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# C-Reactive Protein and Some Hematological, Biochemical Parameters in β-Thalassemia Patients with Cryptosporidiosis

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**ABSTRACT**

Cryptosporidium is a parasite that has the potential to cause complications in Immunosuppressed patients such as beta-thalassemia. Thus, Stool and blood samples were collected from 260 patients with beta-thalassemia who consulted the Thalassemia Center at Azadi Hospital, and from 110 individuals suffering from watery diarrhea, abdominal pain, or any digestive disorder, returning to the pediatrics Hospital and Kirkuk General Hospital, during October 2019 until September 2020. The rate of parasite infection reached (16.92%) in Thalassemia patients, while it reached (14.45%) in the other examined samples, and the rate of infection of males was (17.9%) higher than females (14.4%). The study showed a significant decrease in hemoglobin (Hb), red blood cell count (RBC) and mean cell volume (MCV), and a significant increase in white blood cells (WBC), which may reflect higher neutrophils and lymphocytes in patients with beta-thalassemia infected with the parasite than in other samples. Lipid profile showed a decrease in the level of total cholesterol (TC), low-density lipoproteins (LDL), and high-density lipoproteins(HDL), and a significant increase in the level of triglycerides(TG), liver function enzymes Alanine aminotransferase (ALT), Aspartate transaminase (AST). Besides, Alkaline phosphatase (ALP) enzymes and the level of ferritin (Fe) in thalassemia patients infected with the parasite compared to the other samples. the C-reactive protein CRP level was significantly higher, as a result of infection with the parasite. It was concluded that patients with beta-thalassemia have multiple abnormal complications as a result of multiple blood transfusions, iron overload, and hepatitis. These complications are risk factors causing parasite infection such as Cryptosporidiosis.

**KEYWORDS**

Cryptosporidiosis, β-Thalassemia, CBC, Lipid Profile, Liver Function, Ferritin, Albumin, CRP.

## Introduction

Cryptosporidiosis is one of the causes of diarrhea in humans that results from infection with a parasite of the genus Cryptosporidium, where this parasite is widely spread and is one of the common diseases between humans, animals, reptiles, and birds. (TYZZER, 1907) was the first to describe this parasite, who diagnosed it for the first time in the intestine of mice. However, more than 10 species that belonged to the genus Cryptosporidium have been diagnosed, and C. parvum is the main species responsible for diseases in humans and domestic animals such as cattle, horses, sheep, goats, and pigs (Abdul-Sada, 2015). By means of it has a high epidemic capacity and ability through its short life cycle it spends in a host, and the egg sacs are susceptible to infection and do not need a period and have the ability to resist sterilizers and disinfectants (Laberg et al., 1996). Species of the genus Cryptosporidium are transmitted to various other organisms through water and food contaminated with egg sacs (Steinberg et al 2004). Thus, the parasite infects the intestine epithelial brush border, which leads to severe watery diarrhea with high temperatures, as well as accompanied by Intestinal colic, nausea, and vomiting, especially in children under the age of five years of age, and the disease may last for two weeks or more (Al-Jawasim and Al-khaled, 2019 and Heo *et. al*., 2018). The occurrence of infection may turn into the Chronic type in people with Immunosuppressed, such as patients with thalassemia (Mediterranean anemia). Besides that, beta-thalassemia is the most common and important type of thalassemia, where the child becomes infected with thalassemia when his parents are carriers of the genetic trait responsible for the disease. Its main sign includes chronic hemolytic anemia (severe anemia) that leads to a decrease in the level of hemoglobin, with which the patient needs a continuous blood transfusion that leads to increased iron deposition due to the breakdown of red blood cells. Along with increased absorption of iron in the intestine, which leads to delayed growth, bone deformation, endocrine disruption, heart failure, and viral hepatitis that ends with cirrhosis. It affects in a general and comprehensive way all parts of the body and affects the activity of the organism, which negatively affects its active role as a living organism in society and the condition ends in death under the age of thirty. On the other hand, the host's immune response to cryptosporidium includes components of

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both the innate and adaptive immune system (Xiao, 2010). To understand some of the above, the research aimed to study the prevalence of the above parasite in a sample of beta-thalassemia patients. In addition to evaluating the relationship of some hematological and biochemical parameters represented by the lipid profile and liver function, in addition to determining the percentage of Ferritin, Albumin, and CRP for study.

## Materials and Methods of Work

- **Location and Period of Study**

This study was conducted in the Kirkuk governorate center during the period from the beginning of October 2019 until September 2020, it included 260 patients with beta-thalassemia who consulted the Thalassemia Center at Azadi Hospital. As well as the pediatrics Hospital and Kirkuk General Hospital to receive treatment (110 individuals) who suffering from watery diarrhea, abdominal pain, or any digestive disorder. Coupled with, a control group consisting of 20 healthy individuals who do not have any symptoms, after obtaining official approval from the Kirkuk Health Department. Stool samples were collected and examined to diagnose the parasite by direct wet swab method and using optical microscopy. Then, blood samples were collected using sterile syringes (5 ml) where 5 ml of venous blood was drawn and 2ml of the drawn blood was placed in special tubes containing an anticoagulant substance Ethylenediaminetetraacetic Acid (EDTA) to conduct hematological tests. The remaining amount of blood was separated by a centrifuge at a speed of 3000 rpm for 5 minutes to obtain serum, after which the serum was transferred to several Eppendorf tubes and was frozen at a temperature of (-20 ° C) until the required serological and biochemical tests were carried out. Information about patients was collected by preparing a special questionnaire prepared for this purpose, which included information on age, sex, area of residence, family history of illness, and disease symptoms.

- **Hematological Parameters**

The hematological parameters were measured by a Complete Blood Count (CBC) test using a Swedish Auto Analyzer hematology device called Swelab Alpha, to calculate the RBC and MCV, total and differential WBC count, platelet count, and hemoglobin concentration.

- **Biochemical Parameters**

The level of total cholesterol, TG, and HDL in serum was quantified by colorimetric analysis using an assay kit supplied from the French company Biolabo (Allain et al., 1974; Fossati and Prencipe, 1982, and Friedwald et al., 1972), respectively, while the level of LDL in serum was determined according to the equation below:

$$\mathrm{LDL} = \mathrm{TG} - \mathrm{HDL} - \mathrm{VLDL}$$

VLDL: very-low-density lipoprotein

- **Liver Enzymes**

The activity of the two enzymes ALT and AST were measured by using a bio-Merieux-france assay kit based on color methods (Reitman and Frankel, 1957), while the ALP enzyme was measured depending on its activity in the basal medium (Kind and King, 1954). Serum albumin level was estimated using a 630 nm wavelength spectrophotometer. On the other hand, Ferritin serum was measured using the enzyme-linked immunosorbent assay technique according to the instructions mentioned with the assay kit (Sigma-Aldrich. the USA). Finally, the value of CRP was determined by the automated Cobas CRP Test according to the instructions of the kit supplier.

## Statistical Analysis

The Statistical Packing for the Social Sciences (SPSS) commercial program was used, and by applying the ANOVA system. Furthermore, the differences between the averages were compared using the f-Test, with a probability level of $p < 0.5$ and $p < 0.01$.

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## Results and Discussion

- **Prevalence of Cryptosporidium Parvum Infection**

The study included the examination of 370 stool samples by direct or wet swab method and staining with several regular dyes and concentration methods. Samples were collected from patients whose ages range from one to thirty years and from both sexes. The patients from whom stool and blood samples were taken were suffering from a digestive disorder, where the symptoms appearing on the patients ranged from pain and cramps in the abdomen, flatulence, gas, and diarrhea, especially watery diarrhea and vomiting. This epidemiological study showed that the percentage of infection with the Cryptosporidium parasite in Kirkuk city is 16.2%, where 60 patients were carrying the parasite out of 370 people whose stools were examined, as shown in Table (1) below.

**Table 1.** The percentage of infection with Cryptosporidium parasite according to the studied samples

| **Study samples** | **Total number of examined samples** | **Positive samples (%)** | **Negative samples (%)** |
|---|---|---|---|
| Thalassemia patients Infected with the parasite | 260 | (%16.92) 44 | (%83.07) 216 |
| Patients infected with the parasite only | 110 | (%14.45) 16 | (%85.45) 94 |
| Total | 370 | (%16.21) 60 | (%83.78) 310 |

The current study results were close to what (Al-Kilani 1998) noted about the prevalence of the cryptosporidium parasite in Baghdad, which amounted to 14.6%, (Khalil 2000) stated that the prevalence of this parasite reached 20.5% in some areas of Mosul city. Similarly, a study conducted in Shanghai, Republic of China, showed that the percentage of infection with Cryptosporidium was 13.5% (Yang et al., 2017), while the study conducted in Canada was 15.7% (Iqbal et al., 2015).

- **Factors Affecting Infection with Cryptosporidium Parvum**

**1- Sex**

The current study results showed that the highest percentage of infection with the Cryptosporidium parasite was in males, where the percentage was 17.9%, while the percentage of infection with cryptosporidium in females was 14.4%, as shown in Table (2).

**Table 2.** The percentage of infection with Cryptosporidium parasite according to sex

| **Study samples** | **Sexes** | **Total number of examined samples** | **Positive samples (%)** |
|---|---|---|---|
| Thalassemia patients Infected with the parasite | selaM<br>selameF | 132<br>128 | (%18.18) 24<br>(%15.62) 20 |
| Patients infected with the parasite only | selaM<br>selameF | 58<br>52 | (%17.24) 10<br>(%11.54) 6 |
| Total | selaM<br>selameF | 190<br>180 | (%17.9) 34<br>(%14.44)26 |

This result is consistent with a study conducted by (Kanabe, 2016) in the city of Erbil, where the infection rate in males is higher (13.68%) than females (7.05%) in the rate of infection. It was also consistent with the study of (Al-Hilli 2008) that males are more infected with intestinal parasites than females. Likewise, the percentage in males reached (11.92%) and in females (9.18%) when studying children in the district of Tuwairij in Karbala governorate. It also agrees with the study of (Mahmoud, 2009) that was conducted on patients attending health centers in Al-Dur district of Salah al-Din Governorate. As well as, the study of (Shakir and Hussein, 2015), which was conducted on children with diarrhea in Baghdad. This is because males are more active and mobile than females, and the way they play makes them more susceptible to infection with parasitic pathogens.

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- **Hematological parameters of the study samples**

The current study results showed significant differences in the hematological parameters, as shown in Tables (3). Thus, it was found that the hemoglobin concentration showed a significant decrease in the group of thalassemia with cryptosporidium patients (7.436 ± 0.748 g / dL) and the thalassemia group (7.788 ± 0.625 g / dL), followed by the group infected with the parasite (12.67 ± 1.164), compared to the control group (13.735 ± 0.644 g / dL). Moreover, the results of the study showed a significant decrease in the red blood cell count in the group of thalassemia patients (3.408 ± 0.524 ($10^{\times 12}$/L) and the group of thalassemia patients infected with the parasite (3.452 ± 0.4976 g / dL) compared to the control group (4.66 ± 0484 ($10^{\times 12}$/L) and the group infected with the parasite only (4.462 ± 0.4207 ($10^{\times 12}$/L). The current study results also recorded a significant decrease in the mean corpuscular volume (MCV) in thalassemia patients infected with the parasite (61.85 ± 2.914 fl). Then, in thalassemia patients (62.18 ± 4.135 fl) compared with the control group and the group infected with the Cryptosporidium parasite, who's their MCV are (84.92 ± 5.07 fl) and (85.83 ± 5.39 fl), respectively. Tables (3) showed that there were significant differences in the total platelets count, as it was (364.4 ± 38.30 ($10^{\times 9}$/L) in thalassemia patients and patients infected with parasites only (289.6 ± 98.0 ($10^{\times 9}$/L) and thalassemia patients infected with the parasite (250.7 ± 80.3 ($10^{\times 9}$/L) recorded an affinity with the control group where the platelet count was (227.7 ± 48.7 ($10^{\times 9}$/L)

**Table 3.** The hematological parameters of the study samples

| Parameters | Thalassemia patients infected with the parasite | Thalassemia patients mean ± standard error | Patients infected with the parasite mean ± standard error | Control group mean ± standard error | P value |
|---|---|---|---|---|---|
| Hb g/dl | 0.748 ±7.436$^{c}$ | 0.625 ± 7.78$^{c}$ | 1.164 ± 12.67$^{b}$ | 0.644 ± 13.73$^{a}$ | 0.00007 |
| RBC ($10^{\times 12}$/L) | 0.4976 ± 3.452$^{b}$ | 0.524 ±3.408$^{b}$ | 0.42 ± 4.462$^{a}$ | 0.484 ± 4.66$^{a}$ | 0.0009 |
| MCV fl | 2.914 ± 1.85$^{b}$ | 4.135 ± 62.18$^{b}$ | 5.39 ± 85.83$^{a}$ | 5.07 ± 84.92$^{a}$ | 0.00004 |
| Platelet($10^{\times 9}$/L) | 80.3 ± 250.7$^{bc}$ | 38.30 ± 364.4$^{a}$ | 98.0 ± 289.6$^{b}$ | 48.7 ± 227.7$^{c}$ | 0.0007 |

* Different letters in a row indicate significant differences below the 0.05 probability level.

The above Table showed a decrease in Hb concentration, red blood cell count and platelet count in thalassemia patients as a result of their susceptibility to its complications. Accordingly, the study results of thalassemia patients were close to the results of (Gambhir, 2003) study in India, as well as in the patients infected with the parasite, the results were close to the study results conducted in Basra (Salim, 2018) and with the study results conducted in Spain (Ocaña-Losada *et al*., 2018). The reason for the low level of hemoglobin in those infected with the parasite may be due to poor absorption of sugars, fats, vitamins, folic acid, zinc, and iron caused by the presence of some parasites in the human intestine. Iron deficiency causes anemia because it is included in the composition of hemoglobin, which is the main component of red blood cells. Moreover, the presence of the trophozoite attached to the intestinal microvilli and their absorption of quantities of blood, as well as the hemolytic factors produced by parasites and others all contribute to the occurrence of anemia. (Rey, 2001; Crua, *et al*., 2003 and Gopalakrishnan, *et al*., 2018). In the case of thalassemia patients, the cause of anemia is the most common genetic disorder in the synthesis of hemoglobin, mainly due to the deletion of one or both alpha-globin genes in each locus on chromosome number 16 that leads to an increase in the amount of the beta-globin chain (AL-Haddad 2012). This leads to a decrease in the red blood count in those exposed to normal hemoglobinopathy as a result of reducing the number of natural globin proteins (Alhazidou, *et al*., 2020). (Khawaji et al., 2020) indicated that the decrease in the RBC and the hemoglobin concentration may be attributed to the increased breakdown of RBC in the spleen in those infected with beta-thalassemia. The causes of low red blood cells (microcytosis) volume, this condition is called microcytosis hypochromia, it mainly results from iron and pyridoxine deficiency, and it happens in patients with hereditary anemia or thalassemia. The reasons for the effect of the MCV are due to several factors such as the amount of iron, its ability to divide to penetrate the very thin capillaries, and the concentration of hemoglobin in them. Hemoglobin is an iron and a protein substance, and when an imbalance occurs in one of these factors, the MCV is affected by it, and usually, when examining the MCV, their healthy size in men, women, and children ranges between 80.5 - 99.7 fl,

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and the hemoglobin concentration is between 26 - 32 pg. If these rates decrease, the person will have a small RBC, which is anemia that results from a low level of hemoglobin in the blood and is usually hereditary, which is the hereditary thalassemia anemia (Gretchen and Kristeen, 2017). The current study results are consistent with what was indicated by (Al-Laham *et al*., 2015) in a study on children in Gaza, as well as with the results of (Al-Hasheme et al., 2020) study in Karbala, Iraq. Recent studies indicate that platelets have a major role in innate immunity, inflammation, protozoal infections, parasites, viruses, and the immune response of the host (Domínguez and Toraño, 2001). These platelets aggregate at the site of injury and act to release cytokines and chemokines and the biologically active molecules stored within the granules or that are made upon activation. These cytokines affect both neutrophils, monocytes, and lymphocytes and work to attract and modify the effective response of cells in the immune system. These platelets act on the secretion of adhesion molecules and their accumulation at the site of injury. Besides that these molecules prefer to adhere to blood platelets and after that, a link occurs between platelets, granulocytes, or platelets and non-granulocytes and this will affect the occurrence of inflammation (Sonmez and Sonmez, 2017; 2015 *et al*., Ali).

- **Total and differential WBC count**

The current study results listed in Table (4), showed that there was a significant increase in the total WBC and the rate of Granulocytes in thalassemia patients infected with the parasite (12.772 ± 2.510 ($10^{x9}$/L) and (7.900 ± 0.713 ($10^{x9}$/L), respectively. Likewise, those infected with the parasite (13.665 ± 2.116 ($10^{x9}$/L) (9.060 ± 2.156 ($10^{x9}$/L), respectively, compared with the control group and the thalassemia group. Once studying the number of lymphocytes, significant differences were found among the study samples compared to the control group, where a significant increase was recorded in the thalassemia patient group with cryptosporidium (4.908 ± 1.172 cell / $mm^3$ blood) and those infected with the parasite (3.616 ± 1.641 ($10^{x9}$/L). Followed by the thalassemia group, which had a cell rate (3.828 ± 0.856 ($10^{x9}$/L) compared with the control group (2.745 ± 0.819 ($10^{x9}$/L). No significant changes occurred in monocytes compared to the control group.

**Table 4.** The total and differential WBC count

| **Parameters** | **Thalassemia patients infected with the parasite** | **Thalassemia patients mean ± standard error** | **Patients infected with the parasite mean ± standard error** | **Control group mean ± standard error** | **P-value** |
|---|---|---|---|---|---|
| WBC count ($10^{x9}$/L) | 2.510 ± 12.772[a] | 1437 ±6.842[b] | 2.116 ±13.665[a] | 1.484 ± 6.335[b] | 0.0002 |
| Granulocytes ($10^{x9}$/L) | 0.713 ±7.90[b] | 1.103 ±3.228[d] | 2.156 ± 9.06[a] | 1.068± 4.245[c] | 0.00002 |
| Lymphocytes ($10^{x9}$/L) | 1.172 ±4.908[a] | 0.856 ±3.828[b] | 1.641 ±3.615[b] | 0.819 ±2.745[c] | 0.0006 |
| Monocytes ($10^{x9}$/L) | 0.2273 ±1.500[a] | 0.3041 ±0.880[b] | 0761± 0.995[b] | 0.3080 ±0.830[b] | 0.0005 |

* Different letters in a row indicate significant differences below the 0.05 probability level.

The current study results are in agreement with the results of (Chen et al., 2003) in Taiwan, which indicated that there was an increase in the WBC in those infected with intestinal parasites compared with the control samples, with statistically significant differences. The reason for the high WBC and some differential WBC is due to the response of the immune system to confront parasites and eliminate them. Acidic blood cells play an important role in parasitic infections as these cells move with large numbers of blood towards the site of parasitic infections inside the body. Then, these cells attach to the surfaces of the parasites and begin to remove their granules, then attack the parasites and eventually kill them (Behm and Ovington, 2000). The symptoms emergence of a parasite infection stimulates granulocytes in the bloodstream and collects at the site of infection within hours and is the most important early source of IL-12 production, with the secretion of response molecules, including NO and the reactive oxygen intermediates (ROI). Another important role of neutrophils is the production of chemokines that recruit more cells to respond against the parasite, including monocytes and T cells (Lamb, 2012). The increase in the level of lymphocytes in Cryptosporidium depends on the duration of the infection, as mentioned by (Pantenburg *et al*., 2008), through studying the jejunum tissue taken from patients infected with Cryptosporidium. Therefore, they were found that the

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number of lymphocytes increased on the third day after infection, reaching a peak by day 9, levels fell on day 14. Another study also showed that lymphocytes were higher among the group infected with cryptosporidium compared to healthy people (Juris *et al*., 2014). However, the numerical increase in the total WBC and some of its differential count in thalassemia patients may be attributed to the hemolysis outside and inside the bone marrow, which stimulates the production of the hormone erythropoietin by the kidneys and in turn stimulates the bone marrow to increase the formation of different blood cells, including WBC. Furthermore, the infections in thalassemia patients lead to an increase in the lymphocytes and monocytes cells (Kadhem, 2014).

- **Biochemical Parameters**

**1. Body Lipid Profile**

The results in Table (5) showed statistically significant changes in the lipid profiles, as it was found that levels of (TC) and (LDL) showed a significant increase in those infected with the parasite (151.45±5.31mg/dL) and (103.65 ± 11.25 mg/dL) compared to the study samples. Whereas it showed a higher significant decrease in thalassemia patients infected and not infected with the parasite compared to the control group. Additionally, the current study results showed significant differences in the levels of (HDL), where a highly significant decrease was found in the group of thalassemia patients infected and not infected with the parasite, compared to the control group. Conversely, the study results showed a significant increase in triglyceride levels in the group of thalassemia patients infected and not infected with the parasite, followed by the group of patients infected with the parasite (127.51 ± 10.46 mg/dL) compared to the control group (108.45 ± 8.41 mg/dL).

**Table 5.** The lipid profile in the study samples

| **Parameters** | **Thalassemia patients infected with the parasite** | **Thalassemia patients mean ± standard error** | **Patients infected with the parasite mean ± standard error** | **Control group mean ± standard error** | **P value** |
|---|---|---|---|---|---|
| Cholesterol (mg/dL) | 4.585 ±76.24$^{d}$ | 6.43 ±90.64$^{c}$ | 5.31 ±151.45$^{a}$ | 13.02 ±125.65$^{b}$ | 0.00007 |
| Triglyceride (mg/dL) | 12.98 ±185.6$^{a}$ | 9.83 ±168.44$^{b}$ | 10.46 ±127.51$^{c}$ | 8.41 ±108.45$^{d}$ | 0.00006 |
| Low-density lipoproteins (mg/dL) | 3.883 ±59.08$^{c}$ | 3.84 ±43.480$^{d}$ | 11.25 ±103.65$^{a}$ | 8.34 ±87.950$^{b}$ | 0.00006 |
| High-density lipoproteins (mg/dL) | 2.694 ±36.52$^{c}$ | 2.993 ±28.28$^{d}$ | 7.78 ±43.55$^{b}$ | 4.94 ±53.65$^{a}$ | 0.00003 |

* Different letters in a row indicate significant differences below the 0.05 probability level.

The current study results are consistent with the results of (Yoshikawa et al., 1997) study and other studies on the role of cholesterol in parasitic infections (Bansal et al., 2005 Bahaa AL-Deen, 2018;). On top of the results (Ehrenman, *et al*., 2013) study in Boston, the USA, which indicated a decrease in the level of LDL in those infected with intestinal parasites, the reason is due to the use of these parasites in the reproduction and production of eggs for reproduction as well a food source. Parasites get lipid from the upper parts of the intestine as well as HDL, knowing that the mechanism that parasites follow to do this is not clear and that many scientific studies have shown a change in the lipid profile in most patients infected with parasites. Along with, many Trophozoites stages of intestinal parasites transform in the intestine into cystic stages as a result of factors in the host's body, including the presence of normal flora, pH, and increased bile concentration (Lauwaet *et al*., 2007). The parasite consumes cholesterol during the Encystation process due to the parasite's inability to synthesize it (Bansal *et al*., 2005). As for the low level of TC and LDL in thalassemia patients, it may be due to many factors, including iron overload (high ferritin level), liver injuries, and hormonal disorders, and these factors affect the lipid pattern among these patients. The liver is the main site for iron deposition, as iron is deposited in hepatocytes, free radical production increases and accumulates in the liver, kidneys, and heart and causes damage to these tissues, meaning that it is the sites of deposition (Rameshwar *et al*., 2017). The same as, (Haghpanah et al., 2020) indicated that the pathophysiology of hypocholesterolemia is ambiguous in people with blood disorders, where anemia is a common feature for them. The mechanisms include

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increased RBC formation activity, which leads to increased cholesterol consumption, liver injury due to iron overload, activation of the macrophage system, and release of cytokines. The researcher indicated that lower cholesterol values appear in patients with more severe genotypes, and the low HDL in patients with beta-thalassemia can be considered a predictive value for cardiovascular risk in these patients. However, the high triglycerides are likely due to the lipolysis activity outside the liver.

**2. Liver Function Enzymes**

The current study results recorded a significant increase in the levels of AST, ALT and ALP enzymes in thalassemia patients and not infected with the parasite compared to the control group and those infected with the parasite.

**Table 6.** The levels of liver enzymes in the study samples

| Parameters | Thalassemia patients infected with the parasite | Thalassemia patients mean ± standard error | Patients infected with the parasite mean ± standard error | Control group mean ± standard error | P value |
|---|---|---|---|---|---|
| AST (U/L) | 5.63 ± 66.40 [a] | 4.350 ± 54.44[b] | 1.448 ± 19.41[c] | 3.220 ± 22.50 [c] | 0.00009 |
| ALT (U/L) | 3.520 ± 53.77[a] | 5.07 ± 44.64[b] | 1.662 ± 19.26[c] | 1.930 ± 21.60[c] | 0.00008 |
| ALP (U/L) | 10.42 ± 105. 0[a] | 5.84 ± 86.32[b] | 3.979 ± 59.60[c] | 3.633 ± 53.60[d] | 0.00005 |

* Different letters in a row indicate significant differences below the 0.05 probability level.

The study results agreed with previous studies on the high levels of ALT and AST among thalassemia patients who receive blood transfusions permanently (Mohammad, 2012; Galanello and Origa. 2010). (Hashim et al., 2020) indicated in research in Maysan that the difference in the concentration of Transferases compared to healthy individuals is due to excessive hemolysis. Otherwise, due to the need to manufacture peptide chains through the effectiveness of these enzymes in the transmission of amine groups. Thus, they are more common in many body tissues such as the heart, liver, and kidneys are deposited largely due to the excess amounts of iron in the organs, which leads to the death of cells as a result of the free radical's formation that attacks all vital molecules such as lipids, proteins, and cell DNA. Generally, the increased level of (ALP) may be due to the activity of this enzyme from liver and bone tissues, and because thalassemia patients suffer from damage to these tissues, thus it is deposited into the circulatory system and increases its effectiveness (Shams *et al*., 2010). As for the significant increase in the ALP enzyme compared to the control group in the case of parasite infection, it could be the result of diarrhea and malabsorption, which in turn leads to the loss of many minerals and salts such as calcium and sodium. For this reason, this enzyme has a role in transporting these minerals, therefore, its secretion increases to compensate for the deficiency in those minerals and salts (Lott and Wolf, 1984).

**3. Serum Protein of the Study Samples**

The concentration of CRP significantly increased as a result of infection with the parasite and reached (13.990 ± 1.944 mg / dL) and the group of thalassemia patients infected with the parasite (14.812 ± 2.628 mg / dL) compared with the group of thalassemia patients and the control group (5.952 ± 1.102 mg/dL) and (5.420 ± 0.928 mg/dL) as shown in Table 7. CRP is used as inflammatory indicators such as intestinal inflammation, necrosis, and allergic reactions, and is compared to hematological parameters such as total and differential WBC count. Further, its concentration increases due to the high concentration of Interleukin IL-6 in response to the causes of acute and chronic inflammatory conditions such as parasites, bacteria, and viruses (Pepys and Hirschfield, 1983), which stimulates the phagocytes in the liver to produce this protein. Accordingly, it plays an important role in innate immunity through its binding to phosphocholine on the surface of the cells, which leads to inhibition of the complement system and the enhancement of the phagocytosis by phagocytes, which works to eliminate the pathogens (Thompson et al., 1999 and Bray et al., 2016). The study results showed a significant increase in the level of ferritin as a result of thalassemia infection in thalassemia patients with the parasite (4540 ± 364.6 ng/ml) and the thalassemia group (3478 ± 400.0 ng/ml) compared to the control group (82.8 ± 17.15 ng/ml) and the group infected with the parasite (104.5 ± 43.72 ng / mL).

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**Table 7.** The ratios of CRP, ferritin, the percentage of albumin, and the percentage of blood sugar

| **Parameters** | **Thalassemia patients infected with the parasite** | **Thalassemia patients mean ± standard error** | **Patients infected with the parasite mean ± standard error** | **Control group mean ± standard error** | **P value** |
|---|---|---|---|---|---|
| CRP level (mg / dL) | 2.628±14.812b | 1.102 5.952a± | 1.944±13.990b | 0.928±5.420a | 0.00005 |
| ferritin level in the serum (ng / mol) | 364.6±4540c | 400.0 3478c± | 43.72±104.5b | 17.15±82.8a | 0.000003 |
| Albumin level(g/dL) | 0.1943±3.424a | 0.3525 3.944b± | 0.3164±3.97b | 0.4058±4.495c | 0.0008 |
| Blood sugar level (mg/dL) | 12.72±102.84c | 11.05 98.80a± | 12.61±108.30bc | 4.49±94.85b | 0.001 |

* Different letters in a row indicate significant differences below the 0.05 probability level.

The study results agreed with the study of (Khawaji et al., 2020), which indicated an increase in the level of ferritin in thalassemia patients to more than 2500 ng/ml, and the study of (Akula et al., 2017), which recorded an increase in the level of ferritin in 64% of beta-thalassemia and more than (2000 ng/dL). High ferritin levels make patients more susceptible to iron deposition, which is a complication associated with frequent blood transfusions. Excessive iron is harmful to the body and can lead to death as a result of iron deposition in basic organs such as the heart, liver, kidneys, and spleen. (Munir *et al*., 2013) indicated that the serum ferritin level was used as an indirect indicator to assess iron overload in patients exposed to frequent blood transfusions. The mechanism through which iron overload causes damage to body tissues is not fully understood. Iron has a role that produces reactive oxygen species and free radicals that lead to damage in the organs they operate at the cellular level due to the formation of free radicals and lipid peroxidation leading to damage to mitochondria and the lysosome membrane (Akula *et al*., 2017). This increase in its level puts these patients at great risk of developing heart disease) (Cario, Stahnk, and Kohen. 1999) and its effect on other organ functions. Therefore, evaluation of oxidative stress can be useful to protect beta-thalassemia patients from the risk of disease complications due to iron deposition in various organs. Whereas, (Eshragi et al. 2011) indicated that high levels of it among thalassemia patients indicate irregular treatment practices. The study results showed a significant decrease in the levels of albumin in the group of thalassemia patients and those infected with the parasite compared to the control group. The study results are agreed with the study of (Aljubori, 2019), that the low concentration of albumin in people suffering from cryptosporidium infection and suffer from malnutrition increases the risk of infection if there is acute or chronic diarrhea. Albumin protein is one of the important molecules in maintaining the osmotic pressure and the anti-inflammatory effects that occur in the human body. Moreover, the albumin particles are also associated with the drugs, facilitate the process of transporting them from one site to another, and it is known that infection with the parasite can break the mucous layer of the intestine and a decrease in the level of albumin occurs, thus weakens the absorption process in the intestine (Laodim *et al.,* 2013). Also, the main site for the production of albumin are hepatocytes, therefore, any impairment in liver function, malnutrition, and inflammation can lead to a decrease in the level of serum albumin in people with thalassemia (Akula *et al.*, 2017). The study results showed no significant differences in the blood sugar ratios when comparing the study samples, as shown in Table (7). The lowest value was (4.49 ±94.85c) and the largest value was (12.61±108.30a). All these values are within the normal range for blood sugar. These results are agreed with the study of (Dhefer, Iqbal 2011), which indicated that there were no significant differences in cumulative sugar level among thalassemia sufferers compared to the control group. The cumulative sugar (HbA1c%) reflects blood sugar status for the previous two to three months. Thalassemia patients can show multiple organ dysfunctions, including the pancreas, as a result of excessive iron deposition, which leads to abnormal metabolism of glucose due to the destruction of islet cells, and leads to not synthesis insulin. Besides, excessive fatty acid oxidation resulting from iron accumulation with a decrease in the rate of glycogen use in the body, in addition to the reduced ability of the liver to absorb insulin due to impaired liver function (He LN et al., 2019). However, others indicated that insulin resistance is related to the patient's age and iron level in the body. The study results observed that the high concentration of CRP in the study samples is due to infection with the parasite Cryptosporidium. The current study is consistent with (Barbai et al., 2010 and Juris et al., 2014) as well as the study of (Lee et al., 2005), which indicated an increase in CRP to 18.2 mg / dL among patients infected with the parasite to reflect a type of inflammation found inside the body. Alternatively, (Abrams et al., 2005) reported the relationship of high levels of CRP with parasite density and infection severity.

Also, other studies (Bahaa AL-Deen, 2018) indicated that cases positive for CRP reached 100% in those infected with intestinal parasites (amoeba, giardiasis and blastocystis), and high rates were recorded in those infected with cutaneous leishmaniasis (Hassan et al., 2017). It can conclude from the current study that multiple blood transfusions, increased iron and ferritin levels in patients with beta-thalassemia affect the hematological parameters, and this leads to a defect in the movement and efficiency of phagocytes, in addition to their effect on liver function enzymes and body lipids. These complications are considered risk factors for infection with various pathogens including infection with the Cryptosporidium parasite

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