International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 04 April 2024

Page No: 332-339

DOI: https://doi.org/10.47191/ijpbms/v4-i4-12, Impact Factor: 7.792

Evaluation of Iron Metabolism in a Sample of Children Infected with Pinworm Disease in Anbar Province/ Iraq

Iman Jassam Hammadi Al-Mohammedi[1], Rashied Mohammed Rashied[2], Ashraf Jamal Mahmoud Zangana[3]

[1,3] College Of Edu. For women/Bio. Dep./Tikrit univ./Iraq

[2]College Of Sciences / Biology Dep. / University of Anbar

https://orcid.org/000-0002-3162-0646*[3]

ABSTRACT

Pinworm *Enterobius vermicularis* is a common parasitic worm that infects humans, and its disease is widespread around the world, especially among children. Its infection occurs in areas with poor health and environmental conditions that lack personal hygiene. The study was conducted to determine pinworm infection prevalence in the several areas of Anbar Governorate and the status of some physiological indicators associated with it.

The entire children attending Fallujah Teaching Hospital ages between 4 and 15 years were part of the study, and the study period included November 2022 to November 2023. The total samples comprised 1350 samples from various locations in Anbar Governorate. The infection was diagnosed in this study by direct microscopic examinations with a Logul-iodine-stained swab, and egg concentration methods, namely flotation with salt and zinc sulphate, and ana adhesive tape method. Physiological tests i.e. iron levels, ferironportin, and iron transporters.

Analysis of the study yielded a total pinworm infection rate of 6.28% of all samples. The blood test results showed a marked reduction in hemoglobin level and an increment in eosinophils count in the infected group compared to the healthy controls. Concomitantly, total iron, ferroportin, and diaphoretic transporter levels decreased in the infected group providing an association between infection with the parasite and anemia.

KEYWORDS: physiological criteria; pinworm disease; Iraq.

ARTICLE DETAILS

Published On:
18 April 2024

Available on:
https://ijpbms.com/

INTRODUCTION

Pinworm Enterobius vermicularis is one of the parasitic worms with a vast geographical distribution, Moore et al. (2019), and the global estimates suggest that at least 200 million people, especially Children get infected (Alemu et al., 2019). Pinworm infection cases among humans are associated with consumption of contaminated food, the wrong environmental conditions, and inadequate attention

The highest global prevalence rates of the parasite occur in tropical and subtropical regions. Pinworm infection is also common in temperate climates and in industrialized countries, where it appears at all social levels (Fan *et al*., 2019). On the European continent, several previous studies indicated that the prevalence of the parasite in European countries sometimes exceeded one-fifth of children (Wendt *et al*., 2019), while a study in Iceland revealed that the infection rate of children between the ages of 1 and 5 years was 22.4%, as it was found to be infected (88/392) (Fan *et al*., 2019).

Studies that focused on the relationship of iron concentration and infection with the parasite indicated many results and observations. (Alomashi1 and Al-Shabbani, 2019) concluded that the presence of the parasite contributes to reducing the concentration of iron in the serum, and the same result was indicated by a study (Ismail and Abass, 2022), which was Hemoglobin levels and blood hemoglobin volume among affected children are lower than normal, while the number of white blood cells has increased (Obiad *et al*., 2022; Salem and Soroor, 2022).

The concentration of this ferritin in plasma (or serum) is positively related to the size of the body's total iron stores. A low serum ferritin value reflects depleted iron stores. The remaining iron is stored and preserved for later use in all human cells, but it is mostly stored in bone marrow cells and

liver cells. and spleen cells, and these storage processes are known as ferritin complexes (Ismail and Abass, 2022).

Low levels of ferritin in the blood is the most sensitive test for estimating iron deficiency. However, ferritin levels can increase with any type of chronic inflammation, and since ferritin is an acute phase protein, its concentration rises, but not for a long period when it occurs. Infections or other physiological changes in the body, so it is not possible to rely on estimating ferritin concentration as an indication of pinworm infection.

Total iron binding capacity (TIBC) is the laboratory-measured iron-binding capacity of transferrin (Lanier *et al*., 2018). Transferrin can bind two ferric atoms (Fe3+) with high affinity. This means that transferrin has the capacity to transport approximately 1.40 to 1.49 mg of iron for every gram of transferrin present in the blood (Hung *et al*., 2015).

Ferroportin (Fpn) is a protein that transports iron across the membrane from inside the cell to outside the cell. Ferroportin is the sole recognized provider of iron (Zhang et al., 2018). Intestinal cells absorb dietary iron, and Ferroportin allows the movement of iron from those cells into the blood. Fpn not only facilitates the removal of recycled iron from connective tissues, but also from the spleen and liver (Canonne-Hergaux et al., 2006). The regulation of ferroportin is done by hepcidin which is a hormone synthesized by the liver. Hepcidin attaches to Fpn and suppresses iron efflux activity, as a result reducing plasma iron delivery. Thus, implicates of an interaction between Fpn and hepcidin in the iron homeostasis system (Zhang and Rouault, 2018).

Ferroportin is inhibited by hepcidin, which binds to ferroportin and endocytoses it into the cell (Zhang and Rouault, 2018). This leads to the iron retention in the intestinal cells, liver cells and connective tissue with the consequent reduced levels of the serum iron. This is particularly pronounced in the intestines cells that in the dying process of their lifespan cause the considerable iron leak. Hepcidin is produced in reaction to different cytokines (Ganz, 2011).

Hepcidin is a small cysteine-rich peptide hormone, discovered in 2000, that is the master regulator of iron absorption and homeostasis. It is synthesized and secreted by the liver in response to inflammation, iron stores, oxygen tension, or erythrocyte demands. This hormone binds to ferroportin, leading to its neutralization and phosphorylation. It is broken down inside lysosomes (Rosato *et al*., 2022), and this interaction limits the exit of iron from cells into the blood circulation by reducing the absorption of dietary iron, recycling iron from senescent red blood cells, and releasing iron from the body's iron reserves (Schwartz *et al*., 2019).

DMT1 was cloned by Hediger's group in 1997 through functional DNA screening using duodenal mRNA isolated from mice fed a low-iron diet, and is a mechanically complex transporter with multiple isoforms that functions in diverse environments (Yanatori and Kishi (2019), at least four isoforms of the human DMT1 protein have been found, all of which are similar in the presence of the largest (central) portion of the protein (531 or more amino acid residues) among all isoforms. However, they differ in both The amino and carboxyl termini are a result of variant transcription of the responsible human gene (SLC11A2) located in the minor segment of chromosome 13 (Ingrassia *et al*., 2019).

DMT1 has been proposed to be a link between multiple pathways for epithelial iron transport and cellular iron acquisition in health and disease, and the transport of other metals (such as cadmium and manganese) in disease, where the electrochemical proton potential gradient provides the thermodynamic driving force for the transport of concentrated iron from the extracellular or endosome to the cytoplasm. , which places DMT1 among an important class of proteins called symporters (Song *et al*., 2021).

Haptoglobin is an important protein in blood plasma and is produced mainly in the liver. It functions on the one hand as a transport protein for hemoglobin and on the other hand as one of the acute phase proteins. When red blood cells are destroyed pathologically and abnormally (hemolysis), the red blood pigment (hemoglobin) is released in a free, unbound form, and this free form of hemoglobin can It harms the kidneys. For this reason, haptoglobin binds free hemoglobin and brings it to the so-called reticuloendothelial system (RES) in the spleen and liver within a few minutes. There it is extracted and dismantled (Schaer *et al*., 2014).

Its main role is related to its ability to bind hemoglobin (Hb) and form a soluble complex with Hb, and thus it prevents the filtration of hemoglobin in the kidney and it is broken down in liver (Paul *et al*., 2010), which contributes to preserving the kidney from damage, as well as It reduces inflammation by inhibiting the synthesis of prostaglandins, leukotrienes, and cathepsin B, and reduces the oxidative stress of reactive oxygen derived from iron related to the release of free hemoglobin (Ward *et al*., 2022).

The small intestine, bone marrow, liver, and reticuloendothelial system contribute to iron metabolism, and four main types of cells play key roles in regulating iron homeostasis, including enterocytes, red blood cell precursors, macrophages, and liver cells. Iron is absorbed into the bloodstream through... Intestinal cells (Truman-Rosentsvit *et al*., 2019), the absorbed iron is then bound to the specific systemic iron transport protein transferrin. The majority of the iron bound to transferrin is delivered to red blood cell precursors in the bone marrow to be incorporated into hemoglobin in red blood cells. Newly produced(Arosio, 2022).

The body needs 1-2 mg of absorbed iron per day to replace typical amounts of daily iron lost. The majority of the iron needed to meet high iron requirements (20-52 mg/day) is obtained from daily catabolism of senescent red blood cells. Iron absorption and homeostasis are regulated. Systemic loss and take-up via three iron regulatory hormones: Erythropoietin, Hepcidin, and Erythroferrone (Vogt *et al*., 2021).

Cases of anemia are considered one of the health problems that affect public health, as it turns out that about 50% of children suffer from anemia, especially in developing countries (Tsuyuoka *et al*., 1999).

The aim of the study was the microscopic diagnosis of *E. vermicularis*...to determine the extent of the spread of pinworm infection in some areas of Anbar Governorate, and to determine some biochemical parameters resulting from pinworm infection such as Total Iron Binding capacity hepcidin, ferroportin, divalent metal ion transporter

MATERIALS AND METHODS

1- Sample collection:

1-1: Study area: The current study was conducted in Anbar Governorate, western Iraq, from different regions, and samples were tested in Women's and Children's Hospital in Fallujah, the health center in Saqlawiyah, and the health center in Al-Amriya, in period from November 2022 to November 2023.

In this study, 1350 samples were collected from both sexes, and 86 samples were included and studied in the laboratory to conduct CBC examination and physiological tests for children aged 4-15 years.

1-2: Collecting parasite samples:

 General stool examination was performed using two methods (the direct swab method and the flotation method using saturated salt, and also the flotation method using zinc sulfate) (Forbes *et al*., 2007, and the adhesive tape method was implemented (Li *et al*, 2019).

 Examination, examination and diagnosis of fecal samples and eggs isolated from the adhesive tape under a light microscope using Lugol's iodine solution.

1-3: Blood collection and serum preparation

• 5 ml of the children's blood was drawn.

• Measure the complete blood count using the Sysmex device.

• Hepcidin, Divalent Metal Ion Transporter-1 (DMT1), Total Iron Binding capacity, Ferro protein were measured using a PKL device (PPC POKLER ITALIA 230 Automatic ElISA Reader).

1-4: Physiological tests:

1-4-1: CBC complete blood count analysis

1 ml of blood samples obtained were immediately placed in EDTA anticoagulation tubes to measure hemoglobin and white blood cell concentrations and packed cell volume for both infected and unaffected children using an automatic CBC blood analyzer (Zeki and Harith, 2019).

It was kept in a cool box and was sent within an hour to the laboratory for blood analysis using a Sysmex device.

1-4-2: Hepcidin (Hepc) analysis: The ELIZA kit was used to measure Hepc in blood samples. The ELISA kit uses the Sandwich-ELISA principle produced by Elabscience, USA.

1-4-3: Ferroportin (FPN) analysis: The ELIZA kit was used to measure FPN in blood samples. The ELISA kit uses the Sandwich-ELISA principle produced by Elabscience, USA.

1-4-4: Total Iron Binding Capacity (TIBC)

Take 1 ml of serum, add 1 ml of 10 mg/L Iron Standard solution, mix thoroughly and wait at room temperature for 10 minutes, then add a vial of Reagent 5, mix completely and leave at room temperature for 5 minutes, repeat the mixture. The steps are four times. The supernatant was centrifuged at 2300 g for 10 minutes and the supernatant was taken for detection.

Empty tube: Add 1 ml of double distilled water to a 5 ml EP tube.

Standard tube: Add 1 ml of Iron Standard solution to a 5 ml EP tube.

Add 2 ml of Chromogenic Agent to each tube, mix well using a mechanical mixer and incubate in a 100°C water bath for 5 minutes.

The tubes were cooled with running water, then centrifuged at 2300 g for 10 minutes and 1 ml of supernatant was taken.

The spectrophotometer was set to zero with double distilled water and the OD value of each tube was measured at a wavelength of 520 nm with an amount of 0.5 cm and the result was calculated according to the following equation:

TIBC (mg⁄L)=(ΔA_1)/(ΔA_2)×C_1×f

TIBC (mg/L)=(ΔA_1)/(ΔA_2)×C_2×f

UIBC (μmol/L)=C_4-C_3

i=C_3÷C_4×100%

1-4-5: Divalent Metal Ion Transporter-1 (DMTI) analysis

Drill the plastic sheet for each of the (diluted standard, blank, and sample) and select 7 holes for the standard and one hole for the blank. 100 units of each standard solution, or 100 microliters of samples, were added to the appropriate wells and covered using Plate Covers and incubated for a period of 80 minutes at 37°C.

The liquid was poured into each of the pits, then the solution was withdrawn and washed with 200 μL of Wash Buffer for each pit and left for 1–2 minutes. Then remove the remaining liquid from all the holes completely by wrapping the plate on absorbent paper. It was washed completely 3 times. After the final wash, the remaining Wash Buffer was removed by vacuuming or pouring, then the board was turned over and wiped.

100 microliters of Biotinylated Antibody was added to each well and incubated for 50 minutes at 37°C.

The aspiration and washing step for each group was repeated 3 times as performed in step 2.

100 μl of Streptavidin-HRP solution was added to each well and incubated for 50 min at 37°C.

The aspiration and washing step for each group was repeated 5 times as performed in step 2.

90 μl of Substrate SolutionTMB was added to each hole. They were incubated for 20 minutes at 37°C in the dark. It has been contained

Turn the liquid into blue by adding TMB solution. The Microplate was then heated for approximately 15 minutes before measuring the OD

50 μl of Stop Solution was added. The liquid was turned yellow by adding Stop Solution. Mix the liquid by tapping on

the side of the dish, if the color does not change uniformly, apply gentle pressure on the hole to ensure thorough mixing.

Any drop of water or fingerprint on the bottom of the drill was wiped off and it was then ensured that there was no bubble on the surface of the liquid. The Microplate reader was turned on and the measurement was performed at 450 nm.

1-5: Statistical analysis: The t-test, (X2) Chi-square test, and (Analysis of Variance) were used in the statistical analysis (Pearson, 1900).

RESULTS AND DISCUSSION

Some fecal samples showed *E. vermicularis* worms, and in other samples, pinworm eggs were collected from the perianal area of the infected children. These worms were small, ranging in length from 8 to 13 mm, and resembled small pieces of thread, and they can be seen from Through a magnifying glass, it shows the distinctive shape, pointed tail and sharp front end. These results are consistent with studies (Wendt *et al*, 2019; Fan *et al*, 2019).

Using the direct smear method, the floatation method (saturated salt and zinc sulphate), and the adhesive tape method, the eggs of *E. Vermicularis* were detected, which have an average length of 50-60 micrometers and a diameter of 20-25 micrometers. The eggs appear convex and flat, and the shell is double-walled and smooth. The eggs often contain Embryo. Image (1).

The results showed a significant decrease in hemoglobin level in the infected group (12.09 g/100 ml) compared to the non-infected group (12.58 g/100 ml) with a probability of 0.01. This indicates a relationship between pinworm infection, low hemoglobin levels, and the possibility of anemia.

A higher number of eosinophil cells was observed in the infected group (0.30 cells/mm3) compared to the uninfected group (0.05 cells/mm3) with a probability of 0.00001. This suggests that helminth infection stimulates an inflammatory response that includes an increase in number of eosinophils.

While no significant differences were observed between the two groups in the number of white blood cells, neutrophil cells, mononuclear cells, B cells, and lymphocytes. Which indicates that infection with the parasite may not clearly affect these cells. Figure (1)

The results indicated noticeable changes in the cellular and hormonal immune response following infection with this parasite. Increased activity of the immune system as a result of injury, such as an increase in the number of white blood cells, especially eosinophils, can lead to weak hemoglobin formation and obstruct the absorption of elements necessary for the synthesis of blood components (Al-Daoody A, Al-Bazzaz, 2020; Obaid, 2022).

A study was conducted on 505 children in city of Erbil and found that the overall prevalence of pinworm infection was 27.13%, with a higher rate in females. The study also revealed that infected children showed a significant decrease in levels of total serum protein and iron compared to those who were not infected (Al-Daoody *et al*., 2020).

Early diagnosis of this disease reduces the risk of transmission and avoids further costly diagnostic tests (Schroeder *et al*., 2019).

Another study also indicated the necessity of early examination for pinworm infection in children who suffer from a high number of eosinophils (DI Cicco *et al*, 2023).

The study showed significant differences between children infected with pinworms and the cellular and physiological components of blood. In general, infection with pinworms led to a deficiency of blood components compared to the control group, regardless of the sex and age of children, and despite the fact that pinworms live in the intestines and do not feed directly on blood, but feed on Host waste, but some studies have confirmed its effect on blood components and the appearance of symptoms of anemia in infected children. This is a result of its indirect effect on the one hand, and the secretion of blood-soluble substances on the other hand.

Total iron binding capacity, which is the ability of blood serum to bind and transport iron, was much lower in people infected with parasites compared to healthy people, which indicates a decrease in the efficiency of iron transport in the blood. Hepcidin is a protein that regulates the absorption of iron from intestine and is the main regulator of iron balance in the body. Its levels were Significantly lower in those infected with parasites, which is consistent with a decrease in iron absorption resulting from parasitic infection. Ferroportin, which is a protein that transports iron out of the cells stored in the intestinal absorption cells and microphages, and its levels were also lower in those infected, which reflects the decrease in iron accumulation in them, and when the level of ferroportin is inhibited. Hepcidin prevents the intestinal absorption cells from secreting iron into the hepatic portal system, thus reducing iron absorption. As for the levels of DMT1 (divalent metal transporter), which is a protein that transports iron across the intestinal cell membrane, its levels were observed to decrease in those infected, indicating a defect in the transport of iron to the intestinal cell membrane. Intracellular as a result of parasitic infection.

Patients infected with pinworms have significantly lower levels of total iron binding capacity (TIBC), a basic test used to diagnose iron deficiency anemia and other iron metabolism disorders, which is the ability to bind iron to transferrin. Iron-binding capacity is divided into two types, TIBC and unsaturated iron-binding capacity (UIBC). When iron stores are depleted, blood transferrin levels increase (McDowell *et al*, 2022).

Patients infected with pinworms have significantly lower levels of total iron-binding capacity, and research indicates that infection with the parasites can lead to iron deficiency and anemia caused by chronic bleeding (Laranjiera *et al*, 2019). It is believed that the sharp decrease in the total iron-binding capacity of pinworm patients is automatically due to several factors, including: chronic haemorrhagic iron loss

through the gastrointestinal tract consequent to bleeding and parasite diarrhea associated with the infection, as well as iron absorption from the intestine impaired by some parasite secretions, and increased utilization and storage of iron. Inside the cells of the parasite itself (Movahed, et al, 2016).

Hepcidin has risen as the main iron-regulating hormone over the last 10 years. The 25 amino acid defensin-like peptide is mostly synthesized by liver in response to an excess of iron in plasma or tissue that replenishing the absorption and recycling of the metal in a controlled state limited manner (Bek et al, 2020). A study has also revealed that hepcidin levels drop by 60-90% in people infected with different intestinal parasitic worms (Wang et al, 2016). This reduction is auto linked with some parasites' capacity to disrupt the hepcidin signaling paths within the cell resulting to the inhibition of its synthesis. The parasite has the capability of absorbing iron directly from the serum and thus suppressing hepcidin in a situation of low iron (Pagani et al., 2019).

SNP gene defines ferroportin which is a protein that exports iron from cells into the bloodstream. It plays an essential role in iron homeostasis and has many physiological functions like oxygen utilization, host defense and hematopoiesis. A second expression of such changes in the FPN gene is ferroportin disease (FD) which is an autosomal dominant disease with iron accumulation in the tissue macrophages and reduction of iron to be released in the serum transferrin. Diagnosis on abdominal MRI is the most commonly used (Pietrangelo et al, 2017; Drakesmith et al, 2015).

Patients infected with pinworms have much lower levels of ferroportin, as ferroportin is the main protein responsible for storing and retaining iron inside cells (Richard et al., 2020), and several studies have indicated a close association between low ferroportin levels and intestinal parasitic infections (Abreu et al., 2018), and this is likely due to various mechanisms, including: Iron loss via blood and urine is associated with chronic parasitic diarrhea, reduced iron absorption as a direct consequence of gastrointestinal worm infestation, and consumption and storage of iron within the human host by the parasite itself (Jonker et al., 2013).

Divalent metal transporter 1 (DMT1) is a protein which is a product of the SLCHA2 gene and serves as the major iron transporter in humans. It is involved in the absorption of non-heme iron from the intestine in most cell types and its translocation from endosomes (Burdo et al., 2001).As an iron balance keeper, it provides for proper distribution of iron throughout the body (Skjorringe et al., 2015).

Patients with pinworm disease have much lower levels of the divalent metal transporter protein DMT1. Because it plays an essential role in the transport and absorption of iron across the cell membrane (Montalbetti, *et al*., 2013), it has been shown that there is a significant inhibition in the gene and protein expression of DMT1 during infection with some types of intestinal parasites (Roellig, *et al*., 2015).

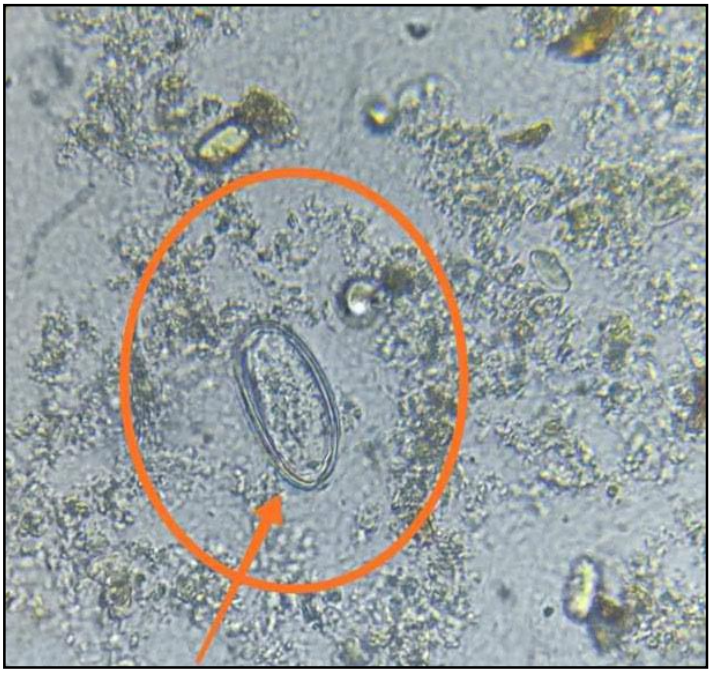

Image 1: A direct stool smear showing distinct planar convex eggs of *E. vermicularis*

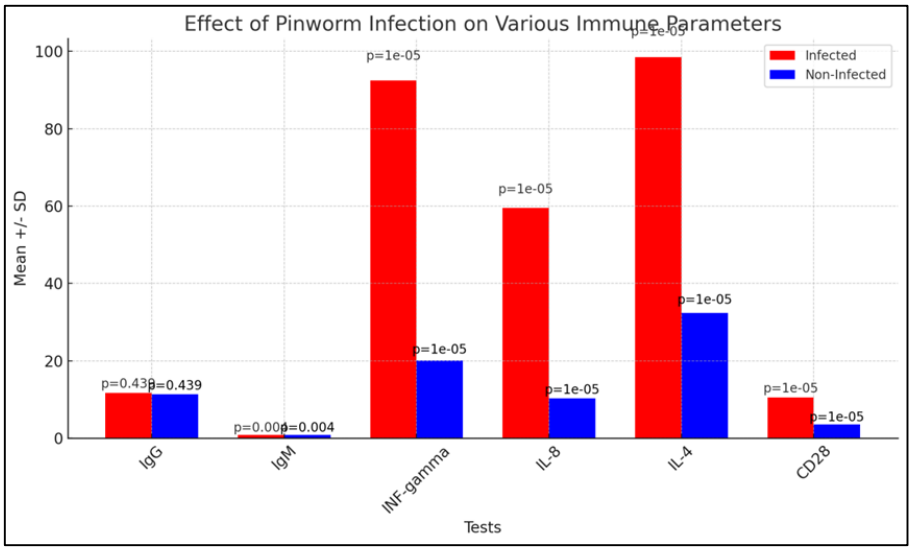

Figure (1): The effect of parasite infection on some components of peripheral blood (hemoglobin, total number of white blood cells, numbers of eosinophils, and platelets)

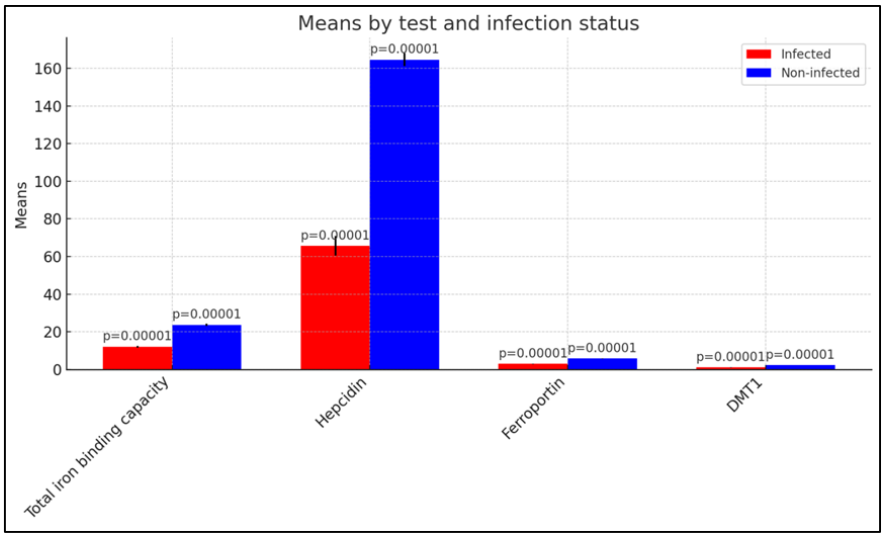

Figure (2): Comparison of physiological parameters between the infection group and the control group

REFERENCES

I. Abreu R, Quinn F, Giri PK(2018) Role of the hepcidin-ferroportin axis in pathogen-mediated intracellular iron sequestration in human phagocytic cells. Blood Adv.;2(10):1089-1100.

II. Al-Daoody A, Al-Bazzaz EN (2020) Impact of *Enterobius vermicularis* infection on biochemical parameters in the blood of children in Erbil Province, Iraq. BMC Infectious Diseases. 2020; 20:336-339.

III. Al-Daoody A, Qadir FM, Tahir AA, Mahmood NH, Majeed AF (2020).Risk factors of *Enterobius vermicularis* infection with symptoms among children in Erbil governorate. Pak-Euro Journal of Medical and Life Sciences.3(2):50-58.

IV. Alemu G, Abossie A, Yohannes Z(2019) Current status of intestinal parasitic infections and associated factors among primary school children in Birbir town, Southern Ethiopia. BMC Infect Dis;19(270):1–8.

V. Alomashi, Ghada&Abd Al-Shabbani, Amal. (2019) Prevalence of Intestinal Parasitic Infestation in Anemic Patients Attended to Al-Diwaniyah Teaching Hospital at Al-Qadisiyah Province/Iraq. International Journal of Pharmaceutical Quality Assurance.

VI. Arosio P. (2022) New Advances in Iron Metabolism, Ferritin and Hepcidin Research. Int J Mol Sci. 23(23):14700.

VII. Bek SG, Üstüner B, Eren N, Sentürk Z, Gönüllü BK.(2020) The effect of hepcidin on components of metabolic syndrome in chronic kidney disease: a cross-sectional study. Rev Assoc Med Bras (1992;66(8):1100-1107.

VIII. Burdo JR, Menzies SL, Simpson IA, Garrick LM, Garrick MD, Dolan KG, Haile DJ, Beard JL, Connor

JR. (2001) Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. J Neurosci Res;66(6):1198-207.

IX. Canonne-Hergaux F, Donovan A, Delaby C, Wang HJ, Gros P. (2006) Comparative studies of duodenal and macrophage ferroportin proteins. Am J PhysiolGastrointest Liver Physiol.290(1):G156-63.

X. Di Cicco M, Bertolucci G, Gerini C, Bruschi F, Peroni DG. (2023) Eosinophilia and potential antibody cross-reactivity between parasites in a child with pinworm and immune dysregulation: a case report. BMC Pediatr 27;23(1):200.

XI. Drakesmith H, Nemeth E, Ganz T. Ironing out Ferroportin. (2015) Cell Metab.;22(5):777-87.

XII. Fan C.K., Chuang T.W., Huang Y.C., Yin A.W., Chou C.M., Hsu Y.T., Kios R., Hsu S.L., Wang Y.T., Wu M.S., Lin J.W., Briand K., Tu C.Y. (2019) *Enterobius vermicularis* infection: prevalence and risk factors among preschool children in kindergarten in the capital area, Republic of the Marshall Islands. BMC Infect Dis.19(1):1–7.

XIII. Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007) Diagnostic microbiology. St Louis: Mosby.

XIV. Ganz T. (2011) Hepcidin and iron regulation, 10 years later. Blood. 117(17):4425-33.

XV. Hung SH, Lin HC, Chung SD (2015) Association between venous thromboembolism and iron-deficiency anemia: a population-based study. Blood Coagul Fibrinolysis. 26(4):368-72.

XVI. Ingrassia, R., Garavaglia, B., & Memo, M. (2019) DMT1 Expression and Iron Levels at the Crossroads Between Aging and Neurodegeneration. Frontiers in Neuroscience, 13, 432714.

XVII. Ismail RM, Abass KS (2022) Evaluation of certain important biochemical parameters in school-age children infected with *Enterobius vermicularis*. InternationalJournal of Health Sciences.6(S6):10558–10563.

XVIII. Jonker FA, Calis JC, Phiri K, Kraaijenhagen RJ, Brabin BJ, Faragher B, Wiegerinck ET, Tjalsma H, Swinkels DW, van Hensbroek MB (2013) Low hepcidin levels in severely anemic malawian children with high incidence of infectious diseases and bone marrow iron deficiency. PLoS One;8(12):e78964.

XIX. Lanier, J.B.; Park, J.J.; Callahan, R.C. (2018) Anemia in older adults. Am. Fam. Physician, 98, 437–442.

XX. Laoraksawong P, Pansuwan P, Krongchon S, Pongpanitanont P,Janwan P. (2020) Prevalence of *Enterobius vermicularis*infections and associated risk factors among schoolchildren in Nakhon SiThammarat, Thailand. Tropical Medicine and Health.48(1):83-90.

XXI. Laranjeira-Silva MF, Hamza I, Pérez-Victoria JM (2020) Iron and Heme Metabolism at the Leishmania-Host Interface. Trends Parasitol;36(3):279-289.

XXII. Li SS, Wang L, Li AH. (2019) [Surveillance of soil-transmitted nematode infections in Zhenjiang City from 2006 to 2018]. ZhongguoXue Xi Chong Bing Fang ZhiZaZhi. 32(1):83-86. Chinese. PMID: 32185933

XXIII. McDowell LA, Kudaravalli P, Sticco KL. (2022) StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL Iron Overload.

XXIV. Montalbetti N, Simonin A, Kovacs G, Hediger MA (2013) Mammalian iron transporters: families SLC11 and SLC40. Mol Aspects Med;34(2-3):270-87.

XXV. Movahed R, Khara H, Ahmadnezhad M, Sayadboorani M. (2016) Hematological characteristics associated with parasitism in Pikeperch Sander lucioperca (Percidae) from Anzali Wetland. J Parasit Dis;40(4):1337-1341.

XXVI. Obaid, M. K., Islam, N., Alouffi, A., Khan, A. Z., da Silva Vaz Jr, I., Tanaka, T., & Ali, A. (2022). Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. Frontiers in Cellular and Infection Microbiology, 12, 941831.

XXVII. Obaid, M. K., Islam, N., Alouffi, A., Khan, A. Z., da Silva Vaz Jr, I., Tanaka, T., & Ali, A. (2022). Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. Frontiers in Cellular and Infection Microbiology, 12, 941831.

XXVIII. Obiad, H.M.; Al-Alousi, T.I.; Al-Jboori, A.H. (2012) The in vivo effect of some medicinal plant extracts on Cryptosporidium parasite. J. Univ. Anbar. Pure Sci. 6, 3.

XXIX. Pagani A, Nai A, Silvestri L, Camaschella C. (2019) Hepcidin and Anemia: A Tight Relationship. Front Physiol;10:1294.

XXX. Paul, W., & Zhu, J. (2010) How helper T cells work. Science, 27(5929), 637–638.

XXXI. Pearson, K. (1900) X. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science, 50(302), 157–175.

XXXII. Pietrangelo A. (2017) Ferroportin disease: pathogenesis, diagnosis and treatment. Haematologica.;102(12):1972-1984.

XXXIII. Richard F, van Lier JJ, Roubert B, Haboubi T, Göhring UM, Dürrenberger F. (2020) Oral ferroportin inhibitor VIT-2763: First-in-human, phase 1 study in healthy volunteers. Am J Hematol;95(1):68-77.

XXXIV. Roellig DM, Salzer JS, Carroll DS, Ritter JM, Drew C, Gallardo-Romero N, Keckler MS, Langham G, Hutson CL, Karem KL, Gillespie TR, Visvesvara GS, Metcalfe MG, Damon IK, Xiao L. (2015) Identification of Giardia duodenalis and Enterocytozoon bieneusi in an epizoological investigation of a laboratory colony of prairie dogs, Cynomys ludovicianus. Veterinary parasitology, 210(1-2), 91-97

XXXV. Rosato, B.E.; Marra, R.; D'Onofrio, V.; Del Giudice, F.; Della Monica, S.; Iolascon, A.; Andolfo, I.; Russo, R. (2022) SEC23B Loss-of-Function Suppresses Hepcidin Expression by Impairing Glycosylation Pathway in Human Hepatic Cells. Int. J. Mol. Sci. 23, 1304.

XXXVI. Salem, K. N. Q., Soroor, H. A. M. (2022) PREVALENCE AND EFFECT OF ASCARIS LUMBRICOIDES, *ENTEROBIUS VERMICULARIS* AND HYMENOLEPIS NANA ON THE ACCOMPANYING BLOOD PICTURE IN IBEN KOLDOON HOSPITAL, LAHAJ-YEMEN. Electronic Journal of University of Aden for Basic and Applied Sciences, 3(3), 222-233.

XXXVII. Schaer DJ, Vinchi F, Ingoglia G, Tolosano E, Buehler PW. (2014) Haptoglobin, hemopexin, and related defense pathways-basic science, clinical perspectives, and drug development. Front Physiol. 5:415.

XXXVIII. Schroeder JC, Jones D, Maranich A. (2019) Peripheral Eosinophilia Found in Pediatric *Enterobius vermicularis* Infections. Clinical Pediatrics;58(1):13-16.

XXXIX. Schwartz AJ, Das NK, Ramakrishnan SK, Jain C, Jurkovic MT, Wu J, Nemeth E, Lakhal-Littleton S, Colacino JA, Shah YM. (2019) Hepatic hepcidin/intestinal HIF-2α axis maintains iron absorption during iron deficiency and overload. J Clin Invest. 129(1):336-348.

XL. Skjorringe T, Burkhart A, Johnsen KB, Moos T. (2015) Divalent metal transporter 1 (DMT1) in the brain: implications for a role in iron transport at the blood-brain barrier, and neuronal and glial pathology. Front Mol Neurosci;8:19.

XLI. Song, P., Zha, M., Yang, Q., Zhang, Y., Li, X., & Rudan, I. (2021). The prevalence of adult attention-deficit hyperactivity disorder: A global systematic review and meta-analysis. Journal of global health, 11.

XLII. Tigabu A, Solomon T, Aynalem M, KasawAdane (2019) Prevalence and associated factors ofintestinal parasitic infections among patients attending Shahura Health Center, Northwest Ethiopia. BMC Res Notes.12:333.

XLIII. Truman-Rosentsvit M, Berenbaum D, Spektor L, Cohen LA, Belizowsky- Moshe S, Lifshitz L. *et al.*, (2018) Ferritin is secreted via 2 distinct nonclassical vesicular pathways. Blood. 131:342– 52.

XLIV. Tsuyuoka R, Bailey JW, Nery Guimarães AM, Gurgel RQ, Cuevas LE. (1999) Anemia and intestinal parasitic infections in primary school students in Aracaju, Sergipe, Brazil. Cad SaudePublica. 15(2):413-21.

XLV. Vogt AS, Arsiwala T, Mohsen M, Vogel M, Manolova V, Bachmann MF. (2021) On Iron Metabolism and Its Regulation. Int J Mol Sci. 22(9):4591.

XLVI. Wang CY, Babitt JL. (2016) Hepcidin regulation in the anemia of inflammation. CurrOpin Hematol.23(3):189-97.

XLVII. Ward, R. J., Dexter, D. T., & Crichton, R. R. (2022). Iron, neuroinflammation and neurodegeneration. International Journal of Molecular Sciences, 23(13), 7267.

XLVIII. Wendt S, Trawinski H, Schubert S, Rodloff AC, Mössner J, Lübbert C. (2019) The Diagnosis and Treatment of Pinworm Infection. Dtsch Arztebl Int 29;116(13):213-219.

XLIX. Wendt S, Trawinski H, Schubert S, Rodloff AC, Mössner J, Lübbert C. (2019) The Diagnosis and Treatment of Pinworm Infection. Dtsch Arztebl Int 29;116(13):213-219.

L. Yanatori I, Kishi F. (2019) DMT1 and iron transport. Free RadicBiol Med. 133:55-63.

LI. Zeki, IsraaNaif, and Harith Saeed Al-Warid. (2019) "The Prevalence of Anemia Among Children Infected With EntamoebaHistolytica in Baghdad". Iraqi Journal of Science 60 (12):2590-99.

LII. Zhang DL, Ghosh MC, Ollivierre H, Li Y, Rouault TA. (2018) Ferroportin deficiency in erythroid cells causes serum iron deficiency and promotes hemolysis due to oxidative stress. Blood.132(19):2078-2087.