hammadi (2012) ,in the Al- Mahmoudyia area in Baghdad, the prevalence of *E. vermicularis* infection was higher among females than males with no statistically significant differences (Al-Yousofi *et al*., 2022)

while in contrast to the present study, other studies reported a higher prevalence among male children ,The result of Lee *et al*. (2002) obtained a total rate 35.3% (38.7% males of and 31% of female) the non-significant differences can be explained as both gender were lived in the same place and had the same chance in playing and eating as well as sharing the same toilets , so the exposure to infectious agent may not varied very much between genders while the high rate of infection in male than in female may be related to that males are spending their times outside home more than females so they have more chance to contact with infected children, thus acquiring the infection. to contaminated waters because they are typically responsible for obtaining water and house working for the family.

Agree with the higher infection in females can be justified by considering that females are more exposed to the infective stages of parasitic infection due to the nature of the chores they perform in the house and their lifestyle explained that the increase in female numbers was due to their daily housework, and thecontact with bed cloth and bed sheet for another infected family member (Merad *et al*., 2018).

E. vermicularis can infect the female genital tract, and for anatomic reasons it can migrate to fallopian tube causing an obstruction of one or both of them and leading to infertility The gravid female worm can move and wander through the female genital tract through the vagina and uterus causing vaginitis, cervicitis, endometritis, myometritis and oophoritis which require surgical intervention

3.3. Distribution of patients with *E. vermicularis* according to age groups .

The frequency distribution of patients with *E. vermicularis* according to age groups was shown in table (3-3). Age groups including (< 4 years, 4-6 years, 6-8 years, 8-10 years and ≥ 10 years) and laboratory assessment. From total eighty seven patients clinical diagnosed as *Enterobiasis* (N=53, 60.9%) were assessed by laboratory examination. The present findings show higher frequency distribution in groups more than 10 years age but the difference was non-significant, (p=0.932).

Table (3.3):Distribution of patients with *E. vermicularis* according to age groups

Age groups	Patients clinically Infected		Patients with lab. examination		χ^2	*P*
	N	%	*N*	%		

< 4 years	3	3.4	2	3.8	0.845	0.932 ¥ NS
4-6 years	11	12.6	6	11.3		
6-8 years	18	20.7	8	15.1		
8-10 years	20	23.0	13	24.5		
≥ 10 years	35	40.2	24	45.3		
Total	87	100.0	53	100.0		

***n*: number of cases; ¥: Chi-square test; NS: non-significant at P ≥ 0.05**

This difference in the rate of infection among age groups may be because the awareness of attention to personal hygiene is much lower among the small children in comparison to older children, as they spend more time outside the house. Therefore, they have more chances to play with dirt and have a greater frequency of physical contact with their friends than do younger children; thus, they have a higher risk of acquiring pinworm infection .Additionally, the anal scratching habit (by the way hand-mouth transmission) is more frequent among small children, facilitating the autoinfection mode of transmission(Khazaal *etal* ., 2020)

A number of studies have identified major risk factors for pinworm infection¸ children aged 4–11 years are particularly frequently affected, with male subjects sometimes being affected more often. A large percentage of children attend kindergarten or primary school during this phase. Close social contact, putting toys or writing utensils in the mouth, as well as nail biting (onychophagia/perionychophagia) play an important Li, role in exposure to *E. vermicularis* at this age(Cranston, *et al.*, 2015 ; *et al.*, 2015). Scratching in the perianal region, unchecked anus-finger-mouth contact, independent and unsupervised personal hygiene practices, as well as low compliance with regard to hand-washing prior to eating are all factors associated with significantly higher infection rates (Chen *et al.*, 2018)

Kim *et al*. (2003) showed in their study that the rate of infection at 6-9 years was 32% while the rate of infection at 9-12 years was 10%. The high rate of infection in school aged children than smaller aged children may be related to that school children are more likely to be in close contact with each other and are exposed to unsatisfactory sanitary environment Several authors have found enterobiasis prevalence to be significantly higher in 6-12 aged children than in children of 2 – 5 years, but the present study shows no statistically significant differences between the age groups, suggesting similar transmission patterns and risk conditions for acquiring pinworm eggs in all individuals.

It was reported that inadequate personal hygiene might increase the risk of pinworm infection among children, and significant factors associated with pinworm infection include playing on the floor, nail biting, failing to wash hands before meals, and living in non-apartment dwellings (fan *et al.*¸ 2021)

3.4. Distribution of patients with *E. vermicularis* according to family history.

The presence of family history is an important contributory factor in *E. vermicularis* infection. This study showed out of 87 participants were recruited in present study, 83 (95.4 %) of infected clinically patients have positive family history in compared to patients with lab. examination which show all patients 53 have positive family history, but the difference between infected clinically patients and patients with lab. examination was non-significant (p = 0.113), table (3-4) .while it was significant between positive family history and negative family history

Table (3.4):Distribution of patients with *E. vermicularis* according to family history

Family history	Patients clinically infected	Patients with lab. examination	χ^2	*P*

	N	%	*N*	%		
Positive	83	95.4	53	100.0	2.508	0.113 ¥ NS
Negative	4	4.6	0	0		
Total	87	100.0	53	100.0		
P	0.001< S					

0.05 ≤*n*: number of cases; ¥: Chi-square test; NS: non-significant at P ≥0.05; S: significant at P (significant difference between positive and negative family history).

Enterobiasis is transmitted from one person to another directly and does not require any intermediate host, it is more likely to spread among members of the same family, especially overcrowded families. In addition, clinically mild cases and asymptomatic infected individuals may provide a hidden reservoir of infection in the family population (Cranston *etal* .¸ 2015)Moreover, by increasing the number of family members, the possibility of bringing the infection from outside to inside of the home increases. *Enterobiasis* is easily transmitted among family members through inhalation, contaminated hands and fomites. Previous studies have shown that it is more prevalent in over-crowded areas or families with many members(Hamarsheh, 2021).

Enterobiasis infection among the family members as well as community and in most cases autoinfection of these parasitic diseases is influenced by the hygiene, sanitation and personal behavior (Stoyanova,2019)

Re-infection is one of the main factors of infection development because *E. vermicularis* has a very simple transmission cycle. It takes only 2–4 weeks for eggs to mature to adult worms. *E. vermicularis* can easily contaminate doorknobs, tables and chairs, toys, school equipment, and even dust. Susceptible children will be infected through close contact with the environment and contaminated children. Although some drugs

can prevent re-infection, hygiene can be useful along with drug administration , Health promotion as well as increasing child and family awareness will reduce the infection rate(Li *et al.* ,2015)

3.5. Distribution of patients with *E. vermicularis* according to treatment history

The frequency distribution of *E. vermicularis* patients according to treatment history has been demonstrated in table (3-5). The present result showed 79 (90.8 %) of infected clinically patients have treatment history in compared to patients with lab. examination 53 (100 %) have treatment history, and the difference was significant (p = 0.023), table (3-5).

Table (3.5):Distribution of patients with *E. vermicularis* according treatment history

Treatment History	Patients clinically infected		Patients with lab. examination		χ^2	*P*
	N	%	*N*	%		
Positive	79	90.8	53	100.0	5.169	0.023 ¥ S
Negative	8	9.2	0	0		
Total	87	100.0	53	100.0		
P	0.001< S					

0.05 ≤0.05; S: significant at P ≤*n*: number of cases; ¥: Chi-square test; S: significant at P (significant difference between positive and negative Treatment history).

Pinworm infection is considered as a group or family infection with all members of the household or institution being infected, especially under crowded conditions(Al-Qadhi.,2011). The whole family is thus treated to prevent re-infection. In pinworm infections, chemotherapy must be combined with education and personal hygiene aimed at preventing autoinfection. Although it is possible to effect a temporary cure, eradication may prove difficult because of re-infection from the contaminated environment or from asymptomatic members of the same household. Eradication may necessitate several repeated courses of

treatment up to a year or more. Further studies are necessary to understand the prevalence, distribution and the physical and psychological impact of pinworm infection in the population(Blake.,2003)

3.6. Distribution of patients with *E. vermicularis* according to history of enuresis

The frequency distribution of *E. vermicularis* patients according to history of enuresis demonstrated in table (3-6). The present result showed only 1 (1.1 %) of infected clinically patients have history of enuresis in compared to 3 (5.7%) patients with lab. examination have history of enuresis, but the difference was non-significant (p = 0.120), table (3-5).

Table(3.6):Distribution of patients with *E. vermicularis* according history of enuresis

History of Enuresis	Patients clinically infected		Patients with lab. examination		χ^2	*P*
	N	%	*N*	%		
Positive	1	1.1	3	5.7	2.415	0.120 ¥ S
Negative	86	98.9	50	94.3		
Total	87	100.0	53	100.0		
P	0.001< S					

0.05; S: significant at P > 0.05 >*n*: number of cases; ¥: Chi-square test; NS: non-significant at P (significant difference between positive and negative History of enuresis)

Nocturnal enuresis is probably due to ano-perineal irritation which provokes a prolonged and severe contracture of bladder sphincter which leads to muscle exhaustation. In sleep time the sphincter relaxes emitting urine.

Result of AL-Qadhi *et al.(*2011), Which study the enterobiasis and its relationship with enuresis they found the infection rate for enterobiasis was (84.31%) ,higher in male than female & significant relationship

between enterobiasis and nocturnal enuresis were investigated, the percentage of children who had pinworm infection and enuresis was 58.82%.

Enuresis may be due to many factors in children such as in physiological and emotional disturbances **(**Al-Shadood .,2015)

3.7. Distribution of patients with *E. vermicularis* according to residence

According to residency, infected clinically patients group included 15 (17.2 %) cases from urban areas and 72 (82.8 %) cases from rural areas, while patients with lab. examination group included 12 (22.6 %) cases form urban areas and 41 (77.4%) cases from rural areas and there was no significant difference in the frequency distribution of patients and control subjects according to residency (P = 0.432), table (3-7)

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Table(3.7):Distribution of patients with *E. vermicularis* according residence

Residence	Patients clinically infected		Patients with lab. examination		χ^2	*P*
	N	%	*N*	%		
Urban	15	17.2	12	22.6	0.617	0.432 ¥ NS
Rural	72	82.8	41	77.4		
Total	87	100.0	53	100.0		
P	0.001< S					

***n*: number of cases; ¥: Chi-square test; NS: non-significant at P ≥0.05 S: significant at P ≥ 0.05 (significant difference between urban and rural residence)**

results of Li *et al.*(2015) confirm that he prevalence of *Enterobiasis* in children from urban areas was 51.05 % , while it was 57.57 % in children from rural areas. However, the difference was not statistically significant ($\chi 2 = 3.341$, $p = 0.068$).

Finding of Chen *et al*.(2018) confirm a significant difference was observed in children who lived in the rural area, but not in other variables such as gender, age, and personal and house hold hygiene, is finding can be explained by that the student number per class in a school located in the rural area is more than that in an urban area,thus, the transmission rate could be high in such a crowded condition

Behavior and environmental factors have been to be important in the transmission of pinworm. A case-control study on school-age children in Central Taiwan revealed playing on the floor, nail biting, failure to wash hands before meals and living in nonapartment dwellings as significant factors(Sung *et al*., 2001). Positive rates in urban regions have been reported to be significantly lower than the suburban and rural counterparts(Kim, *et al*., 2013).

3.8 Immunological study

3.8.1 Serum Interleukin-1 beta (IL-1 B) level in patients with *E. vermicularis* and control groups.

The comparison of serum Interleukin-1 beta level between patients with *E. vermicularis* and healthy controls groups has been carried out and the results were demonstrated in table (3-8) and figure (3-3). Mean levels of serum IL-1 beta in patients with *E. vermicularis* were slightly higher than in comparison the mean levels of control groups, 4545.88 ± 1493.48 pg/ml versus 3439.56 ± 1965.60 pg/ml, but the difference was non-significant (p = 0.782).

Table (3.8.1): Serum Interleukin-1 beta (IL-1 B) level in patients with *E. vermicularis* and healthy control groups

IL-1 b (pg/ml)	*Enterobius vermicularis* patients	Healthy control	*P*
Mean ±SD	4545.88 ± 1493.48	3439.56 ± 1965.60	**0.782 † NS**
Range	2938.1 – 6392.3	1423.43- 6782.12	

0.05≥n: number of cases; SD: standard deviation; †: Independent T test; **; NS: non-significant at P**

The present study was in agree with the finding of Mohsen (2020)that showed high levels of IL-1β was increasing in patients which infected with intestinal parasite compared to healthy controls, but this results was not significantly .so Intestinal epithelial cell production of interleukin (IL-1B) causes an influx of inflammatory cells into the intestinal mucosa with result tissue damage(Zhang *et al*., 2000). Other study shown increasing of serum IL-I β during parasitic infection , The result of Al Gazali *et al*. (2014) showed a highly significant increase in the concentration of IL-1β cytokines in serum of patient infected with *T.vaginalis* compared to healthy control group. Increasing the (IL-1β) level maybe due to increasing the monocyte or macrophages which stimulated by *T.vaginalis* infection caused vaginitis lead to stimulated host immune response cellular and humeral.

It has been reported that helminth infections may induce an evolutionary benefit by preserving host immune tolerance via modulation of immune pathway. Chronic helminth infection induces the activations of Th2 cells and T regulatory cells which shift the activation of adipose tissue macrophage from classical activation into an alternative activation. Alternatively activated macrophages (AAMs) secret anti-inflammatory cytokines such as IL-10, 1L-4, 1L-5, and IL-13 which per se inhibit pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α (Yap and Gause , 2018). At first helminth infection provokes epithelial cells to secret alarming cytokines (IL-25, IL-33) which activate specific innate lymphoid cells (nuocytes) to produce anti-

inflammatory cytokines which initiate Th2 cells activations (Neill *et al*., 2010).

The finding by Abboud (2020) confirm the level of IL-1β in children infected with *E histolytica* was non-significantly increased in compared with control groups .

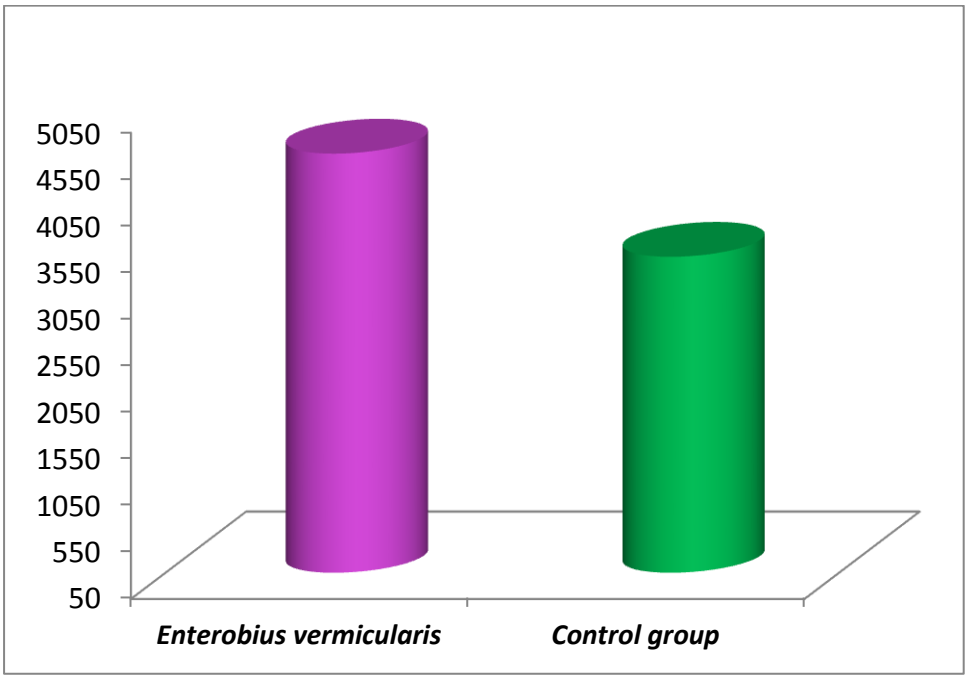

Figure (3-3): Mean difference of IL-1 beta levels according to study groups .

3.8.2 Serum Interleukin-2 (IL-2) level in patients with *E. vermicularis* and control groups.

The comparison of serum Interleukin-2(IL-2) level between patients with *E. vermicularis* and healthy controls groups has been demonstrated in table (3-9) and figure (3-4). Mean levels of serum IL-2 in patients with *E. vermicularis* were slightly higher in comparison the mean levels of

control groups, 971.16 ± 713.51 pg/ml versus 862.93±590.48 pg/ml, but the statistical analysis show No-significant difference (p = 0.459).

Table (3.8.2): Serum Interleukin-2 (IL-2) level in patients with *E. vermicularis* and healthy control groups

IL-2(pg/ml)	Cases –control comparison		
	***Enterobius vermicularis* patients**	**Healthy control**	***P***
Mean ±SD	971.16 ± 713.51	862.93±590.48	**0.459 † NS**
Range	231.64 – 1831.43	290.32- 1460.34	

0.05≥n: number of cases; SD: standard deviation; †: Independent T test; **; NS: non-significant at P**

The finding of Habieb (2021) showed the mean level of IL-2 was decrease in patient ¸infected with Toxoplasmosis ¸that explain there is areduction in the production of IL-2 which appear to mediate ¸the downregulation of T-cell derived cytokines .

Interleukin-2 (IL-2) is an interleukin, a type of cytokine signaling molecule in the immune system. It is a 15.5–16 kDa protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity. IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self". IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes. The major sources of IL-2 are activated CD4+ T cells and activated CD8+ T cells.(Liao *et al*.,2011).

IL-2 has acritical roles in immune system, tolerance and immunity, via its direct effects on T cells In thymus, where T cells mature, it can prevents autoimmune diseases by promoting the differentiation of certain immature T cells into regulatory T cells,. IL-2 enhances activation-

induced cell death (AICD). (Arenas-Ramirez *et al*., 2015) IL-2 also induce the differentiation of T cells into effector T cells and memory T cells when the initial T cell is also stimulated by an antigen, thus helping the body fight off infections.(Liao *et al*.,2011) Together with other cytokines, IL-2 stimulates naive CD4+ T cell differentiation into Th1 and Th2 lymphocytes while it impedes the differentiation into Th17 and Th lymphocytes.(Liao *et al*.,2013).

Previous studies have shown that IL-2, IL-4 and TGFβ are the primary factors that drive differentiation and expansion of Th9 cells , Moreover, this finding shown that Th2 and regulatory cytokines are increased in helminth infection(Kaplan *et al*., 2015).also study of Al Gazali *et al*. (2014) showed a highly significant increase in the concentration of IL-2 cytokines in serum of patient infected with *T.vaginalis* compared to healthy control group

the current work showed elevated the levels of IL-2 in the infected children. The chronic infection of helminth lead to the activations of T helper type 2 cells that transport the macrophage activation of adipose tissue from the classical activation into the alternative activation. The alternatively activated macrophages lead to secret various cytokines like IL-10, IL-2, IL-4 and IL-5.(Yap GS .،2018; AL-Kuraishi .،2020) Also, asymptomatic children with helminthiasis infections have good profile of immunological with a very high antiinflammatory cytokines (Anuradha .، 2016)

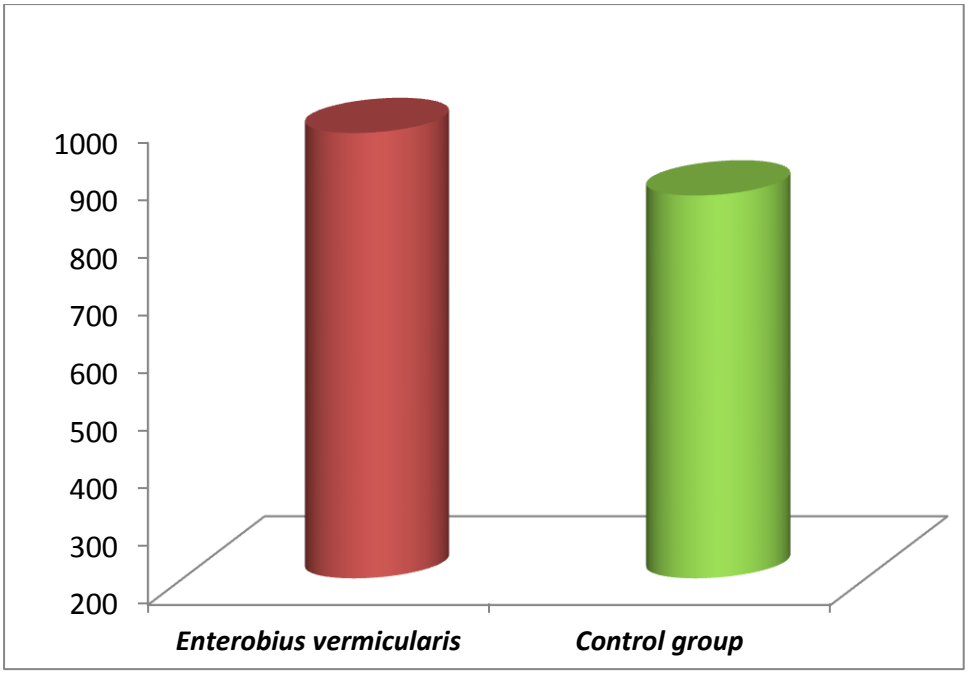

Figure (3-4): Mean difference of IL-2 levels according to study groups .

3.8.3 Serum Interleukin-10 (IL-10) level in patients with *E. vermicularis* and control groups.

The comparison of serum Interleukin-10 level between patients with *E. vermicularis* and healthy controls groups has been carried out and the results were demonstrated in table (3-10) and figure (3-5). Mean levels of serum IL-10 in patients with *E. vermicularis* were increase in comparison the mean levels of control groups, 616.51 ± 418.03 pg/ml versus 517.70±364.43 pg/ml, but the increasing was non-significant (p = 0.264).

Table (3.8.3): Serum Interleukin-10 (IL-10) level in patients with *E. vermicularis* and healthy control groups

	Cases –control comparison		
IL-2(pg/ml)	***Enterobius vermicularis* patients**	**Healthy control**	***P***
Mean ±SD	616.51 ± 418.03	517.70±364.43	**0.264 † NS**
Range	173.23 – 983.13	159.23- 899.23	

0.05≥n: number of cases; SD: standard deviation; †: Independent T test; **; NS: non-significant at P**

Results of Sanchez *etal* .(2015) confirm there were increasing in level of IL-10 in children infected with intestinal helminths in compare with children without intestinal helminth.

IL-10 is an anti-inflammatory cytokine that regulates the inflammatory process, It is produced by many different myeloid and lymphoid cells. During infection, more than 1 population of IL-10 producing cells are induced, resulting in inhibition of the activities of Th1 cells, natural killer cells, and macrophages. In allergic diseases, IL-10 is a down-modulatory factor that functions to induce modified Th2-cell phenotype. The observed elevated levels of IL-10 in preschool- and school-aged children are in line with the reports of Sanchez *et al*.(2015) This observed elevated IL-10 might be necessary to dampen excessive inflammation with a view to protecting host tissues. Schopf *et al*. (2002) shows that IL10 is important in controlling pathology associated with helminth infection, This IL-10 action, however, could facilitate host tolerance to helminth infection and cause immune hyporesponsiveness against chronic helminth infection. Thus, this allows the helminth to live for a long time if not treated.

In support for the current study observation, turner *et al*.(2008) have been shown enhanced production of the anti-inflammatory cytokines IL-10 and TGF-B1 when demonstrated that constatives levels of the regulatory cytokines (IL-10 and TGF-B1) are enhanced in direct relations to the intestinal worm burden and provide evidence that this elevation in anti-inflammtory cytokine secretion in peripheral blood induces immunological hyporesposivness (turner *et al*., 2008).

IL-10 can act directly on CD-4 T-cells, inhibiting proliferation and production of IL-2, IFN-α, IL-4, IL-5 and TNF-α , However, IL-10 can directly regulate innate and adaptive Th1 and Th2 responses by limiting T

cell activation and differentiation in the lymph nodes as well as suppressing proinflammatory responses in tissues, leading to impaired pathogen control and reduced immunopathology (Klüken *et al*., 2003).

Interestingly, helminthes participate and co-evolved with human beings over the centuries due to the mutual relationship benefit between humans and helminthes. Prolong helminthes infection attenuate different metabolic and autoimmune diseases, including asthma, inflammatory bowel diseases, and type diabetes mellitus (Guigas *et al*., 2015). The benefit effect of helminthes infections is through induction of anti-inflammatory effects, which prolong survive of the worms within human host, thus interruption of this mutual relationship between human and worms may increase the risk of allergic and autoimmune diseases(Harnett *et al*., 2017).

This finding was in accordance with AL-Ftlawy (2022) who mentioned that IL-10 is increased in serum obtained from patients with Ascariasis .also previous study by Kumar *etal* .(2020)have confirmed alink between chronic helminth infection and increased TH-2 type immune response .

3.9. Association among Interleukin (IL-1β, IL-2 and IL-10) with age group and gender.

The association between IL-1 beta with age group and gender has been carried out and the results were demonstrated in table (3-11). The present result indicate age group less than 6 years have high levels of IL-1 beta (3974.79 ± 1174.44) in compared with other age groups, but the difference was non-significant (p=0.278). Also the present results show patients with male gender have high levels of IL-1 beta in compared with

patients with female genders, (3859.12 ± 1889.49 vs 3340.32 ± 1152.42), but the difference also was non-significant (p=0.268).

Result of IL-2 indicate age group with 6-8 years old have high levels of IL-2 (1263.85 ± 980.53) in compared with other age groups, but the difference was non-significant (p=0.15). Also the present results show patients with male gender have high levels of IL-2 in compared with patients with female genders, (1065.68 ± 798.15 vs 729.88 ± 357.40), but the difference was non-significant (p=0.082), table (3-12).

Also there were increasing in level of IL-10 in age group with 6-8 years old which have high levels of IL-10 (697.08 ± 529.47) in compared with other age groups (table 3-13), but the difference was non-significant (p=0.399). In addition the present results show patients with male gender have high levels of IL-10 in compared with patients with female genders, (673.45 ± 505.12 vs 415.50 ± 175.94), and the difference was significant (p=0.034).

The current results was close to the findings of Abboud (2020)who confirm there was no significant difference between serum level of IL-1β in age group (1-10)which infected with amoebiasis in compared to healthy controls .Result of Habieb (2021) showed decreasing of IL-2 level in toxoplasmosis infected women with age 15-19 years ¸while level of IL-10 was high in all age patients groups in comparsion with control groups .

In across-sectional study of(Sanchez *etal* .¸2015) in children a negative correlation between intestinal helminths specific IL-10 production and age was found ¸the authors concluded that IL-10 regulatory effects might be more likely in younger persons .

Table (3-11): Association between Interleukin-1 beta with age group and gender.

	Interleukin-1 beta levels	
		Age groups
<6 years	3974.79 ± 1174.44	**0.278 † NS**
6-8 years	3899.63 ± 2178.64	
8-10 years	2870.03 ± 667.01	
≥ 10	3651.08 ± 1602.85	
		Gender
Male	3859.12 ± 1889.49	**0.268 † NS**
Female	3340.32 ± 1152.42	

n: number of cases; **SD**: standard deviation;: †: Independent T test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

Table (3.12): Association between Interleukin-2 with age group and gender.

	Interleukin-2 levels	
		Age groups
<6 years	817.37 ± 304.90	**0.15 † NS**
6-8 years	1263.85 ± 980.53	
8-10 years	658.82 ± 218.40	
≥ 10	855.04 ± 601.87	
		Gender
Male	1065.68 ± 798.15	**0.082 † NS**
Female	729.88 ± 357.40	

n: number of cases; **SD**: standard deviation;: †: Independent T test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

Table (3.13): Association between Interleukin-10 with age group and gender.

	Interleukin-10 levels	
		Age groups
<6 years	504.59 ± 234.65	**0.399 † NS**
6-8 years	697.08 ± 529.47	
8-10 years	413.59 ± 153.24	
≥ 10	518.68 ± 410.56	
		Gender

Male	673.45 ± 505.12	**0.034 † S**
Female	415.50 ± 175.94	

n: number of cases; **SD**: standard deviation;: †: Independent T test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

3.10. The correlations between serum immunological parameters to demographic characteristics.

3.10.1 The correlations between serum IL-1β ¸ IL-2 and IL-10 to demographic characteristics and some immunological parameters.

The correlations between serum IL-1 beta levels and some parameters in patients with *Enterobius vermicularis* are shown in table (3-14)¸(3-15) and (3-16). There was highly significant correlation between serum IL-1 beta and IL-2 and IL-10 in patients with *Enterobius vermicularis* infection.

Table (3.14): The correlations between serum IL-1 beta to demographic and some immunological parameters in patients *with Enterobius vermicularis.*

Characteristic	Serum Interleukin-1 beta level	
	R	***P***
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 (3.15): The correlations between serum IL-2 to demographic and some immunological parameters in patients *with Enterobius vermicularis.*

Characteristic	*Serum Interleukin-1 beta level*	
	R	***P***
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-1 beta levels	0.600	0.001*
IL-10 levels	0.793	0.001*

r: correlation coefficient

Table (3.16): The correlations between serum IL-10 to demographic and some immunological parameters in patients *with Enterobius vermicularis.*

Characteristic	*Serum Interleukin-10 level*	
	R	***P***
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-1 beta levels	0.728	0.001*
IL-2 levels	0.793	0.001*

r: correlation coefficient

The present study found that serum IL-10 levels and age were negatively correlated; *i.e.*, as children's age increased, IL-10 levels tended to decrease. This finding seems unexpected for an endemic

country where helminths exposure -and therefore immune tolerance to helminths- is likely to increase with age.

The results of this study investigate that present significant negatively correlation between age and immunological marker ¸(IL-1β ¸ IL-2 and IL-10) ¸while significant positively correlation among the interleukins togethers.

Other study investigated the correlation coefficients showed a significant moderate negative correlation between IL-10 level and age (r^2=-0.483¸p=0.002) (Sanchez *etal* .¸ 2015)

We proposed that such variations might have been linked to children receiving annual single-dose albendazole treatment, as this regimen is more efficacious for *helminth* . The inverse association between IL-10 and age reported in this study might also be confounded by deworming treatment. Albendazole shortens the duration of infections and/or reduces worm burden, depending on the infecting species (Berner *etal* .¸ 2018)Repeated albendazole treatments have a significant effect in the way children respond to immune stimulus, and an immune modulatory effect by IL-10 is likely more evident in chronic or high-intensity infections(Medeiros *etal* .¸ 2003). In fact, significant differences between chronic helminth infections and elevated levels of IL-10

Conclusion and Recommendation

Conclusions:

Based on the findings of this study ,it could be concluded that :

1. The infection with *E. vermicularis* was widely distribution in children in Babylon province especially in age between 8-10 years
2. The frequency of *E. vermicularis* infection was more in female than male .
3. There was increasing in *E. vermicularis* infection according to family history ,while no correlation between entrobiasis and enuresis .
4. The result showed that entrobiasis was more prevalence in rural than urban area .
5. There was increasing of serum IL-1B and IL-2 in patient with entrobiasis .

6. There was slightly increase in serum level of IL-10 in patient with entrobiasis .
7. There was highly significant positive correlation among IL-1B , IL-2 and IL-10 in patients with entrobiasis.

Recommendations:

1- mass screening should continue, and infected children and their family members should be treated
2- Health promotion as well as increasing child and family awareness will reduce the infection rate
3- Impact of *E. vermicularis* infection on other immunological markers .
4- Further study on coinfection to *E. vermicularis* with other intestinal helminth and it effect on proinflamatory cytokines .

5-Study the immunological modulation and evasion by *E. vermicularis* in children

6-Health education plays an important role in support control software through life cycle clarification parasite and ways of infection and prevention from disease like that cooperation between state institution education ,health and agricultural .

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وزارة التعليم العالي والبحث العلمي

جـــــــامعة بابـــــــل

كـــــــلية الطـــب

تقييم بعض السيتوكينات المحفزة للالتهابات والمضادة للالتهابات في مصل الأطفال المصابين بالدودة الدبوسية

رسالة مقدمة إلى

مجلس كلية الطب/جامعه بابل

وهي جزء من متطلبات نيل شهادة الماجستير في العلوم/

الأحياء المجهرية الطبية

من قبل

اسيل صكر ناجي صكر

بكالوريوس علوم حياة / أحياء مجهرية كلية العلوم للبنات- جامعة بابل (٢٠٠٧)

بأشراف

الأستاذ الدكتورة

هيـــــام خـــــالص المسعـــــودي

٢٠٢٢م **١٤٤٤هـ**

الخلاصة:

أجريت هذه الدراسة خلال الفترة من ايلول ٢٠٢١ إلى آذار ٢٠٢٢ ، وتم اختيار مجموعة من سبعة وثمانين مصاب وثلاثين طفل من الأصحاء كمجموعه ضابطة ، تراوحت أعمارهم بين (٢-١٣ سنة) ، وشملت كلا الجنسين (٣٧ ذكور و ٥٠ أنثى). حيث تم جمع العينات من مستشفى النور للأطفال ، ومستشفى بابل للأطفال ، وقطاع الحلة الثاني ، والقرى والارياف في مدينة الحلة ، وتم إعداد استبيان خاص لكل طفل مشارك في الدراسة وتم ملئه من خلال إجراء مقابلات مع امهاتهم. وتم أخذ عينات الدم من جميع الخاضعين للدراسة الوبائية والمناعية.

وأظهرت النتائج الحالية أن جميع المرضى المصابين سريريا ٨٧ (١٠٠٪) كانت لديهم نتائج تشخيصية إيجابية ، في حين أن الفحص المختبري اعطى ٥٣ (٦٠٪) كانت نتائجهم إيجابية. وكذلك أظهرت النتائج أن ٧٩ (٩٠.٨٪) من المرضى المصابين سريريًا لديهم تاريخ علاجي مقارنة بالمرضى المفحوصين مختبريا و بنسبة ٥٣ (١٠٠٪) كان لديهم تاريخ علاجي ، وكان الاختلاف معنويًا ($p = 0.023$) ، وأظهر أيضًا أن ١ (١.١٪) فقط من المرضى المصابين سريريًا لديهم تاريخ من سلس البول مقارنة بـ ٣ (٥.٧٪) مرضى الفحص المختبري للذين لديهم تاريخ من سلس البول ، ولكن كان الفرق غير معنوي ($p = 0.120$) ، وكان ٨٣ (٩٥.٤ ٪) من المرضى المصابين سريريًا كان لهم تاريخ عائلي إيجابي مقارنة بالمرضى الذين تم اختبارهم. وأظهر الفحص أن جميع المرضى البالغ عددهم ٥٣ (١٠٠٪) لديهم تاريخ عائلي إيجابي ، لكن الفرق بين المرضى المصابين سريريًا والمرضى المختبرين لم يكن كبيرًا.

وبحسب مكان الإقامة فقد اشتملت مجموعة المرضى المصابين سريريا على ١٥ (١٧.٢٪) من الحضر و ٧٢ (٨٢.٨٪) من الريف بينما المرضى المفحوصين مختبريا. اشتملت على ١٢ (٢٢.٦٪) حالة من المناطق الحضرية و ٤١ (٧٧.٤٪) من المناطق الريفية ولم يكن هناك فرق معنوي في تواتر توزيع المرضى وأصحاب المجموعة الضابطة و حسب مكان الإقامة وبنسبة غير معنوية ($P = 0.432$)

كما اشتملت الدراسة على المرضى المصابون سريريا ب٣٧ من الفحص المختبري على ٢١ ذكراً و ٣٢ أنثى (٣٩.٦٪ و ٦٠.٤٪) على التوالي ولم يكن هناك فرق معنوي في تواتر توزيع المرضى المصابين سريرياً والمرضى الفحص المختبري و حسب الجنس ، وكان توزيع الإصابةأعلى في الفئات العمرية اكثر من ١٠ سنوات ، لكن الفرق لم يكن شاسعًا في الفئات العمرية الأخرى. ($P = 0.735$)

أظهرت العلاقة بين انتشار طفيلي الدودة الدبوسية *E. vermicularis* والجنس في الدراسة الحالية أن خطر الإصابة بـ *E. vermicularis* كان أعلى بين الأطفال الإناث ولكن ليس معنويًا ($p \geq 0.05$).

كما أظهرت نتائج المؤشرات المناعية (IL-1 beta و IL-2 و IL-10) أن المرضى المصابين بالدودة الدبوسيه لديهم تراكيز اعلى عند مقارنتهم مع المجموعه الضابطة وبعلاقة غير معنويه E. = 0.782، p = 0.459 و p = 0.264) على التوالي.

تشير النتيجة الحالية إلى أن الفئة العمرية الأقل من ٦ سنوات لديها مستويات عالية من IL-1 beta و IL-2 و IL-10 مقارنة بالفئات العمرية الأخرى ، لكن الفرق كان غير معنوي (p = 0.15) (p = 0.278) (ع = ٠.٣٩٩). بالتتابع. وأظهرت النتائج أيضًا أن المرضى من الذكور لديهم مستويات عالية من IL-1 beta و IL-2 و IL-10 مقارنة بالمرضى من الجنسين ، لكن الفرق أيضًا كان غير معنوي (p = 0.082) (p = 0.268) ، (ع = ٠.٠٣٤) بالتتابع.

أظهرت العلاقة بين المعلمات المناعية في الدم (IL-1 beta و IL-2 و IL-10) للمرضى المصابين بعدوى الدودة الدبوسية (*Æ. vermicularis*) مستوى عالي من الدلالة. وكان الارتباط سلبيا بين مستويات IL-10 والعمر. على سبيل المثال ، مع زيادة عمر الأطفال ، تميل مستويات IL-10 إلى الانخفاض. وكما أظهرت هنالك ارتباط سلبي بين العمر والمعلمات المناعية ¸ (IL-2 ¸ IL-1β و IL-10) - بينما هناك ارتباط إيجابي مهم بين الإنترلوكينات معًا