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Effect of *Enterobius vermicularis* Infection on Some Trace Elements and Immunoglobulins E (IgE) among Children in Babylon Province

A thesis

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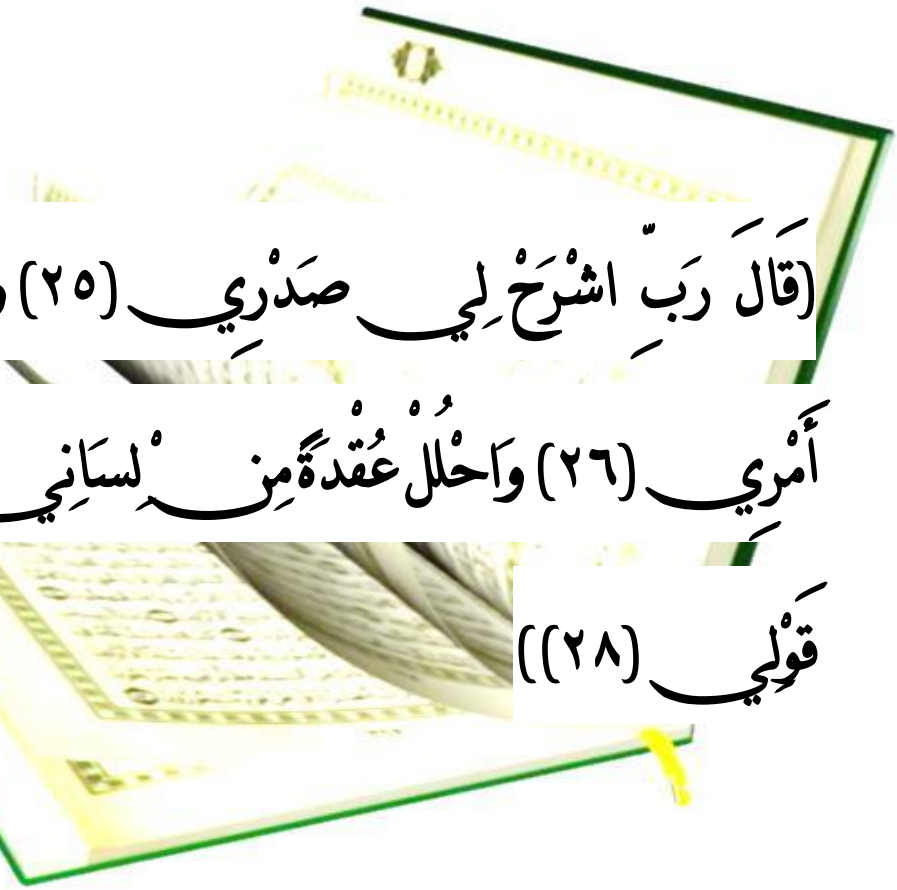

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Dedication

To

Grandson of the Holy People and our guide and leader

Imam AL-Mahdi

Bless upon him

To the pure spirit who gave me her blood, sole and love……..my lovely ***Mother***

To my ideal in life who I carry his name proudly ……..dear ***Father***

To my magnificent husband, who's supported me and with his I face the challenges, with my endless love

To my lovely children who bring the joy to our life

To my brothers and sisters with my love and respect

Rwaa Shaker Saeed Assi

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Summary

Enterobius vermicularis (pinworms) is one of the most common human Nematode parasitic helminthes that may cause enterobiasis, which is common among primary school children in many countries.

This study was conducted to detect the impact of *E.vermicularis* on some trace elements(zinc, copper, and iron). As well as the impact on immunoglobulin E (IgE) in children .

During the period from August 2021 to end of December 2021 , a total of one hundred children participated in this study and their ages group ranged between (2-12) old years , and included both sexes (45 males and 55 females), 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.

The detection of *E. vermicularis* according to laboratory diagnosis results revealed that from one hundred suspected patients suffering from *E. vermicularis* only (60) patients was confirmed as *E.vermicularis* by using cellophane tape technique .

The distribution of patients with *E.vermicularis* according to symptoms results revealed the symptomatic infected children were 25 (41.66%) suffered from perianal itching, and 5(8.33%) have nocturnal enuresis.

The results of the present study show that the percentage of infection with *E. vermicularis* was 45% in male while in female it was 55% ,the age group (8-10),and (10-12) represent higher percentage 30% ,and

23.3% respectively , while the lowest percentage of infection 10% in the age group (2-4) years.

There were no significant differences between the mean age groups of patient and the mean age groups of control. The mean weight of patients were (23.33kg) , it was (28.13kg) in control group there were significant differences between the mean weight of(60) patients and (30)control, while the mean height of (60)patients (117.38cm) and the mean height of (30) controls were (123.07cm), and there were no significance differences. There were significant positive linear correlation between age group and weight and age group and height among study patients.

The mean hemoglobin level of sixty patients were (11.63g/dl) while in control were (13.25g/dl), and the mean PCV levels of sixty patients were (34.30%) , and the mean PCV levels in control were (38.64%).There were significant differences between hemoglobin and PCV according to study group. Also there were significant positive linear correlation between hemoglobin and PCV among study patients.

The results show significant differences between Eosinophil according to study group it was (0.37 $10^{\wedge 3}$/µl) in Enterobiasis patients in compare with (0.18 $10^{\wedge 3}$/µl) in control.

This study included the measuring of IgE level in patients serum; the results reveal a significant increasing in IgE level in patients with Enterobiasis in compare with control group.

The correlation between Eosinophil and IgE among patients with *E. vermicularis* show no significant difference among study .

The present result show that the mean value of serum zinc between patients and control group were (79.34µg/dl), (86.40µg/dl)respectively.

There were slightly decrease in serum level of copper (99.16µg/dl),(103.59µg/dl), and iron(104.80µg/dl),(125.12µg/dl) in patients with *Enterobius* in compare with control group

There were no significant correlation between Zinc, copper and zinc, iron among study patients, but There were significant correlation between copper and iron.

List of Abbreviations

Abbreviation	Meaning
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody- dependent cellular phagocytosis
B cell	B lymphocyte cell
BMI	Body mass index
C°	Centigrate
CD23 or FcεRII	Cluster of differentiate
C.E.	Centuries
CNS	Central nervous system
Cu	Copper
EDTA	Ethylene diamin tetraacetic acid
EETs	Eosinophil extracellular traps
ELISA	Enzyme linked immunosorbent assay
Fc portion	The fragment crystallizable region
FcεRI	High-affinity IgE receptor
Hb	Hemoglobin
IBW	Ideal body weight
Ig	Immunoglobulin
IgE	Immunoglobulin (Epsilon)
IgG	Immunoglulin (Gamma)
IL-13	Interleukin -13
IL-4	Interlukine-4
IL-5	Interlukine-5
MBP-1	Major basic protein-1
MCs	Mast cells
mg	milligram
mg/kg	Milligrams per kilogram
ml	Milliliter
NIH swab	National Institutes of Health swab

nm	Nanometer
No.	Numbers
OD	Optical density
PCR	Polymerase Chain Reaction
PCV	Packed cell volume
pics	Pieces
PPm	Parts per million
RBCs	Red blood corpuscles
rpm	Round per minute
SPSS	Statistical Package of Social Sciences
Th2	T- helper lymphocyte -2
Treg	T cells regulatory
µg/g	Micrograms per gram
µm	Micrometer
ug/dl	Microgram per deciliter
umol/l	Micromole per litre
µl	Microliter
Zn	Zinc

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CHPTER ONE

INTERODUCTION AND LITERATURS REVIEW

1.1.General Introduction.

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) . Pinworm infection can be facilitated by certain factors such as poor personal or group hygiene, and overcrowding in preschools, schools, orphanages, and family groupings (Otu-Bassey *et al*., 2011).

Life cycle of pinworm is simple with fecal-oral transmission of eggs passed in the feces of the host and ingested by same host through contaminated food or water, as well as fomites including hands, clothing, bedding, furniture, and personal care products (Bharti *et al*.,2018) . *E. vermicularis'* eggs hatch into larvae in the small intestine and migrate to the large intestine where they become adults (Hechenbleikner and McQuade, 2015).

Human infection was directly associated with the ingestion of infective eggs through oral routes or from contaminated clothes and bed linens (Fan *et al*.,2021). Additionally, transmission through the respiratory tract has also been speculated by inhaling dust contaminated with eggs (Chang *et al*., 2009).Approximately 40% of affected individuals are oligo- or asymptomatic (Kubiak and Dzika,2017).

The main symptoms are pruritus anal and perianal pruritus, the itching occurs mainly during the night and is caused by the female pinworms migrating to lay eggs around the anus, both the migrating females and the clumps of eggs are irritating, but the mechanisms causing the intense pruritus have not been explained, The intensity of the itching varies. It can be described as tickling crawling sensations, or even acute pain, the itching leads to continuous scratching of the area around the anus (Khubchandani and Bub,2019).

Acid cell hypertrophy(hypertrophy is an adaptive increase in the mass of a cell, tissue, or organ that does not result from cell proliferation, that is, hyperplasia) and elevated IgE levels are characteristic of many parasitic worm infections, and when they are not interpreted , hidden worm disease should be sought. Previous studies have shown that IgE antibodies and eosinophil cells play an important role in the immune response to helminthes (Shin *et al*., 2021).

Immunoglobulin E (IgE) are antibodies produced by the immune system, IgE is thought to be an important part of the immune response against infection by certain parasitic worms, also has an essential role in type I hypersensitivity and anaphylactic reactions has attracted attention for therapeutic interventions (Pritchard *et al*., 2021).

Eosinophils are leukocytes resident in mucosal tissues, were first recognized in association with parasitic infections and have long been thought to function as an end effector cell (Kita,2011). Their presence in association with dead or dying parasites was first noted decades ago, specific granule proteins were noted to possess cytotoxic effects, capable of damaging parasite pathogens, and, in the process, neighboring host tissues .Eosinophil cationic proteins have now been shown to play a role in host defense mechanisms against not only helminths but also viruses and bacterial organisms(Ravin and Loy,2015).

A trace element , also called minor element, is a chemical element whose concentration (or other measure of amount) is very low (a "trace amount"),they are classified into two groups: essential and non essential, essential trace elements are needed for many physiological and biochemical processes in both plants and animals, not only do trace elements play a role in biological processes but they also serve as catalysts to engage in oxidation and reduction mechanisms(Bhattachary *et al*., 2016).

Zinc(zn) is an essential trace elements for all forms of life, clinical zinc deficiency in humans was first described in 1961, Nutritionists have long been concerned that zinc deficiency affects large numbers of women and world wild, zinc deficiency was indicated as a risk factor for immune deficiency(Meydani *et al*.,2007). Zinc is an essential element whose significance to health increasingly appreciated and whose deficiency may play an important role in appearance of diseases, nearly two billion people in the developing world are deficient in zinc, in children it causes an increase in infection and diarrhea (Deshpande *et al.*,2013). Zinc play important roles in growth and development, immune function , neurotransmission, vision, reproduction, and intestinal ion transport (Terrin *et al.*,2015).

Copper (Cu) is an essential trace element for humans and animals, in the human organism،copper exists in two forms – the first and second oxidation form, as most of the copper in the human organism is in the second form, the ability of copper to easily attach and accept electrons explains its importance in oxidative reduction processes and in disposing and removing free radicals from the organism. Although scientists identified copper compounds to treat diseases in 400 BC (during Hippocrates) researchers still discover new information regarding the biochemistry،physiology toxicology, many clinical, laboratory and other indicators of the impact copper in the organism(Angelova *et al*., 2011).

Iron is the most abundant essential trace element in the human body. The total content of iron in the body is about 3–5 g with most of it in the blood and the rest in the liver, bone marrow, and muscles in the form of heme(Prashanth *et al*.,2015). Iron is absorbed in the gut from diet in case of depletion and transported in the form of ferritin, it’s a key element in the metabolism of all living organism , iron is an essential component of

hundreds of proteins and enzymes supporting essential biological function , such as oxygen transport, energy production , DNA synthesis, and cell growth and replication (Bhattacharya *et al*.,2016).

1.1. Aim of the study :

The aim of this study are to check the influence of enterobiasis infections on immunoglobulins E(IgE) level and trace elements (serum zinc, iron, and copper) in children in the Babylon province . This aim was achieved by the following objectives :

1-Diagnosis of *E.vermicularis* by stool sample examination and cellophane tape technique

2-Demographical study including (age group, sex, residence ,and others)

3-Study relationship between infection with *E. vermiculars* and children's nutritional status (height , weight), hemoglobin, and packed cell volume (pcv) level.

4-Estimation the eosinophilia, and immunoglobulins E (IgE) level.

5-Determination of some trace elements such as zinc, copper and iron in patients and control groups, and relationship of these elements with infection with *E.vermicularis* .

1.2. Literatures Review

1.2.1. Historical back ground and literature review :

Biological remains that are excavated from archeological sites are the main source of parasites that existed in ancient times and paleoparasitologists, can take advantage of organic remains to identify ancient parasites of humans and animals, therefore, coprolites, burial soil samples and occasionally suspected parasitic objects are always valuable material to paleoparasitologists (Mowlavi *et al*., 2014).

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).

Evidence of pinworm in ancient populations has been demonstrated in different archaeological sites located in the Americas, Europe, and Asia. In Iran, eggs of *E. vermicularis* have been found in soil samples collected from the Chehrabad salt mine archeological site and dated back to between 1500–2500 years BC (Nezamabadi *et al*., 2013).

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 (Horne, 2002) . Garlic has been used as a treatment in the ancient cultures of China, India, Egypt, and Greece. Hippocrates (459–370 BC) mentioned garlic as a remedy against

intestinal parasites. German botanist Lonicerus (1564) recommended garlic against parasitic worms (Petrovska and Cekovska,2010).

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 parasite eggs were found in a 10,000-year-old human coprolite from Utah, USA, one of the oldest human coprolites ever found and in the mummies' coprolites from several North American archaeological sites . *E.vermicularis* infection was also identified in ancient Andean peoples. The pinworm eggs were observed in ancient coprolites from Chile, Peru, and Argentin(Shin *et al*., 2011).

The discovery of an egg of a parasite of the order Oxyurida in a coprolite from the remains of species of Cynodontia, indicates that the pinworm lineage extends at least as far back as 240 million years, this is the oldest record of an oxyurid nematode yet discovered, and is particularly meaningful since the cynodonts are considered a stem-group of the mammals (Hugot *et al*., 2014).

1.2.2. Classification of *E. vermicularis*

Pinworms (Nematoda: Oxyuridae) are a parasite group occurring in most families and genera of the order Primates including human. Enterobiasis caused by *E. vermicularis* is the most frequently worldwide gastrointestinal diseases reported among preschool children and younger (Valverde *et al*., 2020). Although pinworm infection (enterobiasis) is not commonly serious but usually common parasitic infection (Khadka and Maharjan, 2018).

Kingdom : Animalia

Phylum : Nematoda

Class: Secernentea

Order : Ascaridia

Family : Oxyuridae

Genus : *Enterobius*

Species : *vermicularis* (Despommier *et al*.,1995).

1.2.3. Morphology and ultrastructure of *E. vermicularis*

E. vermicularis is a worm that can enter the mouth of the body through food, air, soil that will nest in the large intestine at night. The adult pinworm (*Enterobius vermicularis*) is small, colored white. The size of the female worm (13mm), is much larger than the male worm (5mm) long. 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 intestine of this worm is also distinctive in shape because it has a large muscular esophagus with a large posterior bulb. The tail of the female worm is straight and pointed while the male has a circular tail. The curved posterior end of male worms has a single copulatory spicule. The males are rarely seen because they die shortly after copulation and are expelled (Sumbele *et al*.,2020).

Enterobius eggs have distinctive characteristics that are exceptionally diagnostic among the helminths, and enable an accurate identification of the parasite to the species *E. vermicularis*, the eggs are oval, elongate, asymmetric, slightly flattened and wider in one of the sides, making a rough "D" shape figure 1: (A and B) . The egg-shell has two recognizable layers when visualized in light microscope and the size of the egg can vary between 50 µm to 60 µm in length and 20 µm to 30 µm in width (Camacho and Reinhard, 2019).

A: Eggs of *E.vermicularis* **B: Adult of *E. vermicularis***

Figure (1-1) : A eggs of *E.vermicularis* and B . Adult of *E. vermicularis* (CDC, 2019).

1.2.4. The life cycle of *Enterobius vermicularis*

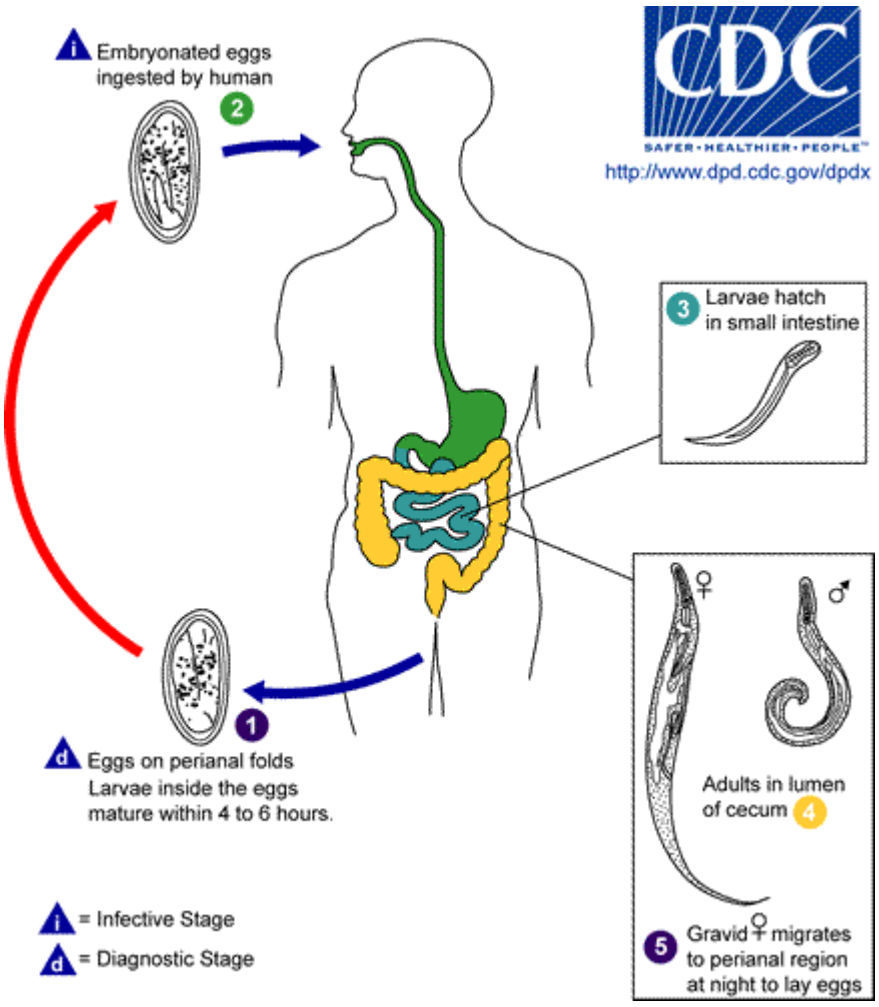
E. vermicularis is an organism that primarily lives in the ileum and cecum ,the entire life cycle, from egg to adult, takes place in the human gastrointestinal tract of a single host, once *E. vermicularis* eggs are ingested, they take about (1 – 2) months to develop into adult worms which happens in the small intestine. At night, gravid females migrate to the anal opening and deposit partially-embryonated eggs in the perianal folds, eggs embryonate quickly and within approximately four houres are already infectious for the next host. Eggs hatch in the small intestine and migrate to the large intestine where they become adults. (Rawla and Sharma, 2019).

When an adult female migrates out of the anus to lay eggs on the skin of the perineum, the cycle begins again; the child scratches and re-infects himself by bringing his hands to the mouth or by inhaling or swallowing the eggs, at that point, the eggs come into contact with the tonsils and with the oral mucosa unleashing an auto-inflammatory reaction (Polese *et al*.,2018).

On rare occasions in female patients, after oviposition, the female worms will accidentally enter the urogenital tract and cause ectopic infection of the vulva, vagina, uterus, fallopian tubes, and ovaries (Choudhury *et al*.,2017).

The embryonic larvae hatch in the duodenum and reach adolescence in jejunum and upper ileum. Adult worms descend into lower ileum, cecum and colon and live there for (7 - 8)weeks. The gravid females, containing more than 10,000 eggs migrate, at night to the perianal region and deposit their eggs there.Eggs mature in an oxygenated, moist

environment and are infectious (3 – 4) hours later Figure(1- 2) (Ghaffar, 2015).



Figure(1- 2) : Life Cycle of *Enterobius vermicularis* (CDC, 2019)

1.2.5.Epidemiology of *Enterobius vermicularis*

Enterobiasis is considered the most prevalent helminth infection, with an estimated 1000 million cases worldwide (Efared *et al*.,2017). However, compared to most intestinal helminths, the prevalence of pinworm infection is underestimated because of parasite migration during the night and the difficulty of egg detection in routine stool examination. The burden of pinworm infection among primary school children worldwide is not well studied. Worldwide epidemiological studies showed the pinworm infection among schoolchildren in Asia, e.g., 54.9% in China (Li *et al*., 2015), 8.8% in Thailand (Tomanakan *et al*.,2020), 47.2% in Myanmar (Chai *et al*.,2015),and 4.4% in South Korea (Kim and Yu, 2014).

The prevalence of *Enterobius* in Africa, 26.3% in Tanzania (Salim *et al*.,2014), 1.7% in Angola (De Alegría *et al*.,2017), and Europe, 17.4% in Germany (Friesen *et al*.,2019), 3.4% in Slovakia Republic (Dudlová *et al*.,2018), and 19.3% in Osh Oblast, Kyrgyzstan (Steinmann *et al*., 2010). The stool examination showed a low prevalence of pinworm infection as the worm's eggs are sticky and adhered to the perianal skin and clothes. A survey compared the prevalence of pinworm infection using fecal analysis (0.4%) to Scotch tape (45.3%) method in the mountainous Qwa-Qwa State, South Africa, among hospitalized children, suggesting that fecal examination underdetermines the true prevalence (Mkhize-Kwitshana and Mabaso, 2012).

In West Bank, Palestine the total rate of infection with pinworms among 384 preschool children , 85 (22.1%) had *E. vermicularis* infestation (Khayyat *et al*.,2021). In children from Lorestan province, Western Iran the results showed that 36 children (9.8%) were infected with at least one or more intestinal parasites, the most prevalent parasites

were *E.vermicularis* (6.8%) (Mahmoudvand *et al*.,2020), while the prevalence of enterobiasis in kindergarten and preschool children of Amol, Mazandaran Province, North of Iran among 462 children, 32 kindergartens of Amol were examined for the prevalence of *E. vermicularis* infection, 2013 was 7.1 % (Afrakhteh *et al*.,2016).

In Keyseri, Turkey the prevalence of enterobiasis among (5 – 7) years old children was 5.1% from 1070 children (Artan *et al*.,2008) . In Al-Jahra and Al-Ahmadi health regions in Kuwait, about 48%, and the least in capital health region 15.8% the most common type of parasite found was *Enterobius vermicularis*, 27.1% (Al-nakkas ,2004).

In Iraq (Erbil city) the total rate of infection with *E.vermicularis* among 505 children was 27.13% (Al-Daoody and Al-Bazza,2020), while in AL-Najaf province (124) children were diagnosed to have Enterobiasis by using direct microscopic examination for the observation the helminthes eggs in the stool, the prevalence rate was high (83.9 %) according to results of Hussein(2015). In Basrah marshes the prevalence of *E. vermicularis* infection of males and females were (72.82%) and (69.16%) respectively according to results of Jarallah and Mansour (2014),while 12.7% rate of infection in Baghdad (Waqar *et al*.,2014).

In Al Diwaniyah province, a study included 122 children from both genders (males, n= 61, and females, n=61) and their ages ranged between (1 – 14) years The prevalence of enterobiasis revealed a high infection rate (73.77%) among the studied population (Dohan and Al-Warid,2022). *E. vermicularis* showed to be the dominant parasites in the other six provinces (Anbar, Baghdad، Basrah, Thi-qar, Dohuk and Muthanna), the percentages ranged between 42-61% (Al-Saqur *et al*.,2020) .

Pinworm infection is linked to age, being most common in children of school age, followed by preschool children, adults are the least common age-group to experience enterobiasis, with the exception of mothers whose children are infected. Children attending day care and other institutions are at greater risk for pinworm because of the high potential for transmission to those who are closely associated with the first case in the household. While some authors assert that pinworm crosses all social barriers, others state that poverty and poor personal hygiene increase the risk of infection (Al-Shadood, 2015).

1.2.6. Pathogenesis of *E. vermicularis*

E.vermicularis (pinworm) is the least pathogenic of all the intestinal helminths. The parasite is cosmopolitan and there is evidence of coexistence with humans that dates back to the Palaeolithic Period (Patsantara *et al*., 2016). The infection with pinworm is more common in temperate than in tropical districts, although recent studies indicated that a prevalence of over 20% is not uncommon in many parts of the world (Kim *et al*.,2013).

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).

Scratching in the perianal region can cause ulceration (excoriation) that shows a tendency toward bacterial super infection, anal dermatitis, perianal folliculitis, or ischiorectal abscess may develop. Very rarely,

pinworms also migrate to the vaginal area, where they can cause vulvovaginitis (Eder *et al*.,2018).

In general, 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 (Altun *et al*., 2017).

Extra intestinal infection patterns in the vagina, urinary bladder, peritoneum, kidneys, liver, and eye have been described in several cases (Kılıç *et al*.,2014);(Babady *et al*.,2011). Enterobiasis can sometimes also overlap with the clinical picture of chronic inflammatory bowel disease (Al-Saffar *et al*.,2015).

E. vermicularis can cause central nervous system infection and this infection could have persisted for long period, linked to immunological response due to malnutrition or repeated reinfection as in poor hygiene or low demographic status (Kandi *et al*.,2019). The risk of developing psychiatric disorders in patients with pinworm infections can increase by *E.vermicularis* (Chao *et al*., 2019). Also there is collaboration between enterobiasis and nocturnal enuresis among children specially in crowded groups like orphanages, and pre-schools (Hussein,2015).

Enterobiasis is one of many parasitic infections that cause malnutrition among children which is major public health problem specially in developing countries (Obaid, 2015). This infection can also

cause 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).

E.vermicularis can cause infection in kidney with histopathological revealed signs of a chronic kidney inflammation with necrotic areas and granuloma formations, these necrotic areas contain multiple ova which found periphery to our knowledge, there have been few reports of the parasite being located in the kidney, probably due to the distance between the kidney and the perianal area (Cateau *et al*., 2010). Male urinary tract infection can occur and the pinworm could reach the urinary tract by urethra which is the only route of infection (Zahariou *et al*., 2007).

Enterobiasis in liver is related by the existence of liver granulomas with a necrotic core, having adult worms or their ova, with relatively light symptom associated with this disease (Arkoulis *et al*., 2012). In intensely rare case *E. vermicularis* detected in eye and nose, it is most likely as a result of direct inoculation of adult female worms from the perianal skin to the eyes by the child's fingers (Babady *et al*., 2011).

1.2.7. Laboratory diagnosis of *E. 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).

National Institutes of Health swab (NIH swab) method is another method for collection of specimens, in both methods the adhesive transparent cellophane portion is used for swabbing by rolling on the perianal area to swabbing it, then the sample sent to the laboratory, where the cellophane portion is separated, spread above a glass slide and examined by light microscope (Paniker and Ghosh , 2013; Ridley, 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).

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 (Shetty *et al*., 2012; Kidambi and Lee, 2018).

Serologic tests are not available for diagnosing pinworm infections, likewise neither blood eosinophilia nor raised immunoglobulin E (IgE) levels are usually prospective due to the low invasiveness of the pinworms (Wendt *et al*.,2019) . The gold standard for diagnosing *E. 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 *Enerobius vermicularis* (Dawood *et al* .,2016).

1.2.8. Immune response to *E. vermicularis*

Parasitic nematodes constitute one of the major threats to human health, causing diseases of major socioeconomic importance worldwide. More than one billion people are infected with parasitic nematodes, and more than a dozen species routinely parasitize humans (Hotez *et al*., 2009).

The vertebrate innate immune response to parasitic nematodes is complex and varies greatly with the species and severity of nematode infection. Invasion of the host tissues by parasitic nematodes activates the complement system, a collection of proteins responsible for identifying pathogens and directing the innate immune response. Leukocytes are recruited to the site of infection and are responsible for enhancing the inflammatory response and release of cytokines, among a variety of other processes (Perrigoue *et al* .,2008).

In vitro experiments demonstrated that eosinophils have the ability to kill helminth in combination with antibodies and complement; importantly, IgE and eosinophil cytotoxicity (antibody-dependent cellular cytotoxicity) has been widely reported as a mechanism for helminth exclusion (Abbas *et al*.,2014). However, the *in vivo* role for IgE and eosinophils is not yet clear because expression of high-affinity FcεRIα is not often found in murine eosinophils, the most common experiment animal model (Yasuda and Nakanishi, 2018).

The parallels between allergy and the immune response to parasitic worms (helminthes) have been noted for some time. Unlike most other inflammatory/infectious conditions, allergy, and helminthes 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).

This normally rare, tightly controlled antibody isotype is greatly elevated in helminth infection. It is widely accepted that IgE, its receptors and distinctive cellular responses did not evolve to target harmless molecules occurring in plant pollen, dust-mites, or animal dander. Instead many believe that the IgE axis evolved to counter metazoan parasites (worms and parasitic arthropods) which are too large to be phagocytosed, and that allergy is a misdirected anti-parasite response in hypersensitive people (Artis *et al*., 2012).

There are however critical differences between the two conditions. Allergy occurs in people with atopy; defined as "a genetic predisposition toward the development of immediate hypersensitivity reactions against common environmental antigens". It is a polygenic disorder linked to polymorphisms in genes of cytokine, cytokine receptors, and transcription factors associated with Th2 immune responses and with the expression of IgE and its receptors (Casaca *et al*.,2013).

In contrast, the elevated Th2 cytokines, IgE and eosinophilia during helminth infection are normal physiological responses to these pathogens(Fitzsimmons *et al*.,2014). Helminth infections are typically associated with hypereosinophilia, considerable IgE production, mucous mastocytosis, and goblet cells hyperplasia . These immune parameters are involved in different effector mechanisms highly depending on where the helminth is localized (Moreau and Chauvin, 2010) .

The innate immune recognition and the immune stimulation and activation of T-helper (Th) cells result in their differentiation to Th1, Th2, Th17 and T-regulatory(Treg) subpopulations and subsequent functions,

as well as in the interaction with B lymphocytes. When activated by the presence of intestinal helminths, the immune system reacts with a type-2 oriented response, which aims to expel them (Bourke *et al*., 2011; Allen and Maizels, 2011);

1.2.9. Role of Eosinophil in *E. vermicularis* infection

Eosinophils were probably observed for the first time by Thomas Wharton-Jones in 1846 (Kay, 2015), The term eosinophil was coined in 1879 by Paul Ehrlich to describe cells in the blood that could be easily identified when stained by the dye eosin (Rodrigo-Muñoz *et al* .,2021). Eosinophils can be easily differentiated from other cells, such as neutrophils and basophils, based on their morphology and brightly brick-red appearance when stained with hematoxylin and eosin (Lacy *et al*.,2014).

Commonly, eosinophils are a kind of white blood cells, measuring 10–20 μm in diameter, with a bilobed nucleus (Dorosz *et al*., 2020) .In homeostatic situations, they circulate in the bloodstream ranging from 0 to 500 eosinophils per microliter (Klion *et al*., 2020). These cells have a very active metabolism, and they are characterized by numerous intracellular secretory granules contained in the cytoplasm, which store the majority of cationic granule proteins and a variety of cytokines, chemokines, and growth factors, and from which they are mobilized and released in response to cell activation (Weller and Spencer, 2017).

Eosinophils are highly versatile immune cells that are capable of acting against a wide range of pathogens, from those as small ones as a virus (Percopo *et al*., 2014) to bigger ones such as parasites(Huang *et al*., 2015) by performing mechanisms of action including cytokine synthesis, classical degranulation, and release of exosomes and eosinophil

extracellular traps (EETs) tools, being classical and recently discovered methods utilized by these resourceful innate cells(Rodrigo-Muñoz *et al*.,2021).

Traditionally, eosinophils were believed to be only involved in parasitic infections and allergic diseases (Kay, 2015).Indeed, several studies have proved that eosinophils and their granule content, combined with their ability to secrete cytokines are major effectors in host defense against parasitic infections, being able to control both *in vitro* as *in vivo*, diverse parasites including *Trichinella* or *Nippostrongylus*, mainly acting during the secondary infection by antibody-dependent cellular cytotoxicity (ADCC) and release of granule enzymes (Huang and Appleton, 2016). Undoubtedly, one of the major features of eosinophils is their granules and their enzymatic content (Acharya and Ackerman, 2014).

The release of the eosinophil granules occurs through exocytosis, cytolysis, or via piecemeal degranulation. The first mechanism described was compound exocytosis, a widely described anti-parasitic mechanism displayed by eosinophils *in vitro*, which consists of the release of their full granule content by the fusion of the granule membrane with the cell membrane .Conversely, piecemeal degranulation allows granule secretion selectively by a cytoplasmic membrane-vesicular tubular network, that transport the granules until they fuse to the cell membrane ,in a process where CD63 is involved (Carmo *et al*., 2016), with an important role in the secretion of MBP-1(major basic protein-1) against *Schistosoma mansoni* infection, proving that eosinophil are able to perform defense mechanisms against parasitic and infection (Dias *et al*., 2018).

Interactions between serum IgE levels, eosinophil count and parasitic infections depend on the duration of infestation and the type of helminth (Solmaz *et al* .,2018).

1.2.10. Role of IgE in *E. vermicularis* infection

Immunoglobulin E (IgE) is a type of antibody (or immunoglobulin (Ig) "isotype") that has been found only in mammals. IgE is synthesised by plasma cells, IgE antibody is one of the widely known antibodies due to its involvement in all types of hypersensitivity reactions as well as it can also participate in protecting the body from parasites(AL-Hashemy *et al* .,2019).

Its Y-shaped and composed of four chains form the basic units of the construction of the antibody(Monomer).These chains are classified into two similar heavy chains called (Heavy chain) and two similar light chains called(Light chains) .The type of heavy chain is Epsilon on the surface of IgE cells is known to be FcεRI, which is high in IgE and CD23 FcεRII, which have a low affinity for IgE ,These receptors are found on the surface of mast cells, Eosinophils and Basophils (Kelly and Grayson, 2016).

IgE antibody is excreted from B and plasma cells (Mukai *et al* .,2016). Despite its half-life of less than a day in the plasma, it can last for several weeks or months when it is bound to the cell surface by the FcεRI receptor, making it a long gatekeeper (Oettgen , 2016) . The main function of IgE is to defend against parasites such as helminths (Fitzsimmons *et al* .,2014).

When the immune system encounters a type of parasite, B-cells class switch to IgE antibodies, which then ‘coat’ the parasite via binding of many antibodies. Following this, effector cells such as eosinophils and

mast cells recognize Immunoglobulin E bound to the helminth and can then bind the Fc portion of the antibody via the Fc-epsilon RI receptor. Once bound, subsequent reactions occur such as further cytokine release and the production of histamine by mast cells, as well as major basic protein and eosinophil peroxidase production by eosinophils. These substances are toxic to the helminth and can thus result in parasitic killing and ultimate clearance of the invader (Sathe and Cusick, 2021).

The physiological roles of IgE in protective immune responses against parasites are well documented, anti-parasitic IgE and IgE loaded on effector cells such as eosinophils have been shown to confer protection against different parasites (e.g., *Schistosoma mansoni*). IgE engaged with FcεRI or CD23 can engender parasite clearance by human eosinophils, platelets and macrophages through ADCC(Antibody-dependent cellular cytotoxicity) and ADCP(Antibody-dependent cellular phagocytosis) (Vouldoukis *et al*., 2011).

Furthermore, high serum titres of parasite antigen-specific IgE have been associated with resistance to infection. Macrophages, eosinophils and mast cells have all been reported to be involved in these protective mechanisms (Sutton *et al*.,2019).

1.2.11.Trace elements

The term trace elements refer to chemical elements present in a natural material at very small amounts. In analytical chemistry, a trace element is an element in a sample that has an average concentration of<100 parts per million (ppm) measured in atomic count or <100 µg/g. In biochemistry, a trace element is a dietary mineral that is needed in very minute quantities for the proper growth, development, and physiology of the organism (Al-Fartusie and Mohssan, 2017).

1.2.11.1 Zinc

Zinc is a nutrient that people need to stay healthy, zinc is found in cells throughout the body. It helps the immune system fight off invading bacteria and viruses and other foreign invaders . The body also needs zinc to make proteins and DNA, the genetic material in all cells, and it is depletion is associated with decline in lymphocyte and thymus functions. Zinc also helps wounds heal and is important for proper senses of taste and smell zinc deficiencies make infants suffer from acute diarrhea (Mayo-Wilson *et al*., 2014).

During childhood, the body needs zinc to grow and develop properly, excess zinc can interfere with the absorption of iron and copper (Funt, 2013). Zinc also helps in the production of antibodies and T-cell and other blood cell activities (Zarebavani *et al*., 2012).

In fact, zinc is the second-most-abundant trace mineral in your body after iron and is present in every cell (Lim *et al*.,2013). Zinc is necessary for the activity of over 300 enzymes that aid in metabolism, digestion, nerve function and many other processes(Zastrow and Pecoraro, 2014).However, a benefit of zinc supplementation has not been shown in people with skin ulcers who have normal blood levels of zinc(Wilkinson,2014)..

Zn is essential for the proper function of the innate and adaptive immune systems; therefore, depletion of whole body Zn content adversely impacts the ability of the host to mount a balanced immune response against invading pathogens(Ibrahim *et al*., 2017). Multiple epidemiologic studies, has been shown that gastrointestinal giardiasis impairs intestinal mucosa function thereby lowering Zn absorption in

young children resulting in Zn deficiency (Astiazarán-García *et al*., 2015).

Although childhood zinc deficiencies are rather unusual, recent studies have suggested that several intestinal parasites may produce these deficiencies (Zarebavani *et al*.,2012) .Also in some investigation showed the mean serum levels of zinc in individuals with enterobiasis were significantly lower than in the control groups (Arbabi *et al*., 2015);(Batta, 2016).

There are limited studies in human concerning zinc level with the parasite infection in Adult or in children but most of the studies are epidemiological, as studies on parasitic infection of children focused on nutritional and growth status, the results of these studies are contradictory about the effect of parasitic infection on the growth status of children ,some authors found that these infections are related to the growth retardation while others reported no relationship (Jawad *et al*.,2011).

1.2.11.2. Copper

Copper, an essential micronutrient in animals and humans، plays an important role in the cellular transporters, called cuproenzymes, that serve as cofactors for a variety of important enzymes, thus, copper is critical in the human body for the proper function of organs and metabolic processes such as hemoglobin synthesis, iron oxidation, cellular respiration, and pigment formation(Prohaska, 2011). Copper is essential for maintaining the strength of the skin, blood vessels,epithelial and connective tissue throughout the body (Osredkar and Sustar, 2011).

Clinical manifestations of acquired copper deficiency are anemia, bone marrow dysplasia, and neuromyelopathy. Copper deficiency may

also lead to abnormally low numbers of white blood cells known as neutrophils (neutropenia), a condition that may be accompanied by increased susceptibility to infection (Bustos *et al*.,2013).

Copper is found in all body tissues and plays a role in making red blood corpuscles and maintaining nerve cells and the immune system(Ware, 2017). Recent mechanistic studies support a role for copper in innate immune response against bacterial infections (Hodgkinson and Petris, 2012).

In patients with *Giardia lamblia* and in enterobiasis found that serum levels of trace elements such as magnesium, zinc, and copper were reduced, These findings were supported by many other researches which clarified the poor absorption of several micronutrients caused by intestinal parasites (Shalaby, and Sayed, 2017).

1.2.11.3. Iron

The control over iron homeostasis is decisive in host–pathogen interaction (Cassat and Skaar, 2013). This is due to the fact that iron is central for several metabolic processes for both, prokaryotic and eukaryotic cells that the metal affects microbial proliferation and pathogenicity and in addition significantly impacts on immune cell plasticity and innate immune responses, these multiple functional aspects of iron are based on its ability to transfer electrons needed during metabolic processes and to catalyze the formation of highly reactive radicals(Koskenkorva-Frank *et al*.,2013).

Many microbes are highly dependent on a sufficient supply of iron and take up this metal by multiple and divergent pathways or steel it from iron deposition sites of the host(Frawley *et al*., 2013). The activation and

expression of such microbial iron acquisition systems is linked to their pathogenicity and proliferation (Andrews-Polymenis *et al*., 2010).

On the other hand, iron plays important roles in anti-microbial host responses, first by synergistic effects towards anti-microbial radical formation(Koskenkorva-Frank *et al*., 2013) but second, by directly altering immune cell proliferation and anti-microbial immune effector pathways (Nairz *et al* .,2014).

Apart from plasmodia, the availability of iron is of importance for other parasitic infection such as Leishmaniosis or Trypanosomiasis. The pathogenicity of *Leishmania* is linked to the expression of different microbial iron acquisition molecules and a sufficient supply of iron (Mittra *et al*., 2013).

1.2.12. Treatment of *Enterobius vermicularis*

The medications used for the treatment of pinworm are either albendazole, mebendazole, or pyrantel pamoate. Single doses of albendazole (400 mg), mebendazole (100 mg), or pyrantel pamoate (11 mg/kg up to 1 g) are highly effective. A second dose is given two weeks later because of the frequency of reinfection and autoinfection. Repeated infections should be treated using the same method as that used during the first infection, in households where more than one member is infected or where infections are repeated, symptomatic infections occur; thus, it is recommended that all household members be treated at the same time(Laoraksawong *et al*.,2020).

Treatment of *Enterobius* infection in pregnancy should be reserved for patients who have significant symptoms, in pregnant patients, pyrantel pamoate is preferred over other medications(Rawla and Sharma,2021).

Other medications that have been used to treat enterobiasis are ivermectin and piperazine, although the latter has lower efficacy and higher toxicity.

1.2.13. Prevention and control of *E. vermicularis*

Washing your hands with soap and warm water after using the toilet, changing diapers, and before handling food is the most successful way to prevent pinworm infection, in order to stop the spread of pinworm and possible re-infection, people who are infected should shower every morning to help remove a large amount of the eggs on the skin, showering is a better method than taking a bath, because showering avoids potentially contaminating the bath water with pinworm eggs. Infected people should not co-bathe with others during their time of infection (Muliawati *et al*.,2020).

Also, infected people should comply with good hygiene practices , they should also cut fingernails regularly, and avoid biting the nails and scratching around the anus. Frequent changing of underclothes and bed linens first thing in the morning is a great way to prevent possible transmission of eggs in the environment and risk of reinfection, these items should not be shaken and carefully placed into a washer and laundered in hot water followed by a hot dryer to kill any eggs that may be there (Mathieu, 2011).

CHAPTER TWO
MATERIALS AND METHODS

2.Materials and methods

2.1.Materials :

2.1.1. Laboratory equipments and instruments

The equipments and instruments that used in the present study with their manufacturer and origin are shown in table (2-1)

Table (2-1) : Equipments and Instruments.

No.	Instruments	Company	Origin
1	Absorbent paper	Deltalab	Spain
2	Balance weight	Iscale	China
3	Centrifuge	Hettich	Germany
4	Cover slips	WKM	India
5	Cotton	MAY	Turkey
6	Cotton swab	AFCO	Jordan
7	Cool box	Cool box	India
8	Distilled water	Super pure	India
9	Disposable pipette tips	Biobasic	Canada
10	Elisa reader	Biocheck	USA
11	Elisa washer	Biocheck	USA
12	Eppendrof tubes (1.5)	CAPP	Germany
13	EDTA tubes	AFCO	Jordan
14	Gloves	Sempermed	Thailand
15	Gel tubes	AFCO	Jordan
16	Glass slide	WKM	India
17	Hematology analyzer	Sysmex	Japan
18	Incubator	Memmert	Germany
19	Length measuring tabe	Uxcell	China
20	Microscope	Lenovo	China
21	Microplate reader	Elabascience	USA
22	Micropipette	Slamed	Germany
23	Plain tubes	AFCO	Jordan
24	Precision pipette	Calbiotech	Italia
25	Rack	AFCO	Jordan
26	Refrigerator	Hettich	Europe
27	Scotch tape	Scotch	USA
28	Syringes (5cc)	Medijecte	China
29	Spectrophotometer	Aquaris	England
30	Timer	Citoclase	China

2.1.2. Laboratory Kits

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

Table (2-2) : laboratory kits

No.	Kits	Company	Origin
1	Human immunoglobulin E ELISA kit	BT	China
2	Zinc fluid monoreagent	Centronic Gmbh	Germany
3	Copper(colorimetric test with Dibromo-PAESA)	LTA	Italy
4	Iron direct method (Ferene)	Biolab	France

2.1.3.Ethical Approval

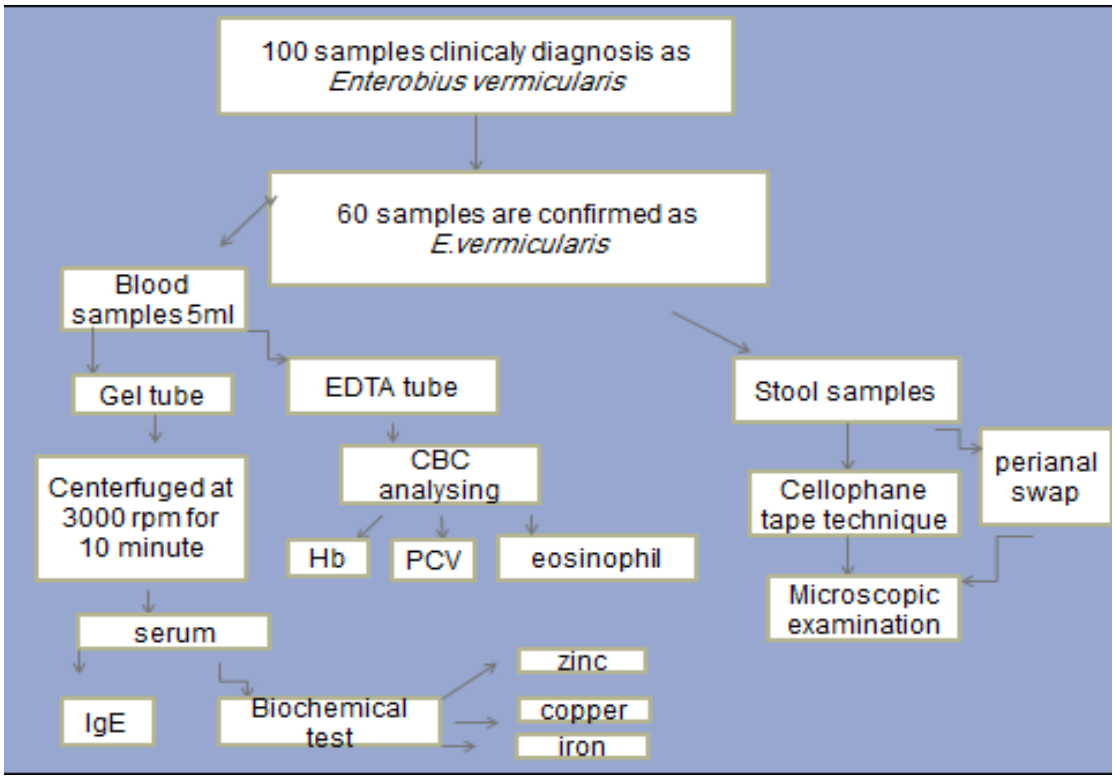
The necessary ethical approval was obtained by verbal consent from family of children. This study was approved by the committee of publication ethics at college of medicine , Babylon university , Iraq. This study consider a case – control study .

2.2. Methods

2.2.1. Samples collection

During the period from August 2021 to end of December 2021 , a total of one hundred children participated in this study and their ages group ranged between (2-12)years old , and included both sexes (45 males and 55 females), 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. A special questionnaire form was prepared for each participant child in the study and filled out by interviewing their mothers.

2.2.1.1.Study design



2.2.1.2. Cellophane tape sample collection.

The samples were collected 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, this procedure were done by taken 10 cm of the transparent adhesive tape fold it on the tongue depressor with the adhesive side out , press the adhesive side of tape over the perianal area and cover the maximum area, then the tape was transferred to glass slide with sticky side down : a drop of toluene was added for clearing and examined under light microscope (Shyamasundari, and Rao, 2019).

2.2.1.3.Blood sample collection .

Five milliliter from venous blood were collected from each child suspected with *E.vermicularis* and healthy (control group) by using disposable syringe (5 ml) .The blood samples were placing in EDTA tube and 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 portions 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-20C° until being analyzed for *E. vermicularis*. After completing the sampling , all samples were simultaneously extracted from the freezer and tested. The EDTA tubes were used to anticoagulant whole blood for hematological investigations as the complete blood count (CBC) to measured haemoglobin (Hb) ,packed cell volume (PCV), and eosinophil (Erhabor *et al*.,2019).

2.2.2.Inclusion and Exclusion Criteria

The inclusion criteria were all children were infected with *E. vermicularis* while exclusion criteria included children with non-intestinal protozoal infections, and chronic diseases and did not take vitamins or mineral supplements for the last 3 months as well as immunocompromised patients.

2.2.3. Serological Test

2.2.3.1.ELISA kit of IgE

A. Assay Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA).The plate has been pre-coated with human IgE antibody. IgE present in the samples added and binds to antibodies coated on the wells. And then biotinylated human IgE Antibody is added and binds to IgE in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IgE antibody**,** after incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IgE. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B. Reagent Provided

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

Table (2-3): Components of reagent of ELISA kit of IgE.

Components	Quantity
Standard solution (2400ng/ ml)	0.5 ml x 1
Pre – coated ELISA plate	12*8 well strips x 1
Standard Diluent	3 ml x 1
Streptavidin - HRP	6 ml x 1
Stop Solution	6 ml x 1
Substrate Solution A	6 ml x 1
Substrate Solution B	6 ml x 1
Wash Buffer Concentrate (25x)	20 ml x 1
Biotinylated human IgE Antibody	1 ml x 1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pics

C. Assay Procedure

1. All reagents were prepared , standard solutions and samples as instructed. All reagents save in room temperature before use. The assay was performed at room temperature**.**

2 . The number of strips required for the assay were determined. The strips in the frames were insert for use.

3 . A 50µl standard was added to standard well.

4. A 40µl sample was added to sample wells and then a 10µl anti-IgE antibody was added to sample wells, then a 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. The plate with a sealer was covered. Incubated 60 minutes at 37C°.

5 . The sealer was removed and the plate was washed five times with wash buffer. Wells were soaked with at least 0.35ml wash buffer for 30 seconds to one minute for each wash. For automated washing, the wells were aspirated all and washed five times with wash buffer, the wells were overfilling with wash buffer. the plate was blot onto paper towels or other absorbent material.

6.A50µl substrate solution A was added to each well and then a 50µl substrate solution B was added to each well. Incubate plate covered with a new sealer for 10 minutes at 37C° in the dark.

7.A50µl Stop Solution(H2SO4) was added to each well, the blue color would change into yellow immediately.

8.Determine the optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minuets 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 (Figure 2.1).

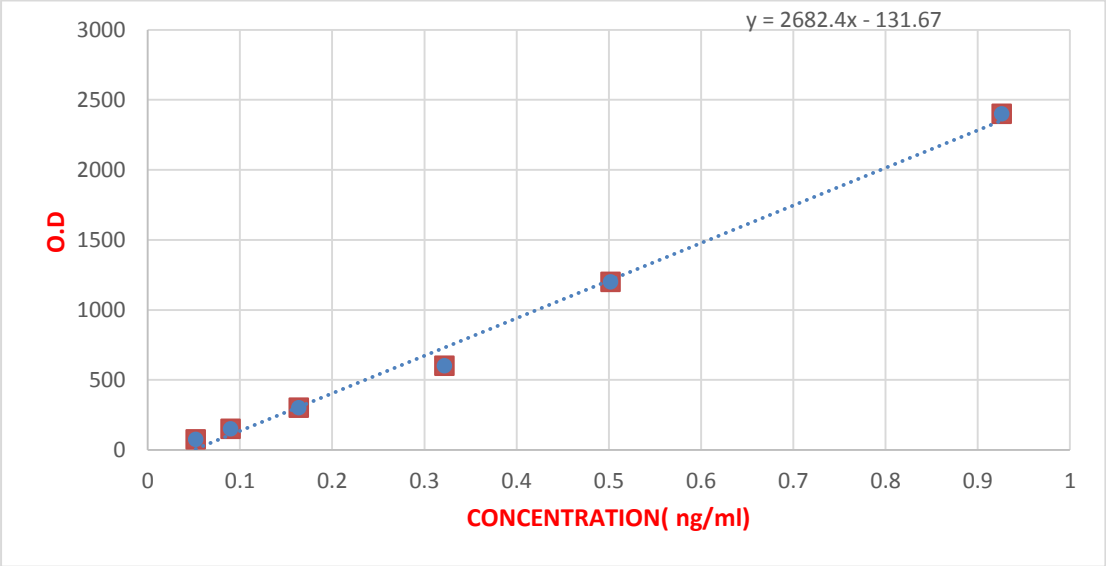


Figure 2.1 : Standard Curve for IgE.

2.2.3.2. Iron direct Method (Ferne)

A. Principle:

After dissociation of iron –transferrin bound in acid medium, ascorbic acid reduces Fe^{+3} iron into Fe^{+2} iron then form a coloured complex with 3-(2-pyridyl)-5, -6-difuryl-1,-2,-4-triazine-disulfonate (Ferene) . The absorbance thus measured at 600 nm (580-620) is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent tom prevent the copper interference. This test was done by a spectrophotometer .

B. Manual Procedure:

The reagents and specimens was stored at room temperature.
Prepared 2 sets of tubes according to following boards:(table2-4 and 2-5).

Table (2-4) : Manual procedure of Iron method (A1).

Blank tubes	Blank	Standard	Assay
Reagent R1	1 ml	1 ml	1 ml
Specimen			200ul
Standard		200 ul	
Distilled water	200 ul		

Mixed gently and stand for at least three minute at room temperature .

A1 absorbance was recorded at 600 nm (580-620) against blank.

Table (2-5) : Manual procedure of Iron method (A2).

Assay tubes	Blank	Standard	Assay
Working reagent	1 ml	1 ml	1 ml
Specimen			200 ul
Standard		200 ul	
Distilled water	200 ul		

Mixed gently and stand for five minutes at room temperature .

A2 absorbance was recorded at 600 nm (580-620) against blank .

Color stable for one hour.

C. Calculation of results

The result was calculated as follows:

Result = (A2 - A1) Assay / (A2 – A1) Standard x standard concentration.

2.2.3.3. Copper

A. Principle test :

The chromogen 3,5-Di-Br-PAESA react with cupric ions anf forming a blue – violet compound , which intensity is proportional to the copper concentration in the sample. The method does not require de-proteinization of the serum nor the blank sample. The copper test was achieved by a spectrophotometer.

B. Reagents:

Reagent A : Acetate buffer 0.1 M pH 4.9 ; reducing agents and preservatives .

Reagent B : 3,5-Di-Br-PAESA.

Standard: Ion copper 200 ug / dI ; preservatives.

C. Procedure :

Kind of analysis :	End point
Reading time :	10 minutes
Colour stability:	30 minutes
Wavelength:	580 nm (570-590)
Temperature :	20-25 0 c
Light path :	1 cm
Zero :	Blank reagent

Table (2-6) : Procedure of copper .

Reagents	Blank	Standard	Sample
Working reagent	1 ml	1 ml	1 ml
Distilled water	66 ul	…..	…..
Standard	…..	66 ul	…..
Sample	…..	…..	66 ul

Mix and wait for ten minutes then read the absorbances against the blank at 580 nm. The colour is stable for thirty minutes.

D. Calculation of results :

Copper ug/dl = A(sample) /A(standard)x 200

Copper umol/l = A(sample) / A(standard) x 31.47

2.2.3.4.Zinc colorimetric :

A. Principle :

Zinc forms with 2-(5- Brom-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)-phenol a red chelate complex. The increase of absorbance can be measured and is proportional to the concentration of total zinc in the sample. Zinc test was done by a spectrophotometer.

B. Assay procedure .

Assay procedure of zinc colorimetric test was shown in table (2-7).

Table (2-7): Assay procedure of zinc test.

Wavelength temperature	560 nm 25 °c / 37 °c	
	Standard	sample
Reagent	1000µl	1000µl
Serum/Plasma/Urine	……	50µl
Standard	50µl	…….

Mixed and incubated for eight minutes at 25 c◦ or five minutes at 37 c◦ . The absorbance of the sample A(sample) and of the standard a(standard)were measured against the reagent blank A(RBL).

δ A(s) = A(sample)-A(RBL)

δ A(STD) = A(standard)- S(RBL).

C. Calculation of results :

With standard:

C = 200 × δA(s) / δA(STD) (μ g/ dl)

2.2.4. Statistical 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. Pearson correlation coefficient was used to find the relationship between two continuous variables. A p-value of ≤ 0.05 was considered as significant (Daryanto, 2020).

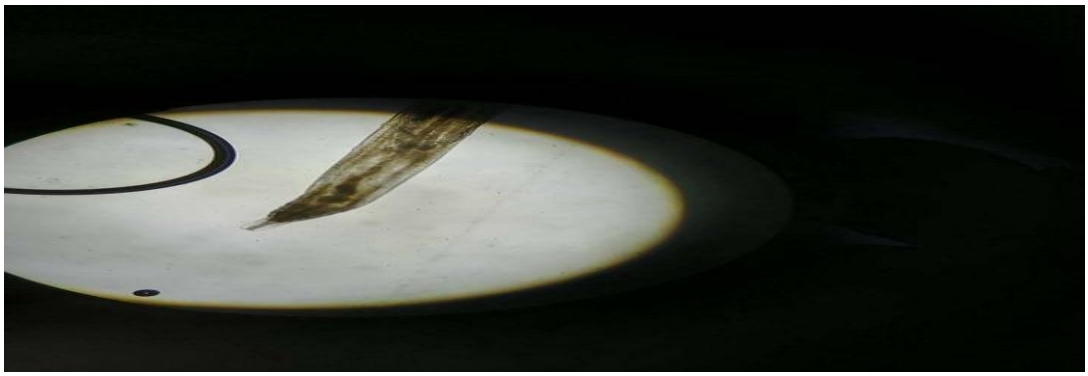
CHAPTER THREE
RESULTS AND DISCUSSION

3.Result and discussion

3.1. Detection of *Enterobius vermicularis* according to clinical and laboratory diagnosis

This study included one hundred patients clinically suffering from *E. vermicularis* attended to some hospitals in Hilla city (Al- Noor hospital for children , Emergency Babylon Hospital for Maternity and Children , and Al - Hilla Second Sector) from total of (100) patients only (60) patient were confirm as *E. vermicularis* infection by laboratory diagnosis when used direct stool examination and cellphone tape techniques . (Figure 1: A , B, and C) and table (3-1) .

A: Eggs of *E. vermicularis*



B:Enterior end of *E. vermicularis*

C:Posterior end of *E. vermicularis*

Figure (3-1) : *E. vermicularis*

This figure was examined under (4X) power of light microscope , and was taken by a mobile phone camera.

Table(3-1): Detection of *E. vermicularis* according to suspected and laboratory diagnosis

Age (years)	Suspected diagnosis as *E. vermicularis*	Laboratory confirmed cases of *E. vermicularis*	%
2-3	14	6	42.8%
4-5	13	7	53.8%
6-7	15	8	53.3%
8-9	25	18	72%
10-11	20	14	70%
≥ 12	13	7	53.8%
Total	100	60	60%

Table (3-1) shows children suspected with *E. vermicularis* , from total one hundred patients suspected as *E.vermicularis* , only (60) patients were confirmed as *Enterobiasis* by laboratory examination when using cellophane tape test.

A previous study indicated that behavioral changes are frequently shameful and inferior due to having worm, however, children's discomfort is often overlooked by parents, despite pinworm infection possibly causing developmental and/or health problems, in particular,

mothers can become distraught when they think their child has been infected by ‘worms’ .Although adult worms directly seen by the naked eye and microscopic detection of eggs from feces allow a confirmative diagnosis, this is impractical, because adult worms are uncommonly seen around the anal area or in the stool, and eggs are only found in the stool of 5% of infected persons ; thus, the scotch-tape test can serve as a quick and sensitive way to clinch a diagnosis (Fan *et al* .,2019).

Although a history of perianal itching, irritability, and insomnia may suggest a pinworm infection, diagnosis depends on demonstrating the eggs or adult worms. This is normally achieved by sampling the perianal and perineal skin by using adhesive cellulose tape (scotchtape) (Gunawardena *et al*.,2013).

Result of Gunaratna *et al* .(2020) confirm that the best diagnosis test for *E. vermicularis* was ‘cellophane tape’ or ‘scotch tape’ test method. Direct application of clear cellophane or tape to the peri-anal region and then transfer onto to a standard glass slide can be submitted to microscopic examination to detect egg . To obtain better results specimens should be collected in early morning prior to washing the perianal area. The sensitivity of three consecutive tape collections is about 90%.

The ratio between anal swab results and actual *Enterobius* infection is affected by several factors, including the size of the parasite’s brood, time intervals between reinfection, and the distribution of the *E. vermicularis* burden in the surveyed community (Remm and Remm, 2009).

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

Sixty patients infected with *E. vermicularis* who confirm by laboratory examination were suffering from some clinical symptoms (table 3-2).

Table(3-2): The distribution of patients with *E. vermicularis* according to symptoms

Symptoms	Number of patients	%
Perianal itching	25	41.66%
Abdominal pain	8	13.33%
Nocturnal enuresis	5	8.33%
Irritability	12	20%
Lose appetite	10	16.66%
Total	60	99.98%

This results revealed the symptomatic infected children were 25 (41.66%) suffered from perianal itching . Anal itching or perianal itching is the most common symptoms were children suffering from it. Also there were 12 (20 %) suffering from Irritability,10 (16.66%)child suffering from lose appetite , while 8(13.33%) suffering from abdominal pain, and 5(8.33%) have nocturnal enuresis . Infections are characterized by intense perianal itching (pruritus ani) caused by host sensations and reactions to female worms depositing sticky eggs on the skin. Patients vigorously scratch themselves attempting to relieve the itching. Heavy infections in children may cause abdominal pain, irritability, lose appetite, and bed-

wetting (Mahmud *et al*.,2017). The present results were agree with results of other study, Al-Qadhi *et al*.(2011) and Taylor *et al*. (2018).

3.3. Distribution of patients with *E. vermicularis* according to gender

The results of the present study show that the percentage of infection with *E. vermicularis* was (45%) in male while in female it was (55%) table (3-3).

Table(3-3): The distribution of patients with *E. vermicularis* according to gender

Gender	Number of infected patients	%
Male	27	45%
Female	33	55%
Total	60	100%

Table (3-3) show sixty patient confirmed as *E. vermicularis* by laboratory examination were the number of infected males were (27)with percentage 45% while the number of infected females were (33)with percentage 55%. Although there was no significance relationship between the gender and frequency of *E. vermicularis* infection.

The present study showed that the infected with enterobiasis in female slightly higher than that in male . The female may be more frequently exposed to infection than male due to connected with their daily house work, contact with bed sheet and cloth for infected person (s) of family and this is agree with many studies such as study by Ali *et al*. (2014) were the majority of cases 69.6% were females while males 30.4% ,study by Al-Bazzaz (2020) in Erbil Province, confirm that the frequency of

infection with *E. vermicularis* among females were higher than that among males at rates of 28.85% (75/260) and 25.31% (62/245) respectively, and Doğan *et al*. (2008) were the overall prevalence of intestinal parasitic infection rate was 3.6%, of these patients, 52.5% were female and 47.5% male as well as also the present study is accept with the study by Fan *et al*. (2021) were the prevalent of pin worms in children under age eight years were higher among girls (13.17%, 22/167) than boys (11.17%, 20/179) .Study of Khazaal *et al*. (2020) confirm there was no significant different between gender in pinworm infection . Also there were many studies disagree with present study as in study by Kadir and Amin (2011) the rate of infection in males were (26.57%) higher than females (22.83 %). Disagreement also found with study by Hussein (2015) in Najaf province , proved the rate of infection in males were (59.4%) higher than females (40.6%) also study of Dohan and Al-Warid(2022) show a significant relation that noticed between gender and the occurrence of *E. vermicularis*, the most positive cases were diagnosed in males, this is possibly because males are more involved in outdoor activities.

3.4. Distribution of patients with *E. vermicularis* according to age group

The results of the present study show that the age group (8-10) was the highest group of infected children with *E. vermicularis* worm with percentage (30%) , while the age group (2-4) was the lowest group with percentage (10%) as shown in table (3-4) :

Table (3-4) The distribution of patients with *E. vermicularis* according to age groups

Age group in years	Infected patients	%
2-3	6	10%
4-5	7	11.7%
6-7	8	13.3%
8-9	18	30%
10-11	14	23.3%
≥12	7	11.7%
Total	60	100.00 %

The prevalence of enterobiasis in children of the age school was high , and this is may be due to that the children in this age often they have less self-awareness, and may could not be able to manage their hygiene practices and care themselves, and may ignored cleaning their hands with soap after the use of toilet, also hands wash neglecting before meals, and some of them have nails biting habits or bite pencils habit, this make the children in this age in the risk of acquiring enterobiasis infection. Study of Khazaal *et al*. (2020) revealed Enterobiasis infection were be found higher among children of the age group 6-8 years that include 17 (2.49%) of 53 infected children divided into 9 (1.32%) males and 8 (1.17%) females. In Venezuela, the prevalence of enterobiasis found in (5-14)years old children was 19.1% (Devera *et al*.,1998) . In school age

children, in Thailand, in(5- 10)years old children, the prevalence was 21.91% (Nithikathkul *et al*., 2001).

3.5. Body characterization (Age, weight , and hight) of patients that infected with *E. vermicularis* related with study groups

Table (3-5) show the mean differences of study variables including (age, weight, and height) according to study group including (patients infected with *E. vermicularis* and compared with control group).

Table(3-5): The mean differences of study variables (age , weight, and height) according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Age (year)	Patients	60	8.03	2.94	-0.397	0.692
	Control group	30	8.30	3.13		
Weight (kg)	Patients	60	23.33	8.08	**-2408**	0.018*
	Control group	30	28.13	10.40		
Height (cm)	Patients	60	117.38	17.54	-1.477	0.143
	Control group	30	123.07	16.47		

P value ≤ 0.05 was considered as significant

The mean age of sixty patients were (8.03) while the mean age groups of thirty control were (8.30). There were no significant differences between the mean age of patient (8.03± 2.94) and the mean age groups of control (8.30± 3.13) (t = - 0.397, p-value = 0.692).

The mean weight of (60) patients were (23.33) ,and the mean weight of (30) controls were (28.13), and there were significant differences between the mean weight of patients (23.33± 8.08) and the mean weight of control (28.13±10.40) (t = -2408, p- value = 0.018).The reason of the

lose weight as the most common symptoms in Enterobiasis are reported to be abdominal pain, weight loss and diarrhea. Also this may be due to effected of the parasite on the integrity of the gut due to the irritation of the mucosal lining leading to malabsorption, decreased appetite , dyspepsia, abdominal discomfort(Nicki *et al* .,2010).

This result were in constant with the study of Ali *et al* .(2014) , he reported the weight for age and notice there was significant difference in the growth parameters between the cases and controls ($p<0.001$) .

Study of Çeliksöz *et al* . (2010) who stated that weight were found to be lower in the infected children than the non infected group(25.93 ± 0.56), (33.95 ± 0.33) respectively. Study by Değerli nad Kuzu (2016) , When students were compared according to their weight and height, it was found significant to be statistically difference ($p=0,04$, $p< 0,05$), and that was hold with the recent study about significant differences in weight,but not height.

The results of this study was disagreement with the study by Aziz Kadir and Amin (2011), in Sulaimania, who confirm that non significant differences between infected children(249) and non infected children (37) with enterobiasis ($P>0.05$).

The mean height of sixty patients were (117.83± 17.54),and the mean height of controls were (123.07 ± 16.47) (t = - 1.477, p- value = 0.143) there was no significant differences between mean height of the patients and controls .This is may be due to one of the complications that occur during pinworms infection which is growth retardation (Doni *et al*.,2015).

3.6. The correlation between (age and weight) and (age and height) among patients that infected with *E. vermicularis*

There were significant positive linear correlation between age group and weight and age group and height among study patients.

Table (3-6): The correlation between (age and weight) and (age and height) among patients that infected with *E. vermicularis* (N=60).

Study variable	Number	Mean	SD	r	p-value
Age(years)	60	8.03	2.94		
Weight(kg)	60	23.33	8.08	**0.799**	**<0.001***
Age(years)	60	8.03	2.94		
Height(cm)	60	117.38	17.54	**0.915**	**<0.001***

P value ≤ 0.05 was considered as significant

Infections with some gastrointestinal parasites are associated with the reduction of nutrients and minerals uptake from the intestine by destructing the intestinal mucosa, some intestinal parasite like *Schistosomes*, and *Ascaris lumbericoides* display adverse properties on weight gain , which may cause poor appetite and metabolic and clinical disturbance (Grab and Mbofung,2010). Weight and length or height measurements and comparisons of weight to ideal body weight (IBW) are integral to pediatric healthcare. They are used to monitor growth and nutrition status but more importantly to screen for deviations from an established growth pattern that may signal a developing problem and the need for intervention (Phillips *et al*.,2007). Anthropometric measurements remain a simple, acceptable indicator of wellness, maturation and development at different age groups in humans(Chinedu *et al*., 2013). Increase in stature is part of the

normal human development and maturity processes; females are known to attain physical maturity earlier than males of their age. Average BMI (body mass index) was low at childhood, normal at adolescence and at early adulthood and high at middle and advanced adulthood (Chinedu *et al* .,2017).

Enterobiasis usually follows a less acute course than the bacterial and/or viral infections and has less lethality, patients with enterobiasis are often asymptomatic, and their growths are affected (Aciouml *et al*.,2010). On the other hand, the weight for age and the height for age were found to be lower in the infected children than the non-infected group .

3.7. The mean differences of hemoglobin and Packed cell volume (PCV) according to study group

Table (3-7) show the mean differences of hemoglobin concentration and PCV according to study group including (patients with *E. vermicularis* and control group).

Table (3-7) : The mean differences of hemoglobin and PCV according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Hb(g/dl)	Patient	60	11.63	1.22		
	Control	30	13.25	1.32	-5.779	**<0.001***
PCV(%)	Patient	60	34.30	2.89		
	control	30	38.64	3.76	-0.604	**<0.001***

P value ≤ 0.05 was considered as significant

The mean hemoglobin level of sixty patients were (11.63± 1.22) while the mean hemoglobin level of thirty control were (13.25± 1.32), (t= - 5.779, p= <0.001*)figure (3-2) and the mean PCV of sixty patients

were (34.30±2.89) , and the mean PCV of thirty control were (38.64 ±3.76), (t= - 0.604, p= <0.001*). There were significant differences between hemoglobin and PCV according to study group figure(3-2) (3-3).

Infection with intestinal parasites is significantly correlated with low levels of hemoglobin and the packed cell volume in blood (Alum *et al*.,2010).This result may be due iron deficiency in the lumen of the gut and impaired the synthesis of hemoglobin and subsequently the synthesis of red blood corpuscles. It had been found that the adult parasites such as *Giardia intestinalis* and *Entamoeba histolytica* produced hemolysin that consume the RBCs and lead to anemia and reduced levels of hemoglobin and PCV (Ali *et al*.,2014).

Study by Aziz Kadir and Amin(2011) in Iraq found that there was significant differences between the mean hemoglobin in patients and control .Also study of Dohan and Al-Warid (2022) in Iraq, investigate the hemoglobin concentrations were decreased significantly (P< 0.05) in *E. vermicularis* (+ve) ,(12.34 ± 1.15 g/dl) children compared with *E. vermicularis*(–ve) children (13.25 ± 0.7 g/dl) significant differences (P < 0.05).

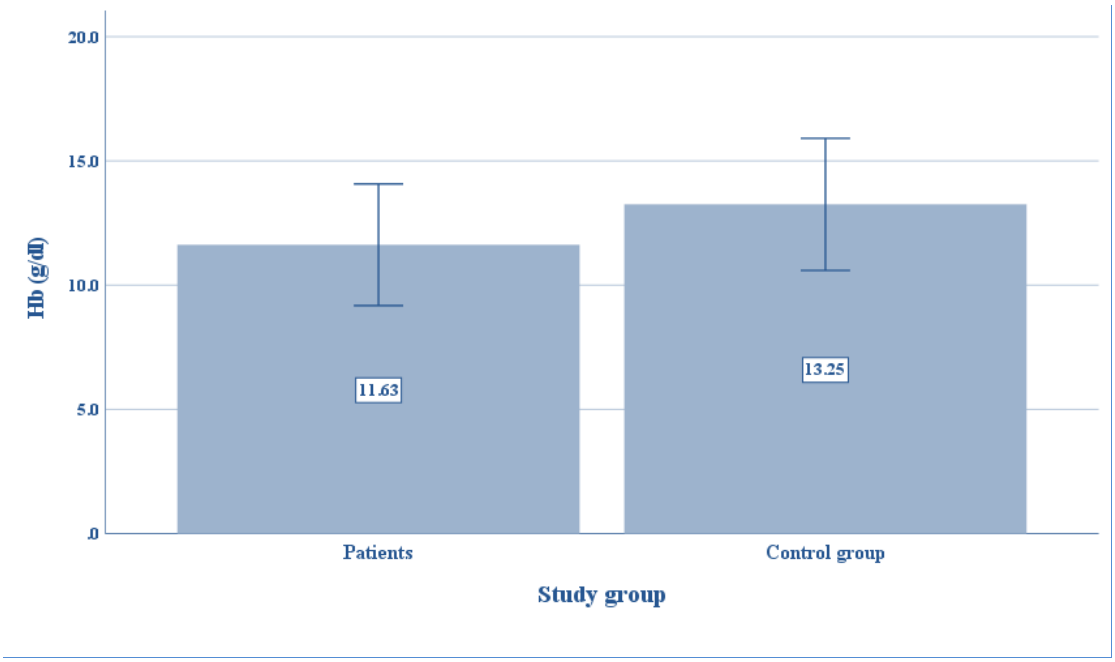


Figure (3-2): Mean differences of Hb (g/dl) according to study group (P<0.001*)

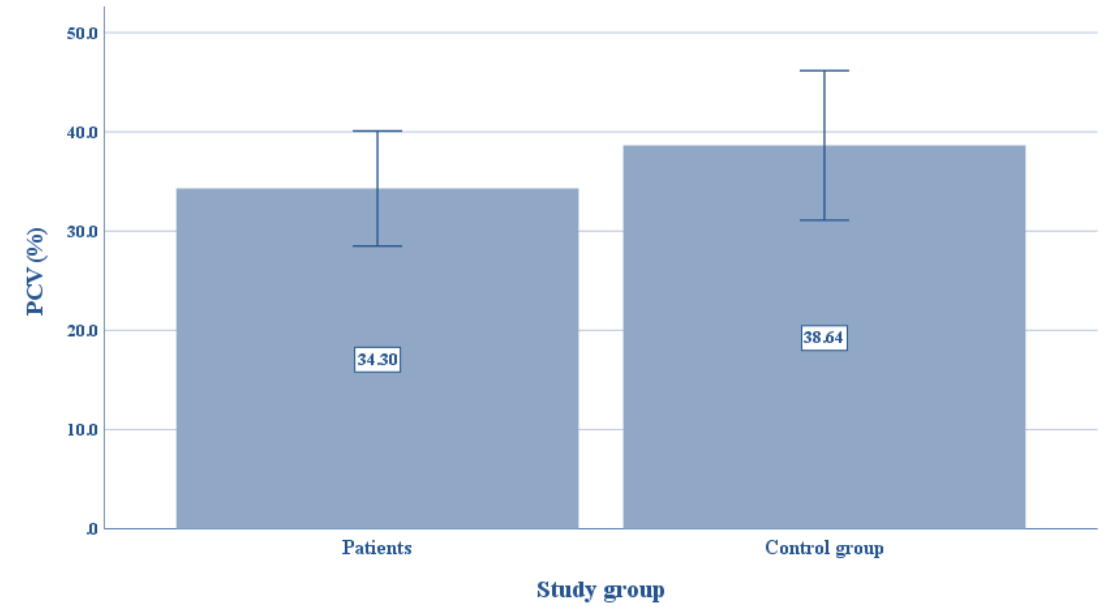


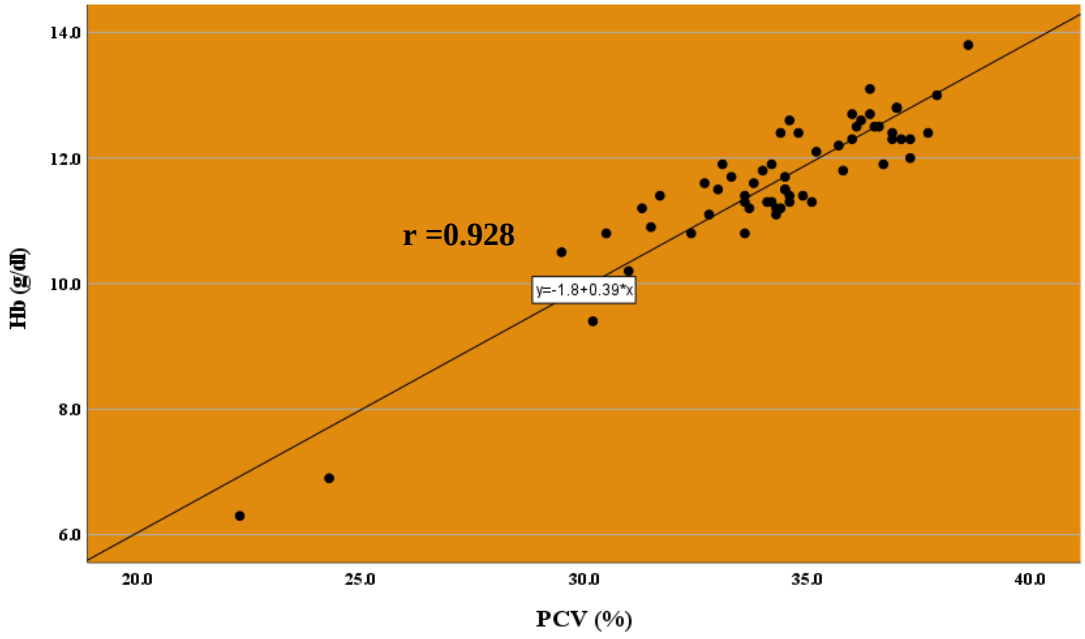
Figure (3-3): The mean differences of PCV (%) according to study group (P<0.001*)

The eggs of *E. vermicularis* pass into the digestive system and hatch in the small intestine, from the small intestine, pinworm larvae go to the large intestine, where they live as parasites (with their heads attached to the inside wall of the bowel) (Baron, 1996). Intestinal nematode infections are most often seen as common chronic infections of children

that lead to relatively mild sickness, intestinal nematode infections in adults can lead to anemia depend on the intensity of infection, intestinal nematode infection is known to contribute to iron-deficiency anemia, the relative contribution depends on many factors, including the intensity of infection, iron status, the quality and quantity of iron sources in the diet, the absorption properties of the gut and the presence of other illnesses(Guyatt,2000). This result may be due iron deficiency in the lumen of the gut and impaired the synthesis of hemoglobin and subsequently the synthesis of red blood cells.

3.8. The correlation between hemoglobin and PCV among patients with *E. vermicularis*

Figure (3-4): The correlation between hemoglobin and PCV among patients with *E. vermicularis*.



There were significant positive linear correlation between hemoglobin and PCV with occurrence of enterobiasis among study patients. (N=60, r =0.928, P<0.001*) this significant correlation is likely because of the effect of parasitism, it is expected that intestinal parasite will compromise

nutrient intake and absorption (Marques *et al*., 2020) . This results similar to finding of Dohan and Al-Warid (2022) who confirm a significant correlation between anemia and the infection with *E. vermicularis*.

Hemoglobin and Packed cell volume regarded as one of the important hematological parameters which used in the diagnosis and follow up of anemia and polycythemia (Ciesla, 2007). Hemoglobin is the molecule responsible for transport of oxygen under physiological condition with a molecular weight of 64.500 Dalton and consists of four poly peptide chains each carrying a heme group. The packed cell volume (PCV) can be used as a simple screening test for anemia. The PCV is about three times the Hb expressed in g/l .In conjunction with estimations of HB and RBCs it can be used in the calculation of red corpuscles indices. However used in under resourced laboratories may be limited by the need for a specialized centrifuge and a reliable supply of capillary tubes (Shaweeish and Masood, 2014).

3.9.The mean differences of Eosinophil according to study group

Table (3-8) show the mean differences of Eosinophil according to study group including (patients with *E. vermicularis* and control group). the mean value of Eosinophil in sixty patients were (0.37 ± 0.27) , while the mean value in control group was (0.18 ± 0.09),(t = 4.703),(p = <0.001*).There were significant differences between Eosinophil according to study group.

Table(3-8): The mean differences of Eosinophil according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Eosinophil ($10^{\wedge 3}$/µl)	Patients	60	0.37	0.27	**4.703**	**<0.001***
	Control group	30	0.18	0.09		

P value ≤ 0.05 was considered as significant.

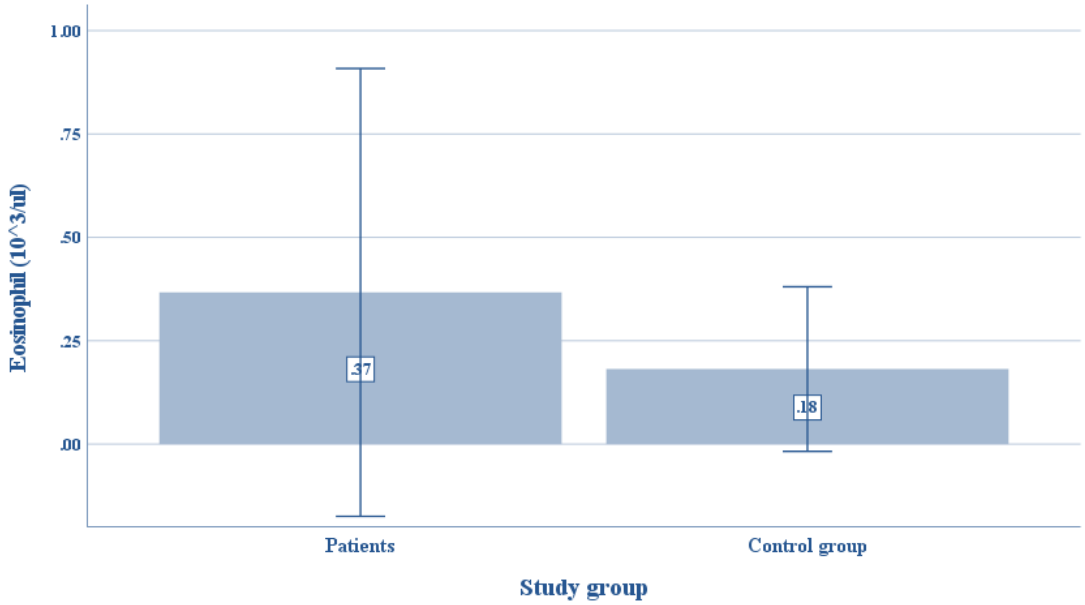


Figure (3-5): The mean differences of Eosinophil ($10^{\wedge 3}$/ul) according to study group (P<0.001*)

Study by Kara and Volkan (2018) found that patients with *E. vermicularis* exhibited higher eosinophilia levels (p=0.001). Also study by Vasjari *et al*., (2014) is through with recent study.

The increase in the number of Eosinophils leads to the hypothesis that eosinophilia is concomitant to the immune response induced by parasitic worms(Lee *et al*.,2010). High IgE levels and eosinophilia have been shown to generally occur during tissue migration or the harboring of parasites, and especially when the parasite invades the bowel mucosa , Eosinophils frequently accumulate in tissues soon after helminth invasion (Panggabean *et al*., 2016). This happens also in hosts immunologically naive to the invading parasite, a fact suggesting that eosinophils play a role in innate defense against this type of pathogen, this rapid and non-specific eosinophilic response might be a barrier limiting mechanism active against the invasion of many tissue-dwelling helminthes (Löscher and Saathoff, 2008).

Solmaz *et al*.(2018) reported elevated eosinophil levels in patients with *E. vermicularis* compare with control (0.56 ± 0.04),(0.37 ± 0.12) respectively .

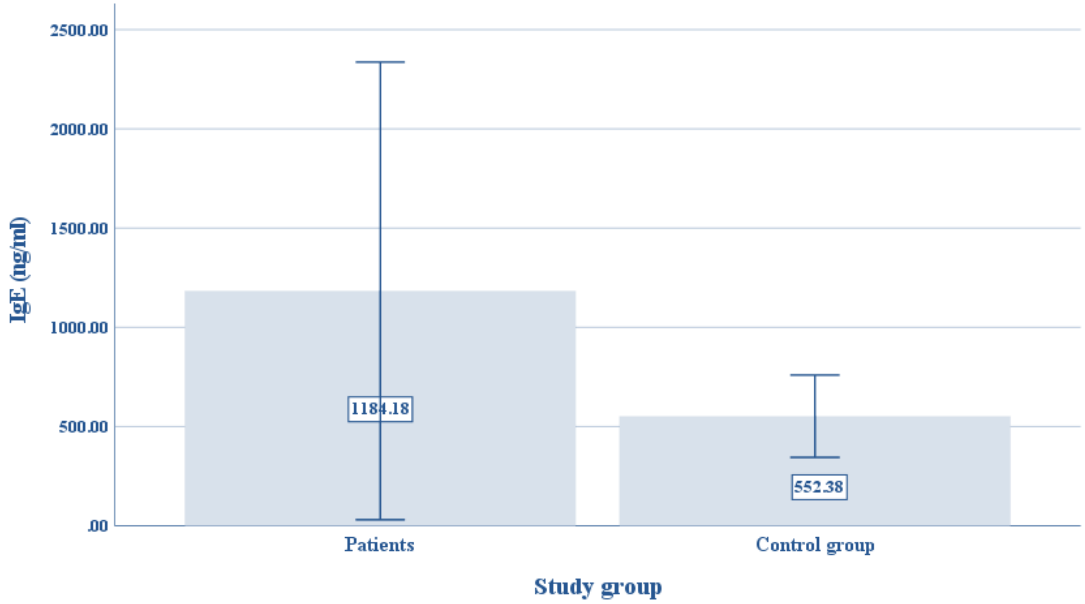
3. 10. The mean differences of IgE according to study group

This study included the measuring of IgE level in patients serum by using IgE ELISA kit; the results reveal a significant increasing ($p \leq 0.05$) in IgE level in patients with Enterobiasis in compare with control group (table 3-9. Figure 3-6).

Table (3-9) : The mean differences of IgE according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
IgE(ng/ml)	Patients	60	1184.18	576.33		
	Control	30	552.38	103.61	**8.23**	**<0.001***

P value ≤ 0.05 was considered as significant



Figure(3-6): The mean differences of IgE (ng/ml) according to study group (P<0.001*)

There is increasing in the mean differences of IgE according to study group including (patients with *E. vermicularis* and control group). The mean value of IgE in sixty patients were (1184.18 ± 576.33) while the mean value in control group was (552.38 ± 103.61),(t= 8.23),

(p=<0.001*) .There were significant differences between IgE according to study group .

The results of recent study were positively with other result (Qhatan, 2019) his study revealed that among those with the *E.vermicularis* 39 parasite , 55.71% had a positive examination of IgE with a mean (227.09 IU), while 31, 44.28% of patients with a negative examination IgE (37.87 IU) compared with the results of the control group negativity for IgE examination with mean (48.66 IU).The results of the statistical analysis showed the presence of very clear significant differences indicating the high level of IgE among those with the *E.vermicularis* parasite compared with the control group at the probability level 0.05.

The immune response include the release of IgE and as result of the binding of IgE with the worms, histamines and other intermediates are released triggering an anaphylactic response(Vasjari *et al*.,2014). Parasitic infections not only stimulate the production of IgE antibodies to the parasite but also the synthesis of non-specific polyclonal IgE (Nagarji *et al* .,2004).The reason may be due to the moral elevation of the concentration of antibody IgE in sero-infected children with intestinal parasites, compared with non-infected children as a result of the immune response and host defense mechanism against the parasite and its venom as it can vary depending on the type of parasite that cause infection (Mukai *et al*., 2016).

The defense against many parasitic infections by stimulation helper T cells type II (Th2), leading to the production of antibodies IgE and activating the role of eosinophils (Amancio *et al*., 2012). As the increase in levels of high IgE receptors on the surface such as mast cell (MC) and Basophils and activation followed by these influential cells to produce

and release mediators biologically active such as histamine and others amines , which have an important role in the expulsion of the parasite, another mechanism of parasite expulsion is antibody dependent cell mediated cytotoxicity via receptors IgE or IgG (Mukai *et al*., 2016).

As for the possible roles of IgE in host defense against toxins, tissue infection appears to be the main cause of T-cell immune responses (Th2) (Kelly and Grayson , 2016). In mammals , including human Th2 responses may lead to a public function in the defense of the host , including the preventive mechanism against toxins and other harmful substances as well as identifying acquired immunity to worms and possibly other pathogens (Hamid and Al-Waaly, 2019). Most of the toxins is a complex mixture of active amines biologically peptides and enzymes often have activity toxic or toxic to nerve cells, however, many of the toxins contain compounds that cause damage to tissues and thus the tissue damage caused by the toxin can generate major danger signals that are felt the immune system, which begins immunity type II and directs the development of antibodies IgE (Mukai *et al*., 2016).

3.11. The correlation between Eosinophil and IgE among patients with *Enterobius vermicularis.*

There were no significant between Eosinophil and IgE among study patients. (N=60, r = -0.082, P =0.532).

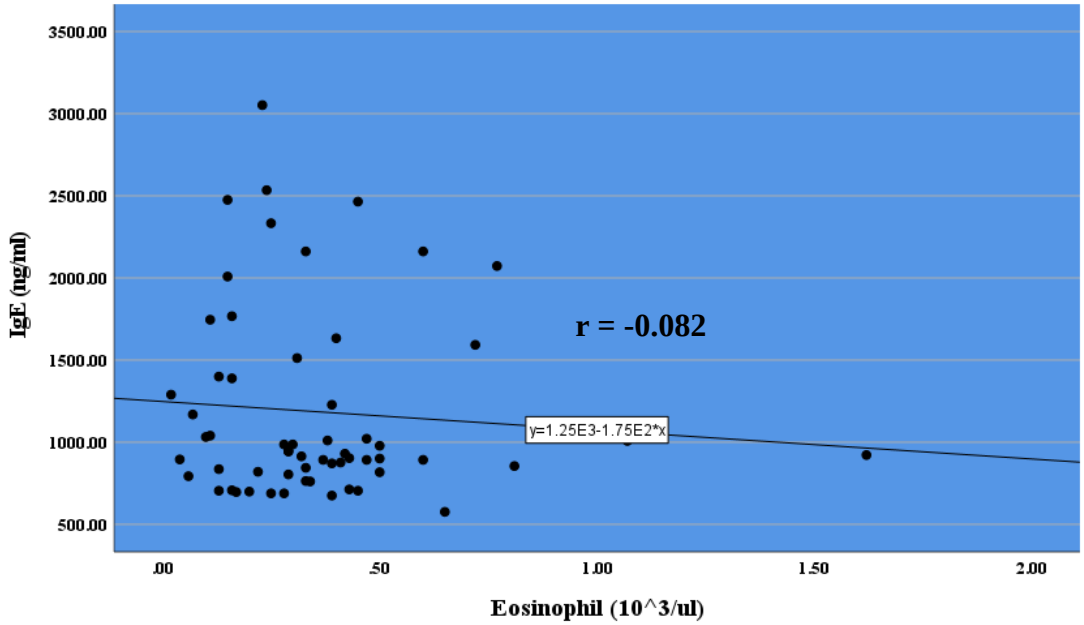


Figure (3-7): The correlation between Eosinophil and IgE among patients with *E. vermicularis* (N=60)

Elevated levels of serum IgE and eosinophilia are the indicators of atopy or allergy , but intestinal parasitic infections may also play role in modulation of those parameters ,in atopic persons, the secretion of IgE rises depending on type 1 immune response, human and animal studies have uncovered a relationship between allergy and parasitic infections(Solmaz *et al*., 2018). High serum total IgE levels appear either chronic parasitic infection or atopy. Interactions between serum IgE levels, eosinophil count and parasitic infections depend on the period of infestation and the type of helminth. This is common in the population with endemic helminthic infections (Rîpă *et al*.,2010).

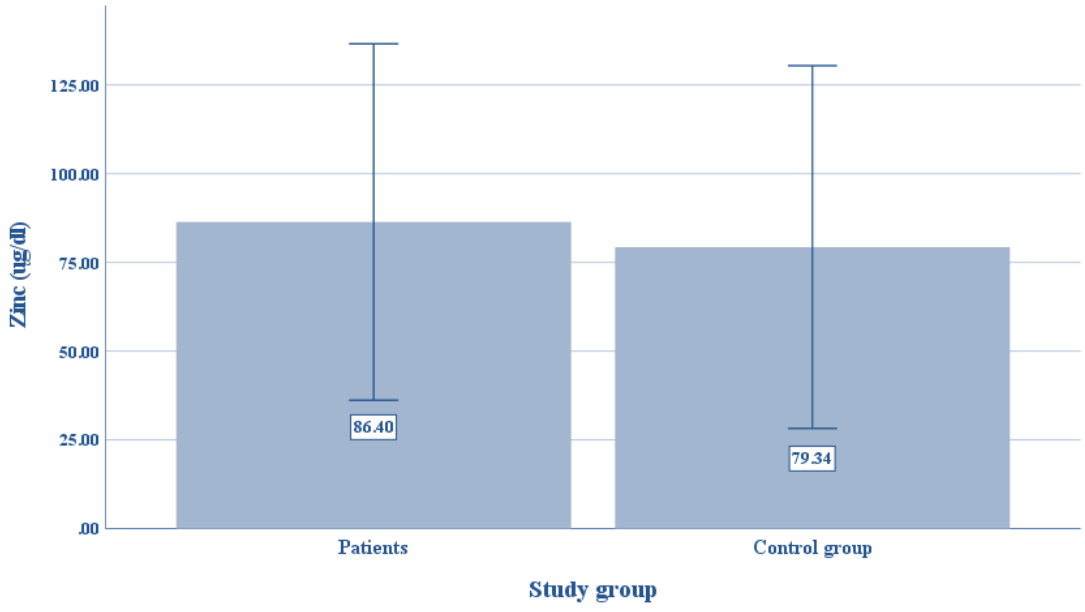
3.12. The mean differences of Zinc according to study group

The results in table (3-10) show the mean differences of Zinc level according to study group including (patients with *E. vermicularis* and control group).There were slightly decreasing in zinc level in patients with *E. vermicularis* but the statistical analysis show no significant differences between Zinc according to study group.

Table (3-10) : The mean differences of zinc according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Zinc(µg/dl)	Patients	60	79.34	25.09		
	control	30	86.40	25.54	1.252	0.214

P value ≤ 0.05 was considered as significant



Figure(3- 8): The mean differences of Zinc (µg/dl) according to study group (P= 0.214)

The present result show that the mean value of serum zinc between patients and control group were (79..34 ± 25.09), (86.40 ± 25.54),(t=

1.251, p-value= 0.214) respectively. The present study was accept with the study by Arbabi *et al*. (2015) he found that the levels of zinc in children with *E. vermicularis* was(72.7 ± 17.92) and in control group (80.66 ± 23.58) , (p- value = 0.05).

Study of Sadraei *et al*.(2007) show a significant difference in serum zinc level in patients with *E. vermicularis* intestinal infection comparing to the control group. Also the present study were in accordance with those recorded by Al-Daoody and Al-Bazzaz (2020) who confirm the mean of serum zinc level in the Enterobiasis positive group were not significantly lower than those in the Enterobiasis negative group.

Zinc from animal sources has higher bioavailability compared to zinc sourced from plant products, people who abstain from eating red meats, vegetarians, vegans, and people living in developing country who rely mainly on plant-based foods are at higher risk of developing zinc deficiency due to inadequate zinc intake (King *et al*.,2015).

Zinc is not stored in the body in large amounts, serum zinc levels could easily decline during infections in children with low zinc intake. However elevation of serum zinc levels could cause acute infections due to the immediate emptying of body stores (Ertan *et al*., 2002).

Insufficient intake of nutrients which the body needs،in particular meats, vegetables, fruits, and other foods، has an adverse effect on levels of essential elements (Koltas *et al*., 1997).

Zinc deficiency was reported to potentially lead to immune dysfunctions that consequently worsen responses toward parasites, and the lack statistical significance may be attributed to numeric problems rather than biologic significance (Atasoy and Bugdayci, 2018). During infection the mucosal epithelium has a high turnover rate and functional

immaturity of enzyme and transport systems. Thus, it is hypothesized that the increased intestinal absorption of zinc associated with anti-Giardia treatment may be explained by the restoration of the impaired intestinal mucosa as a result of the infection , another hypothesis has suggested that zinc deficiency may result from organ redistribution of zinc, from plasma to the liver, as part of the acute phase response of the host; apparently, the immune response of the host leads to activation of the synthesis of metallotionein in the liver and other tissues, altering the hepatic uptake of zinc (Astiazarán-García *et al*.,2015).

3.13. The mean differences of copper according to study group

The present study show the mean differences of copper according to study group including(patients with *E. vermicularis* and control group).There were no significant differences in copper according to study group(Table 3-11), and figure (3-9)

Table(3- 11): The mean differences of copper according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Copper(µg/dl)	Patients	60	99.16	36.23	-0.544	0.587
	Control group	30	103.59	36.52		

P value ≤ 0.05 was considered as significant.

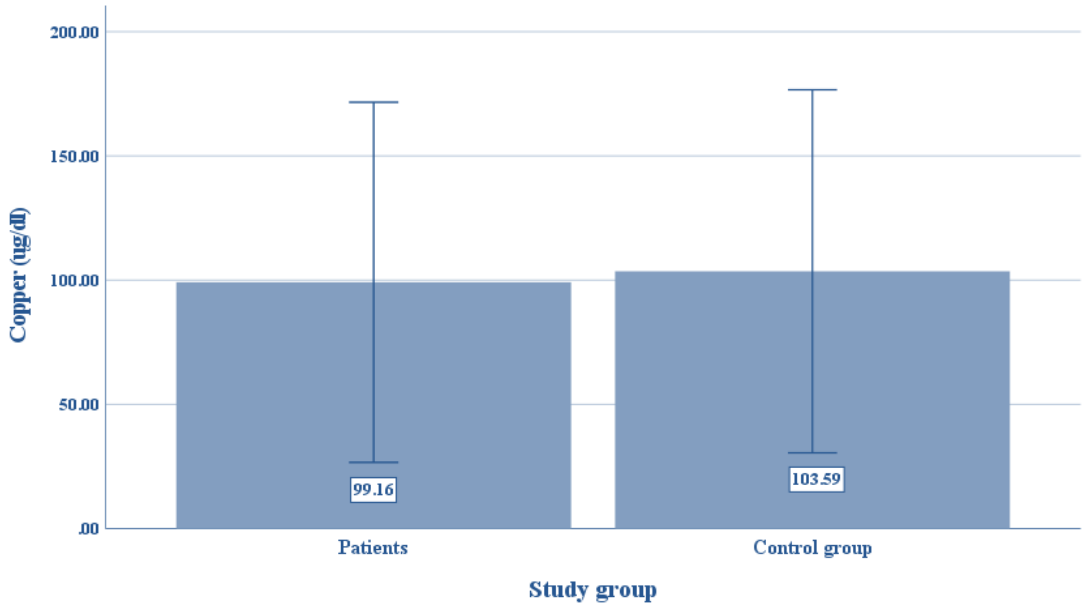


Figure (3-9): The mean differences of Copper (ųg/dl) according to study group (P= 0.587)

The mean value of serum copper in patients were (99.16 ± 36.23) and in control group were (103.59 ± 36.52),(t= - 0.544, p-value = 0.587). study by Çulha and Sangün (2007) in Turkey found that the levels of serum copper in patients were decrease than that in control group as he reported in his results (9.35±0.71),(12.45±2.46) ,(p= 0.112) respectively.

Sadraei *et al*. (2007)show conformed with the study in the level of serum copper ,decreased in patients group than that in control group (94.75 ± 12), (127.75 ± 12. 02)

Copper deficiency usually presents as anemia and neutropenia. If zinc is ingested in high enough amounts, copper malabsorption may ensue since copper binds avidly to metallothionein, over an extended time period of months or years, this may lead to copper deficiency(Gabreyes *et al*.,2013).One of the causes of copper deficiency are consuming too much zinc, as zinc competes with copper to be absorbed (Duncan *et al*.,2015).

The path physiology is not clearly understood; however, micronutrients deficiencies may be linked to malabsorption due to mucous affection (Shalaby *et al*.,2017).

The present study were in acceptable with those recorded by Al-Daoody and AL-Bazzaz (2020) who confirm the mean level of serum copper in the Enterobiasis positive group were not significantly lower those in the Enterobiasis negative group. Intestinal parasite use food sources of the host, such as carbohydrate, minerals, vitamins and lipids as essential energy source for their life cycle (Ramana, 2012).

3.14. The mean differences of Iron according to study group

The mean differences of iron according to study group including (patients with *E. vermicularis* and control group) was shown in table (3-12) and figure (3-10).

Study by Çulha and Sangün (2007) in Turkey revealed that the levels of the serum iron in patients were (128.9±17.9), and in control group were (293.2±62.57), ($p= 7.7\times10-6$; $p<0.01$) and that was in with the recent study . Also the result of Al-Daoody and Al-Bazzaz, (2020) be through with the recent study about the level of serum iron in patient and control group were he reported as (68.74 ± 32.01),(86.40 ± 42.59), (p-value = 0.036) respectively . Study of Dohan and Al-Warid, (2020)also confirm that serum iron was significantly decline in patients infected with *E. vermicularis*.

Iron levels decreased due to the malabsorption (Saboor *et al*.,2015). There were negative effectors reduce iron absorption or compete for/inhibit absorption, such as manganese, zinc, lead, and calcium.

Conversely, positive effectors are fructose, copper, vitamin A enhance absorption of iron (Briguglio *et al*.,2020).

Serum iron declined in *E. vermicularis* infected group as compared to non- infected group but There were no significant differences in iron levels according to study group. The result show that the mean value of serum iron between patients with enterobiasis and control group were (104.80 ± 125.12), (t= - 1.774, p- value= 0.08) respectively. The decrease in serum iron concentration is likely because loss of appetite that may be a result of *E. vermicularis* infection. The pathogenic mechanism is not clear; this could reflect a more affected intestinal mucous and iron deficiency secondary to malabsorption (Olivares *et al*., 2004).

Table (3-12) : The mean differences of Iron according to study group

Study variables	Study group	N	Mean	SD	t-test	p-value
Iron(µg/dl)	Patients	60	104.80	50.26	-1.774	0.08
	Control group	30	125.12	53.14		

P value ≤ 0.05 was considered as significant

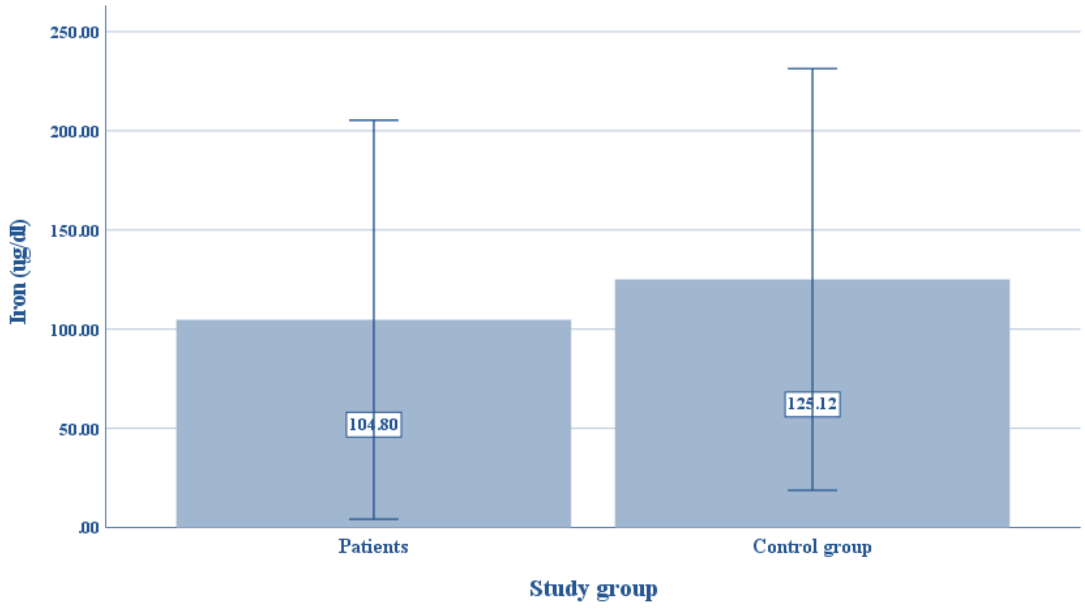


Figure (3-10): The mean differences of Iron (µg/dl) according to study group(p=0.08)

The amount of iron that body need each day depends on age, sex, and whether a consume a mostly plant-based diet, vegetarians who do not eat meat, poultry, or seafood need almost double amount of iron because the body doesn't absorb non heme iron in plant foods as well as heme iron in animal foods ,humans require iron for energy production, oxygen transport and utilization, cellular proliferation, and pathogen destruction. (Lynch *et al* .,2018).

3.15. The correlation between Zinc, copper and iron among patients with *E. vermicularis*

From the result in table (3-13) the present study confirm there is a positive correlation between serum zinc and copper also between serum zinc with iron but these correlation was not significantly ($p > 0.05$) . While there were significant correlation between copper and iron(p= 0,051). Copper, like iron, is required for normal erythropoiesis; copper deficiency causes an iron-deficiency-like anemia. Iron-copper interactions in biological systems may be attributed to their positive charges, similar atomic radii, and common metabolic fates. For example, dietary iron and copper are both absorbed in the proximal small intestine (Gulec andCollins, 2014).

Also, iron and copper must be reduced before uptake into enterocytes and further, both metals are oxidized after (or concurrent with) export into the interstitial fluids (enzymatic iron oxidation may occur while copper oxidation is likely spontaneous), moreover, both metals are involved in redox chemistry in which they function as enzyme cofactors, and both can be toxic when in excess (Ha *et al*.,2016). Furthermore, a reciprocal relationship between iron and copper has been established in some tissues, for example, copper accumulates in the liver during iron

deficiency, and iron accumulates during copper deficiency .Copper levels also increase in the intestinal mucosa and blood during iron deprivation(Gulec and Collins, 2014).

Table (3-13): The correlation between Zinc, copper and iron among patients with *E. vermicularis* (N=60).

Study variables	Number	Mean	SD	R	P-value
Zinc (µg/dl)	60	79.34	25.09	0.089	0.5
Copper (µg/dl)	60	99.16	36.23		
Zinc (µg/dl)	60	79.34	25.09	0.224	0.086
Iron (µg/dl)	60	104.79	50.26		
Copper (µg/dl)	60	99.16	36.23	**-0.253**	**0.0 51**
Iron (µg/dl)	60	104.79	50.26		

P value ≤ 0.05 was considered as significant.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Based on the obtained results, it is possible to reach the following conclusions:

- The most common symptoms were children suffering from it was perianal itching.
- The present study showed that the infected with enterobiasis in female slightly higher than that in male .
- There were significant differences between the mean weight of patients and the mean weight of control.
- There were significant positive linear correlation between age groups and weight and age and height among study patients.
- There were significant differences between hemoglobin and PCV according to study group. There were significant positive linear correlation between hemoglobin and PCV among study patients.
- Patients with *E. vermicularis* exhibited higher eosinophilia levels. The results reveal a significant increasing in IgE level in patients with Enterobiasis.
- There were no significant differences in zinc, copper, and iron. As well as There were no significant correlation between Zinc, copper and zinc, iron among study patients.

Recommendation

The presented conclusion may permit to suggest the following recommendations:

1- Conducting studies of detergents that help eliminate the eggs of *E. vermicularis* , and appropriate treatment for pinworms and urinary enuresis.
2- Expanding the circle of research for *E. vermicularis* infection and inflammation of the urinary tract and rectum.
3- Expanding the studies to investigate the relationship of the infection of enterobiasis and its impact on the appendix.
4- Study the impact of *E. vermicularis* on some immunological marker such as IL-10, IL-4, IL-6, and IL-17A.
5- Conducting a molecular study to detect infections by PCR or RT-PCR .
6- Studying the genetic map or genetic tree of the pinworm and recording it in NCBI .
7- Studying the negative effectors , and positive effectors that effect on iron absorption during the infection with *E. vermicularis* .

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الخلاصة

تعد (الدودة الدبوسية) واحدة من أكثر الديدان الطفيلية البشرية شيوعًا والتي قد تسبب داء الدبوسيات ، وهو أمر شائع بين أطفال المدارس الابتدائية في العديد من البلدان.

اجريت هذه الدراسة للكشف عن تأثير الدودة الدبوسية على بعض العناصر النزرة (الزنك والنحاس والحديد). وكذلك تأثيره على الغلوبيولين المناعي(E) عند الأطفال.

شارك في الدراسة الحالية مائة طفل تتراوح أعمارهم بين (٢-١٢) سنة ، وشملوا كلا الجنسين (٤٥ ذكر و ٥٥ أنثى).

تم الكشف عن الدودة الدبوسية وفقاً لنتائج التشخيص السريري والمختبري حيث ان (١٠٠) مريض كان مشكوكا بأنهم مصابون بالدودة الدبوسية بينما تم تأكيد (٦٠) مريضاً فقط على أنها دودة دبوسية بالفحص المختبري.

أظهر توزيع المصابين بالدودة الدبوسية حسب الأعراض أن ٢٥ (٤١.٦٦٪) من الأطفال المصابين كانوا يعانون من حكة حول الشرج ، و ٥ (٨.٣٣٪) يعانون من سلس البول الليلي.

اظهرت نتائج الدراسة الحالية أن نسبة الإصابة بالدودة الدبوسية كانت (٤٥٪) في الذكور بينما كانت في الإناث (٥٥٪) ، والفئة العمرية (٨-١٠) ، و (١٠-١٢) تمثل نسبة أعلى. وكانت النسبة المئوية (٣٠٪) ، و (٢٣.٣٪) على التوالي ، بينما أقل نسبة إصابة (١٠٪) في الفئة العمرية (٢-٤) سنوات.

لم تكن هناك فروق ذات دلالة إحصائية بين متوسط عمر المريض ومتوسط عمر السيطرة. بلغ متوسط وزن (٦٠) مريضا (٢٣.٣٣ كغم) ، وكان (٢٨.١٣ كغم) في مجموعة السيطرة ، وكانت هناك فروق ذات دلالة إحصائية بين متوسط وزن

المريض ومجموعة السيطرة ، في حين بلغ متوسط طول (٦٠) مريضا (١١٧.٣٨سم) ومتوسط الطول بالسيطرة كانت (١٢٣.٠٧سم) ، ولم يكن هناك فروق ذات دلالة إحصائية ، وكانت هناك علاقة ارتباط خطية موجبة معنوية بين العمر والوزن والعمر والطول بين مرضى الدراسة.

كان متوسط مستوى الهيموجلوبين لستين مريضاً (١١.٦٣غم/ديسيلتر) بينما كان في السيطرة (١٣.٢٥ غم/ديسيلتر) ، ومتوسط مستويات حجم الخلية المرصوصة لستين مريضاً (٣٤.٣٠%) ، ومتوسط مستويات حجم الخلية المرصوصة في السيطرة (٣٨.٦٤%) حسب مجموعة الدراسة. كما وجدت علاقة ارتباط خطية موجبة بين الهيموجلوبين و حجم الخلايا المرصوصة بين مرضى الدراسة.

أظهرت النتائج وجود فروق معنوية بين الحمضات في مجموعة الدراسة اذ كانت (٠.٣٧ ١٠^٣٨/ مايكروليتر) في مرضى داء الدبوسيات مقابل (٠.١٨ ١٠^٣٨/مايكروليتر) في مجموعة السيطرة .

تضمنت هذه الدراسة قياس مستوى الغلوبولين المناعي (E) في مصل المرضى(١١٨٤.١٨) ومصل السيطرة (٥٥٢.٣٨). كشفت النتائج عن زيادة كبيرة في مستوى الغلوبولين المناعي (E) في المرضى الذين يعانون من داء الدبوسيات مقارنة مع مجموعة السيطرة.

العلاقة بين الحمضات و الغلوبولين المناعي (E) بين مرضى داء الدبوسيات لا تظهر فروق ذات دلالة إحصائية بين الدراسة.

أظهرت النتائج الحالية أن متوسط قيمة الزنك في الدم بين المرضى ومجموعة السيطرة كانت (٧٩.٣٤ ميكروغرام / ديسيلتر) ، (٨٦.٤٠ ميكروغرام / ديسيلتر) على التوالي. كان هناك انخفاض طفيف في مستوى النحاس في الدم (٩٩.١٦ ميكروغرام / ديسيلتر) ، (١٠٣.٥٩ ميكروغرام / ديسيلتر) ، والحديد (١٠٤.٨٠

ميكروغرام / ديسيلتر) ، (١٢٥.١٢ ميكروغرام / ديسيلتر) في المرضى الذين يعانون من الدودة الدبوسية بالمقارنة مع مجموعة السيطرة.

لم يكن هناك ارتباط معنوي بين الزنك والنحاس والزنك والحديد بين مرضى الدراسة ولكن توجد علاقة ارتباط عكسية معنوية بين النحاس والحديد.

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