DOI: 10.24996/ijs.2025.66.6.7

ISSN: 0067-2904

Hormonal and biochemical factors among chronic liver disease men infected with Toxoplasmosis and some protozoan intestinal parasites

Buraq Abdualameer Fingan[1, 2], Harith Saeed Al-Warid[1], Hayder Jamal Al-Sultan[3]
[1] *Department of Biology, College of Science, University of Baghdad, Al-Jadriya Campus, Baghdad, Iraq*
[2]*National Center for Teaching Laboratories, Medical City, Ministry of Health and Environment, Baghdad, Iraq*
[3]*Gastroenterology and Hepatology Teaching Hospital, Medical city, Ministry of Health and Environment, Baghdad, Iraq*

Received: 17/2/2024 Accepted: 3/7/2024 Published: 30/6/2025

Abstract

Chronic liver disease (CLD) can potentially cause disruptions in the normal functioning of various endocrine organs responsible for producing hormones. As a result, individuals suffering from CLD may experience fluctuations or imbalances in the levels of certain hormones within their bodies. As well as they frequently have suppressed immune systems making them more vulnerable to parasite infections. The primary objective of this study was to investigate the association between Toxoplasma gondii infections and liver function by analyzing the interplay between these parasites and hormones. This study was conducted in Baghdad, Iraq from December 2021 to May 2022. One hundred and twenty male patients with Chronic liver disease (CLD) (age:14-75 years) and 120 control males (age: 24-70 years) participated in this study. Stool and serum samples were collected from all individuals and were then analysed for intestinal protozoan parasites and anti-*Toxoplasma* antibodies respectively. Hormonal tests were conducted for all participants which included (Cortisol, testosterone, prolactin, insulin, and thyroid-stimulating hormone TSH). Biochemical tests included (Prothrombin time PT, international normalized ratio INR and albumin); liver enzymes were (aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphatase ALP and gamma-glutamyl transferase GGT) and interleukins (Interleukin 13 IL-13 and transforming growth factor TGF). The findings indicate that among the control group participants, 34 individuals, which constitute 28.33% of that group, tested positive for protozoan parasitic infections. In contrast, a higher proportion, 69 individuals or 57.5%, of the participants diagnosed with CLD were found to be positive for protozoan parasites. Four sub-groups were formed in response to prior results: Control-parasites positive, control-parasites negative, CLD-parasite positive and CLD-parasite negative. The status of the protozoan parasites did not affect the hormones levels. The results of liver enzymes showed that parasite positive status was significantly related to all enzymes among CLD patients except the GGT. As well as parasite positive status was not correlated with the other biochemical (PT, INR, and albumin) and immunological parameters (IL-13 and TGF). There was no correlation between the positive status of parasites and cortisol, testosterone, insulin, prolactin, or TSH. The liver enzyme results showed a high correlation ($p<0.05$) between the presence of parasites and all of the enzymes among CLD patients, with the exception of the GGT enzyme. PT, INR, albumin, and the other biochemical and immunological markers (IL-13 and TGF) did not correlate with the presence of parasites.

Keywords : Cryptosporidium, Entamoeba histolytica, Giardia lamblia, Hormones, Liver disease, males, Toxoplasma gondii

* Email: harith.saeed@sc.uobaghdad.edu.iq

العوامل الهرمونية والكيميائية الحيوية بين مرضى أمراض الكبد المزمنة من الرجال المصابين بداء المقوسات الكوندية و بعض الابتدائيات المعوية الطفيلية

براق عبد الامير فنجان[2،1] , حارث سعيد الورد[1], حيدر جمال السلطان [3]

[1]قسم علوم الحياة, كلية العلوم, جامعة بغداد, الجادرية, بغداد, العراق

[2]المركز الوطني للمختبرات التعليمية، دائرة مدينة الطب، وزارة الصحة و البيئة، بغداد، العراق

[3]مستشفى امراض الجهاز الهضمي و الكبد التعليمي، دائرة مدينة الطب، وزارة الصحة و البيئة، بغداد, العراق

الخلاصة

يمكن أن تسبب امراض الكبد المزمنه اضطرابات في الأداء الطبيعي لمختلف أعضاء الغدد الصماء المسؤولة عن إنتاج الهرمونات. ونتيجة لذلك، قد يعاني الأفراد الذين يعانون من من امراض الكبد المزمنه تقلبات أو اختلالات في مستويات بعض الهرمونات داخل أجسامهم .بالإضافة إلى أنهم في كثير من الأحيان يعانون من تثبيط في الجهازالمناعي مما يجعلهم أكثر عرضة للإصابة بالعدوى الطفيلية. هدفت هذه الدراسة إلى تحديد تأثير الإصابة ببعض الطفيليات الأولية على وظائف الكبد من خلال فحص العلاقة بين هذه الطفيليات والهرمونات . اجريت هذه الدراسة في المدة الزمنية المحصورة بين كانون الاول من العام 2021 و ايار من العام 2022 في مستشفى امراض الجهاز الهضمي و الكبد التعليمي، بغداد–العراق. تم شمول 120 مريضاً من الذين يعانون من امراض الكبد المزمنة (العمر :14–75 سنة) و 120 شخصا من غير المصابين بامراض الكبد المزمنه (العمر :24–70 سنة).

جُمعت عينات البراز والمصل من جميع الأفراد وتم تحليلها للتأكد من وجود الطفيليات المعوية والأجسام المضادة لداء المقوسات الكوندية على التوالي. أجريت الاختبارات الهرمونية لجميع المشاركين والتي شملت (الكورتيزول، التستوستيرون، البرولاكتين، الأنسولين، والهرمون المحفز للغدة الدرقية)، الفحصوصات الكيموحياتية (زمن البروثرومبين، النسبة المعيارية الدولية و الالبومين) , فحوصات انزيمات الكبد (ناقل امين الاسبارتات, ناقل امين الالنين, ,انزيم الفوسفاتيز القلوي و ناقل الببتيد غاما غلوتاميل) و فحوصات المدورات الخلوية (انترلوكين–13 و عامل النمو المحول). تشير النتائج إلى أنه من بين المشاركين في مجموعة السيطرة ، كان اختبار 34 فردًا، أي ما يشكل 28.33% من تلك المجموعة، إيجابيًا لفحوصات الطفيليات. في المقابل، وجد أن نسبة أعلى، 69 فردًا اي مايشكل 57.5%، من المصابين بامراض الكبد المزمنه كانت نتائجهم إيجابية بالنسبة لفحوصات الطفيليات. .تم تشكيل أربع مجموعات فرعية استجابةً للنتائج السابقة: مجموعة السيطرة الموجبين لفحوصات الطفيليات ، طفيليات السيطرة السالبين لفحوص الطفيليات، مرضى الكبد الموجبين لفحوصات الطفيليات و مرضى الكبد السالبين لفحوص الطفيليات. لم تؤثر حالة الاصابة بالابتدائيات الطفيلية على مستويات الهرمونات. أظهرت نتائج إنزيمات الكبد أن الحالة الإيجابية للابتدائيات الطفيلية كانت مرتبطة بشكل معنوي ($p<0.5$) بجميع الإنزيمات لدى مرضى الكبد باستثناء ناقل الببتيد غاما غلوتاميل فضلا عن أن الحالة الإيجابية للطفيليات لم تكن مرتبطة بالعوامل الكيموحياتية (زمن البروثرومبين، النسبة المعيارية الدولية و الالبومين) و فحوصات المدورات الخلوية (انترلوكين–13 و عامل النمو المحول). لم يكن هناك ارتباط بين الحالة الإيجابية للطفيليات والكورتيزول، التستوستيرون، الأنسولين، البرولاكتين، أو الهرمون المحفز للغدة الدرقية .أظهرت نتائج إنزيمات الكبد وجود علاقة عالية بين وجود الطفيليات وجميع الإنزيمات لدى مرضى الكبد، باستثناء إنزيم ناقل الببتيد غاما غلوتا .لم يرتبط زمن البروثرومبين، النسبة المعيارية الدولية و الالبومين، الفحصوصات الكيموحياتية و فحوصات المدورات الخلوية انترلوكين–13 و عامل النمو المحول بوجود الطفيليات.

.

1. Introduction

Chronic liver disease" (CLD) denotes long-standing deterioration of liver function persisting over a duration of six months or more. The decline in liver function impairs its

capacity to synthesize clotting factors and other proteins, detoxify toxic metabolic byproducts, and secrete bile, leading to a range of complications and reduced overall health. Fibrosis and cirrhosis are the results of the ongoing inflammation, degradation, and regeneration of the liver parenchyma in CLD. Chronic liver disease has many different etiologies, including as exposure to toxins, long-term alcohol consumption, infections, autoimmune diseases, genetic disorders, and metabolic problems. The last stage of chronic liver disease, known as cirrhosis, is characterized by disturbance of the architecture of the liver, creation of extensive nodules, vascular reorganization, neo-angiogenesis, and extracellular matrix deposition. At the cellular level, fibrosis and cirrhosis are caused by the recruitment of fibroblasts and stellate cells; parenchymal regeneration is dependent on hepatic stem cells. The focus is on the common etiologist, clinical symptoms, and management of chronic liver disease, a clinical condition that is exceedingly frequent. The most significant health issues nowadays are chronic liver disorders and cirrhosis, according to the most recent gastroenterology literature [1]. In many cases of CLD, when the amount of the liver damage is parallel to the immunological disturbances, hyperglobulinemia and reduced cell-mediated immunity are prevalent [2]. Due to a decrease in humoral and cell-mediated immunity, hepatic cirrhosis is characterized by impaired antigen handling [3]. Exhaustion, mood swings, and low libido are examples of symptoms of hormonal imbalance that can appear in people with liver problems. Unexpected weight increase or loss is a sign that there might be hormonal imbalances. Mood: weariness, restlessness, anxiousness, and annoyance. Chronic liver disease can lead to malfunction in most endocrine organs, such as the pituitary, thyroid, and other glands. The most common endocrine manifestations of CLD include short stature, hepatic osteodystrophy, delayed puberty, hypogonadism, relative adrenal insufficiency, and sick euthyroid syndrome. Patients with cirrhosis usually have normal or slightly elevated levels of estradiol, lower levels of testosterone and dihydroepiandosterone, and a higher estrogen/androgen ratio, depending on the severity of the liver disease. Individuals with liver illness may show significant immune system dysfunction. This illness encompasses alterations to both the systemic and localized immune defences of the cirrhotic liver, changes that are integral to explaining the disease's pronounced susceptibility to infections and considerable infection-connected mortality rate. The increasing prevalence of extensively or multidrug-resistant pathogens, which are linked to higher mortality, longer hospital stays, and higher healthcare-related costs when compared to infections caused by susceptible strains, is another worrying aspect of infections in cirrhotic patients. Among the infectious pathogens that may be linked to CLD are protozon parasites. According to [4, 5], *Toxoplasma gondii* and certain intestinal protozoan parasites have become prominent opportunistic parasites that can infect immunocompromised people fatally. *Giardia lamblia* (*G. lamblia*), *Entamoeba histolytica* (*E. histolytica*), *Blastocystis hominis* (*B. hominis*), *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, and *Microsporidia* are among the many parasitic infections that can affect patients with chronic liver disease (CLD) [5]. Unsanitary practices, such as eating and drinking contaminated food and water, or feco-oral rout, are common ways in which these parasites are spread. When a parasite infection is successfully identified and treated in patients with chronic liver disease (CHD), problems such as electrolyte imbalance, dehydration, and the development of hepatic encephalopathy can be prevented [5]. On other hand immunological and hormonal disorders associated with CLD can deteriorate the general health condition of the CLD patients. The purpose of the study was to identify common parasites among CLD patients as well as assess the hormonal and immunological state of these individuals.

2. Materials and Methods

2.1 Individuals and Study Design

This study was conducted as a case control in the Gastroenterology and Hepatology Teaching Hospital, Medical City in Baghdad, Iraq, between December 2021 and May 2022. A

total of 240 adult males were involved in this study. One hundred and twenty of them were diagnosed as Chronic Liver Disease (CLD) patients. Some clinical and biochemical tests performed by a gastroenterologist served as the basis for the diagnosis of CLD. The study procedure was approved by the Ethics Committee at the College of Science, University of Baghdad, as confirmed by the relevant confirmation letter (CSEC/1221/0081).

2.2 Serum preparation

A venipuncture procedure, which involves drawing a blood sample, was performed on each participant in the study. Blood samples were put in gel activator tubes, they were centrifuged for ten minutes at 6500 rpm after coagulating for roughly thirty minutes at 25 °C. Each serum sample was divided into portions equally. Serum was utilized to screen for anti-*Toxoplasma* antibodies and to assess immunological, biochemical, and hormone levels.

2.3 Protozoan parasite screening

2.3.1 Anti-*Toxoplasma* Screening

Anti-*Toxoplasma* was screened according to the manufacturer instruction available with the ELISA kit (My BioSource, China) for IgM and IgG [6].

2.3.2 Collection and examination of stool for intestinal parasites

Using screw-cap containers, stool samples were taken from the individuals. On every sample, the date of collection and the identification number were inscribed. The samples were concentrated using the formalin-ether method [7]. Each deposit was taken in a drop form and applied to a glass slide using a sterile pipette. A modified acid-fast approach was employed to stain a second smear [8]. A light microscope with a ×100 objective was used to examine each smear.

2.4 Hormones analyses

The hormones cortisol, testosterone, prolactin, insulin, and thyroid stimulating hormone (TSH) were measured in both control and CLD patients. Sandwich enzyme-linked immunosorbent test (ELISA) was used to evaluate each of these hormones. Every assessment was carried out following the guidelines provided by the manufacturer, which can be found with each hormone's ELISA kit (My BioSource, China). In a 96-well plate, each sample was evaluated. The plate was then analysed using an ELISA reader that operated at 450 nm wavelength [6].

2.5 Biochemical analyses

2.5.1 Liver enzymes:

Liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were all assessed using liver enzymes kit (Abbott, USA). All of the enzymes were measured using ABBOTTc400 chemistry analyzer (Abbott, USA) based on the manufacturer's instruction found in the kits. As well as, Gamma glutamyl Transferase (GGT) was measured for all participants using ELISA kit (My BioSource, China) according to the instructions available with the kit. Then, all samples were analyzed and read at 450 nm wavelength [9].

2.5.2 Prothrombin time (PT) and International normalized ratio (INR) analyses

Both Prothrombin time (PT) and International normalized ratio (INR) were evaluated for all participants using BCS XP analyzer (Siemens, Germany). The assessment procedure was done according to the instructions that came with the kit [10].

2.5.3 Plasma albumin measurement

Based on the manufacturer's instructions included in the kits, the ABBOTTc400 chemical analyzer (Abbott, USA) was used to quantify the plasma albumin concentration for each participant [6].

2.5.4 Interleukin 13 (IL-13) and Transforming Growth Factor (TGF)

Plasma interleukin 13 (IL-13) and Transforming Growth Factor (TGF) were both assessed using a sandwich enzyme-linked immunosorbent assay (ELISA) in accordance with the IL-13 and TGF kits' manufacturer's instructions (FineTest, ELISA kit, China). In a 96-well plate, samples were subjected and screened [11, 12].

2.6. Statistical Analysis

The Statistical Analysis System, (SAS) version 9.1, was used to statistically analyze the data. Chi-square was used to analyze the distribution of protozoan parasites among CLD patients and the control group. Using the ANOVA test all parameters including cortisol, testosterone, prolactin, insulin, TSH, AST, ALT, ALP, PT, INR, albumin, IL-13 and TGF were compared between the CLD and the control group. The post hoc Tukey test was employed to assess the statistical significance of the differences observed between the mean values of different pairs of groups. P-values less than 0.05 were regarded as statistically significant. Data were stated as the mean and standard deviation (mean ±SD).

3. Results

3.1 Protozoan parasites screening

The results of protozoan parasites showed that sixty-nine (57.5%) of the CLD patients were infected with protozoan parasites while only 34 (28.33%) individuals of the control group were infected with protozoan parasites. Results showed T. gondii antibodies were present in 27.5% of patients with CLD, versus 25% of the control group. Moreover, 23.8% of CLD patients exhibited both T. gondii antibodies and intestinal parasite infection, noticeably higher than the 3.33% that demonstrated this combination in the control group. Single infection of protozoan intestinal parasites was recorded in (5.83%) while no infection with protozoan intestinal parasites was detected in control group. Only one CLD patients had double infections with protozoan parasites versus no double infection in control (Table 1). Statistical analysis showed that the occurrence of protozoan parasites was significantly ($p<0.05$) higher in CLD patients versus the control group.

Table 1: Parasites positive and negative status among CLD patients and control group

Group	*Toxoplasma* positive n(%)	*Toxoplasma* positive +intestinal protozoan parasites n(%)	Intestinal Protozoan parasites (single infection) n(%)	Intestinal Protozoan parasites (double infection) n(%)	Total n(%)	X^2(p-value)
CLD-patients (n=120)	33(27.5%)	28(23.8%)	7(5.83%)	1(0.83%)	69(57.5%)	16.11(0.001)
Control (n=120)	30(25%)	4(3.33%)	0(0%)	0(0%)	34(28.33%)	

Intestinal protozoan parasites (single infection): infected with one of the following: *G.lamblia*, *E.histolytica* and *Cryptosporidium* spp

Intestinal protozoan parasites (double infections): infected with two of the following: *G.lamblia*, *E.histolytica* and *Cryptosporidium* spp

3.1 Hormones levels

This study measured hormone levels in CLD patients as well as controls. It found that CLD patients positive for parasites, CLD patients negative for parasites, control subjects positive for parasites, and parasite-negative controls all exhibited distinct hormone levels. There were significant increases for some of these hormones and significant decreases in others. Table 2 presents all of the hormones' effects. The current study included four groups, each with varying amounts of cortisol. Significant variations in cortisol were revealed by statistical analysis using the ANOVA test ($p<0.05$). The patients who tested positive for CLD parasites and those who tested negative for CLD parasites had the highest cortisol levels, measuring 29.29±3.5 ng/ml and 27.17±0.9 ng/ml, respectively. The control groups with positive and negative parasites had the lowest cortisol levels, measuring 15.82±0.08 ng/ml and 15.76±0.05 ng/ml, respectively. There were no significant differences ($p<0.05$) between patients with CLD parasites and those without them, according to post hoc Tukey analysis. While the significant differences ($p<0.05$) were noticed between CLD-parasites positive group and Control –parasite positive group. The levels of testosterone were decreased in CLD-*parasite* positive patients (1.67±01.2 ng/ml) and CLD-*parasite* negative patients (1.68±0.013 ng/ml) while the testosterone levels were higher in control-*parasites* positive patients (2.99±0.05 ng/ml) and control-*parasites* negative patients (2.93±0.04 ng/ml). The post hoc Tukey analysis revealed no significant differences when comparing CLD patients who tested positive for parasites with CLD patients who tested negative for parasites, as well as when comparing control subjects who tested positive for parasites with control subjects who tested negative for parasites. However, significant differences ($p<0.05$) were noticed between CLD-parasites positive group and Control –parasite positive group. Prolactin levels also varied among the four groups. The highest prolactin levels were noticed in CLD-*parasite* positive patients (68.49±7.5 ng/ml) and CLD-*parasite* negative patients (64.21±1.2 ng/ml). Prolactin was lower in control-*parasite* positive group (45.47±0.5 ng/ml) and control-*parasite* negative group (44.98±0.3 ng/ml). Post hoc Tukey analysis showed significant differences ($p<0.05$) in prolactin level between CLD-parasites positive group and Control-parasite positive group. Insulin levels also differed significantly among groups. The levels of insulin were higher in both CLD-parasite positive patients (15.77± 0.29 ng/ml) and CLD-*parasites* negative patients (15.37±0.25 ng/ml) while the levels of insulin were lower in control-*parasites* positive subjects and control-*parasites* negative subjects as their levels were (9.36±0.17 ng/ml) and (9.29±0.12 ng/ml) respectively. Post hoc Tukey analysis showed no significant differences in insulin levels between CLD-parasite positive patients and CLD-*parasites* negative patients. While significant differences ($p<0.05$) were recorded between CLD-parasites positive group and Control-parasites positive group. ANOVA analysis showed that TSH levels differed significantly ($p<0.05$) among groups. TSH levels were lower in CLD-*parasites* positive patients (0.64± 012 IU/ml) and CLD-*Toxoplasma* negative patients (0.65 ±0.17 IU/ml) while control groups (*parasites* positive group and parasites negative group) showed a higher level of TSH levels (0.92±0.36 IU/ml) and (1±0.36 IU/ml) respectively.

Table 2: The levels of cortisol, testosterone, prolactin, insulin and (TSH) hormones in CLD patients and control group.

Hormones (Mean ±SD)	CLD patients (n=120)		Control group (n=120)		F-test (P-value)
	Parasite infected (n=69)	Parasite uninfected (n=51)	Parasite infected (n=34)	Parasite uninfected (n=86)	
Cortisol (ng/ml)	29.29±3.5 ^{A}	27.17±0.9 ^{A}	15.82± 0.07 ^{B}	15.76± 0.05 ^{B}	12.7 (p<0.05
Testosterone (ng/ml)	1.67± 1.2 ^{A}	1.68 ±0.13 ^{A}	2.99± 0.5 ^{B}	2.93± 0.4 ^{B}	244 (p<0.05
Prolactin (ng/ml)	68.49± 7.5 ^{A}	64.21± 1.2 ^{A}	45.47± 0.5 ^{B}	44.98± 0.3 ^{B}	8.5 (p<0.05
Insulin (ng/ml)	15.77± 0.29 ^{A}	15.37± 0.25 ^{A}	9.36± 0.17 ^{B}	9.29 ±0.12 ^{B}	259.7 (p<0.05
TSH (IU/ml)	0.64 ±0.12 ^{A}	0.65 ±0.17 ^{A}	0.92± 0.36 ^{B}	1.00± 0.36 ^{B}	29.5 (p<0.05

Means with a different capital letter in same raw significantly different (P<0.05).

3.2 Liver enzymes

Liver enzymes were also measured in this study in both CLD (*parasites* positive patients and *parasites* negative) and control group (*parasites* positive subjects and *parasites* negative subjects). Table 3 illustrates the results of liver enzyme tests, which revealed significant (p<0.05) differences between groups for all enzymes assessed. The highest level of AST enzyme was noticed in CLD-*parasites* positive group (96.99 ± 7.11 IU/L). The levels of this enzyme decreased in the following groups, CLD-parasite negative, control-*parasite* positive group and control-parasite negative group respectively as their levels were (52.10 ± 10.03 IU/L), (22.33 ± 1.115IU/L) and (23.35±0.7 IU/L). Post hoc Tuckey analysis showed that AST levels in CLD-*parasites* positive were significantly (p<0.05) higher than the other groups. AST levels were significantly related to parasites positive status in CLD patients. Results showed that the levels of ALT and ALP enzymes were significantly (p<0.05) higher in CLD-*Toxoplasma* positive group (103.27 ± 7.91 IU/L) and (216.53±32.9 IU/L) respectively compared to other groups. These enzymes were significantly related to parasites positive status in CLD patients. The Levels of GGT enzymes were also differed significantly (p<0.05) among groups. The higher GGT levels were recorded in CLD- parasite positive (20.26 ±1.7 IU/L) and CLD *parasite* negative groups (16.73± 0.9 IU/L) compared to control- *parasite* positive (7.06 ± 0.07 IU/L) and control parasites negative groups (6.9± 0.06 IU/L). The high level of this enzyme was related significantly to parasites positive status.

Table 3: The levels of liver enzymes (AST, ALT, ALP and GGT) in CLD patients and control group. **(P-value** <0.05**)**

Liver enzymes (Mean ±SD)	CLD patients (n=120)		Control group (n=120)		F-test (P-value)
	Parasite infected (n=69)	Parasite uninfected (n=51)	Parasite infected (n=34)	Parasite uninfected (n=86)	
AST (IU/L)	96.99 ± 7.11 ^{A}	52.10 ± 10.03 ^{B}	22.33 ± 1.15 ^{C}	25.35±0.7 ^{C}	35.15 (p<0.05
ALT (IU/L)	103.27±7.91 ^{A}	45.32±9.17 ^{B}	41.41±12.8 ^{B}	32.77±5.6 ^{B}	17.57 (p<0.05
ALP (IU/L)	216.53±32.9 ^{A}	135.33±19.31^{B}	97.33±4.01 ^{B}	93.02±2.5 ^{B}	8.5 (p<0.05
GGT (IU/L)	20.26±1.7 ^{A}	16.73±0.9 ^{A}	7.06±0.07 ^{B}	6.9±0.06 ^{B}	40 (p<0.05

Means with a different capital letter in same raw significantly different (P<0.05).

3.3 Prothrombin time (PT), International normalized ratio (INR) and *serum* albumin

Results showed that PT values were not differed significantly among groups. All values were illustrated in Table 4. On the other hand, INR showed significant differences (<0.05) among groups. The highest INR values were noticed in CLD-parasites positive (1.21 ±0.04) and CLD-parasites negative patients (1.23±0.05). While both control-parasite positive and control-parasite negative subjects showed low INR values (0.9±0.04) and (0.8±0.05) respectively. Post hoc Tukey analysis showed significant differences (<0.05) between the following pairs of groups (CLD- *parasites* positive versus control *parasites* positive), (CLD-*parasites* positive versus control *parasites* negative), CLD- parasites negative versus control *parasites* positive) and (CLD- *parasites* negative versus control parasites negative). Significant differences (<0.05) in albumin levels were also recorded among groups. Albumin decreased significantly in CLD- *parasites* positive (3.63 ±0.09 g/dl) and CLD-*parasites* negative patients (3.65 ±0.08 g/dl) compared with control- *parasites* positive subjects (4.17 ±0.07 g/dl) and control-*parasites* negative subjects (4.15±0.04 g/dl). Post hoc Tukey analysis revealed no significant differences between the following pairs of groups (CLD- *parasites* positive and control- *parasites* negative) and (control- *parasites* positive and CLD- *parasites* negative).

Table 4: The values of prothrombin time (PT), International randomized ratio and albumin in CLD patients and control group.

P-arameters (Mean ±SD)	CLD patients (n=120)		Control group (n=120)		F-test (P-value)
	Parasite infected (n=69)	Parasite uninfected (n=51)	Parasite infected (n=34)	Parasite uninfected (n=86)	
PT (second)	12.44±0.33	12.07±0.44	12.08±0.12	11.09±0.14	0.6 (0.5) NS
INR (%)	1.22±0.04 ^{A}	1.23±0.05 ^{A}	0.9±0.04 ^{B}	0.8±0.05 ^{B}	27 (p<0.05
Albumin (g/dl)	3.63±0.09 ^{A}	3.65±0.08 ^{A}	4.17±0.07 ^{B}	4.15± 0.04 ^{B}	15.46(p<0.05

Means with a different capital letter in same raw significantly different (P<0.05).

3.4 Interleukin 13 (IL-13) and Transforming Growth Factor (TGF)

Results showed that IL-13 differed significantly (<0.05) among groups. CLD- *parasites* positive and *parasites* negative patients had the highest IL-13 levels as their values (258.74±23.7 pg/ml) and (233.23±22.2 pg/ml) respectively. While control- *parasites* positive and control- *parasite* negative had the lowest IL-13 levels as their values (88.77±1 pg/ml) and (90.78±2.3 pg/ml) respectively. Post hoc Tuckey analysis revealed no significant differences between the following pairs of groups (CLD- *parasites* positive and control- *parasites* negative) and (control- *parasites* positive and CLD- *parasites* negative). The analysis revealed no statistically significant differences among the groups in terms of TGF values. Although, TGF was higher in CLD- *parasite* positive (162.04±17.4 pg/ml), control- *parasites* negative group (160.5±87.2 pg/ml) and CLD- *parasites* negative patients (152.9±9.8 pg /ml). While TGF values were lower in control- *parasites* positive group (77.33±1.19 pg/ml) Table 5.

Table 5: The levels of IL-13 and TGF in CLD patients and control group

Parameters (Mean ±SD)	CLD patients (n=120)		Control group (n=120)		F-test (P-value)
	Parasite infected (n=69)	Parasite uninfected (n=51)	Parasite infected (n=34)	Parasite uninfected (n=86)	
IL-13(pg/ml)	258.74±23.7 ^{A}	233.88±25.2^{A}	88.7±1 B	90.78±2.3 ^{B}	9.08 (p<0.05
TGF(pg/ml)	162.04±17.4	152.9±9.8	77.33±1.9	164.5±87.2	0.3(0.8) NS

Means with a different capital letter in same raw significantly different (P<0.05).

4. Discussion

4.1 Protozoan parasites screening

The study found that intestinal protozoan parasites and antibodies against Toxoplasma were more prevalent among patients with CLD compared to the control group. However, there is a paucity of research exploring the potential association between liver disease patients and protozoan parasitic infections in the context of Iraq. The reason why CLD patients have more anti-*Toxoplasma* antibodies than the control group could be that the latent infection that reactivates later on is causing patients with chronic infections to have fewer humoral and cell-mediated immune responses. Our results are in line with the results of Ghanam *et al*. [13], which found that patients with acute and chronic liver diseases had a large seroprevalence of *T. gondii* antibodies (65.5%), while the group of healthy control participants had a seroprevalence of 27%. Furthermore, our results supported those of other studies that linked liver problems to toxoplasmosis [5, 13]. Our results, on the other hand, were inconsistent with those presented in [14], who found no evidence linking liver problems to seropositivity to toxoplasmosis, despite a comparable seroprevalence of *Toxoplasma* antibodies in both CLD patients and control individuals. The results obtained were similarly consistent with the findings of Younes *et al*. [15], who investigated the incidence of recurrent diarrhea in CLD patients and discovered that 21.5% of cases had *E. histolytica* and 23.3% had *G. lamblia*. But according to Hegab *et al*.,[5], who examined 80 CLD patients ranging in age from 6 months to 14 years, *G. lamblia* was the most prevalent organism, appearing in 45% of cases, followed by *E. histolytica* in 37.5% of cases. Regarding *Cryptosporidium*, the results were consistent with those of Shrestha *et al., [*16], who examined 30 cases with obstructive and non-obstructive hepatic lesions to determine the role of parasite infection in patients with chronic diarrhea. They discovered that 10% of the cases contained *Cryptosporidium.* This finding, however, was at odds with that of [15], who found that no *Cryptosporidium* was present in any of the CLD patients. Benamrouz *et a*l. [17], did not find *Cryptosporidium* in any of the individuals they evaluated who had schistosomal liver disorders. This intriguing discovery assisted us in identifying the cause of the infection, which pointed to contaminated water sources and a practice of poor hygiene.

Hormones

Liver disease may cause an imbalance in hormones. Males or females with liver illness may experience alterations in their hormones [18]. The findings of this study on hormone levels support the notion that patients with CLD and the control group exhibit distinct differences. These differences resulted in an increase in some hormones and a decrease in others. Cortisol levels increased in the groups with CLD parasites positive and negative compared to the groups with control parasites positive and negative. Certain theories suggest that cortisol levels correlate with the severity of liver failure and may be a sign of bad prognosis. This establishes the foundation for using cortisol levels to evaluate the degree and likelihood of illness. Our findings indicate that cortisol plays a critical role in the development and course of liver failure, with higher levels associated with improved prognosis for patients. The cortisol levels in this study were unaffected by the positive results for *Toxoplasma gondii* and other intestinal protozoan parasites. Nevertheless, an increase in this hormone can impair immunity, increasing the likelihood that people with liver issues will contract parasites. This outcome was consistent with previous study [19], which demonstrated that elevated cortisol levels can impair the immune system's capacity to combat diseases such toxoplasmosis. The findings also indicated that intestinal protozoan parasites were present in a subset of CLD patients, which may have a connection to malnourishment. A major source of stress, malnutrition raises cortisol levels has a catabolic effect on the body. Additionally, a lack of meals decreases the insulin-dependent tissue synthesis's anabolic activity [20]. However, there were notable differences in testosterone levels between the groups. CLD patients had decreased testosterone levels

compared to greater levels in control groups. Gonadal failure, a central hypothalamus-pituitary mechanism, and enhanced peripheral androgen aromatization could all contribute to this result [21, 22]. The finding that males with advanced liver disease have lower testosterone levels than controls was supported by this research [23]. Furthermore, the study's findings on testosterone were in line with other recent research showing that, regardless of the underlying cause of the disease, low testosterone is associated with an increased risk of mortality and is significantly more common in men with end-stage liver disease [23, 24]. A statistically significant difference was not observed between the groups that tested positive or negative for CLD-*Toxoplasma*, according to the study's findings. In contrast, previous research [25] was unable to determine if testosterone concentration is affected by *Toxoplasma* infection or whether individuals with low or high testosterone levels are more likely to become infected. It is accurate to say that toxoplasmosis alters testosterone levels. Lower testosterone levels may represent an adaptive response of infected mice to *Toxoplasma*-induced immunosuppression, according to the idea that increased testosterone concentrations have immunosuppressive effects [26]. Based on direct and indirect data, some specialists claim that persons with toxoplasmosis had higher testosterone concentrations. On the other hand, an infection caused by gastrointestinal parasites has been associated with malnutrition, insufficient intake of essential micronutrients, and poor dietary habits. Low testosterone levels can result from any of these conditions [27]. Prolactin concentrations differed in the CLD and control cohorts. Prolactin increased as compared to the control. Different types of amino acids enter the central nervous system in patients with CLD due to decompensated liver function. It has been demonstrated that increased levels of circulating aromatic amino acids can cause the synthesis of false neurotransmitters, such as octopamine and phenylethanolamine [28]. These synthetic neurotransmitters may impede the release of dopamine, leading to hyperprolactinemia. Furthermore, a researcher documented instances of hypogonadism in cirrhotic individuals associated with hyperprolactinemia [29]. The current study's prolactin results were in line with those of [9] investigation, which discovered that people with liver cirrhosis had noticeably higher prolactin levels [9]. Conversely, during the genesis and effector phases of the anti-*Toxoplasma* response, prolactin (PRL) is one of the most important hormones engaged in immunoregulation and various complicated interactions with the endocrine system. Inflammation is brought on by protozoa parasites, which also increase the secretion of pro-inflammatory cytokines. PRL stimulates immune system cells' anti-parasitic activities on several levels, either by boosting phagocytosis and T helper cytokine 1 (Th1) immunological responses or by raising NK cytolytic activities. Conversely, some parasites that have PRL receptors and may use PRL to enhance their growth, motility, or reproduction may also cause infections due to PRL [30]. The current study's findings demonstrated a noteworthy difference between those with CLD-positive parasites and those with CLD-negative parasites. The study revealed that prolactin levels were significantly elevated in individuals who tested positive for parasitic infections compared to those who did not have any detected parasites. Patients with CLD who did not test positive for parasites had significantly lower prolactin levels, although they were still higher than those in the non-CLD group (control). These results were consistent with [31], who showed that prolactin hormone levels were greater in *T. gondii* infection patients than in the control group. Inflammation is brought on by protozoa parasites, which also increase the secretion of pro-inflammatory cytokines. PRL stimulates immune system cells' anti-parasitic activities on several levels, either by boosting phagocytosis and T helper cytokine 1 (Th1) immunological responses or by raising NK cytolytic activities [30]. This study also found that insulin levels among those with CLD surpassed the insulin readings of the control group. Hyperinsulinemia in liver disease patients is caused by increased hepatic insulin resistance, as observed in those with CLD who do not have both significant hepatic parenchymal cell loss and portal-systemic shunting. Another study found that cirrhosis patients had more pancreatic islets that proliferate and have a reduced amount of apoptosis in comparison to those who have chronic liver disease [31]. Our

findings are consistent with recent research suggesting that hyperinsulinemia in cirrhotic people may be the consequence of the adaptive response of pancreatic beta cells to elevated insulin resistance [32]. Thus, prolonged blood sugar levels, elevated urine sugar levels, inadequate insulin synthesis, and chronic pancreatitis have all been associated with toxoplasmosis [33]. An infection with *T. gondii* may cause pancreatic tissue necrosis [34]. Therefore, compared to those who do not have the infection, those who have a *T. gondii* infection may be more likely to develop diabetes. Unquestionably, insulin has been shown to promote *T. gondii* multiplication in vitro [34]. Additionally, a study showed that insulin and D-glucose have a synergistic, dose-responsive boosting effect on *T. gondii* tachyzoite reproduction in vitro. On the other hand, compared to high TSH levels in controls, TSH significantly decreased in CLD patients [35]. Prior research has demonstrated a robust correlation between thyroid dysfunction and hepatic disease in individuals with liver disease. The current investigation's low TSH level was in line with a previous study's discovery that liver failure patients had much lower TSH concentrations, indicating that euthyroid sickness syndrome may have evolved in these people [36]. Overall, it showed that blood thyroid hormone levels and the severity of liver malfunction were correlated, which is consistent with other studies on cirrhotic liver diseases [36]. The TSH was slightly lower in the CLD-*Toxoplasma* positive group as compared to the CLD-*Toxoplasma* negative group. Thyroid disease and *T. gondii* infection may be directly connected, according to study. Increases in thyroid peroxidase autoantibodies and autoimmune thyroid diseases have been related to *T. gondii* infection. Moreover, lowered thyroid function has been connected to mice toxoplasmosis. Given the low TSH levels found in this study, it is possible that this parasite either prevents thyroid dysfunction or does not contribute to thyroid dysfunction. Regarding the former, it is possible that a small percentage of individuals with *T. gondii* would infect their thyroid gland; as a result, any inflammation or tissue damage that causes thyroid dysfunction may only occasionally be observed [37]. The potential protective impact of exposure to *T. gondii* and other infections against atopy, asthma, thyroid dysfunction in general, and hypothyroidism in particular, is uncertain. To support or refute *T. gondii's* protective function and any underlying mechanisms, more research is needed. This result supported the hypothesis that toxoplasmosis was associated with a little decrease in TSH levels [38]. In contrast, intestinal protozoan parasite infections can lead to deficiencies in electrolytes and other essential micronutrients. These disruptions impact the body's osmotic regulation system and influence thyroid hormone transmission across all body cell membranes [39].

Liver enzymes.

Both blood AST and ALT activities are useful markers of hepatocellular injury [24], while serum ALT activity is more specific than serum AST for assessing liver injury [28]. The significantly elevated serum activity of aminotransferases in the toxoplasmosis cases in this experiment that tested positive for the disease serologically are consistent with prior studies. The findings of this study aligned with the results obtained from previous research investigations conducted on experimental animal models [40]. These increases imply that liver cells are involved. A known side effect of toxoplasmosis is hepatic necrosis, which can cause focal necrosis of the liver cells, increased endothelial cells, round cell infiltration in the portal areas, and cholestasis. Despite the significant rise in AST and ALT activity when compared to the controls, the levels are still within normal ranges, suggesting a minimal impact on the liver. Serum ALP activity in the sick group was notably higher than in the control group. Since hepatic ALP is reported to be present on the canalicular and luminal domain of bile duct epithelium [28], this finding is consistent other findings [41] and may be explained by the presence of *Toxoplasma gondii* parasites in the bile duct cells. Consistent with previous findings, serum GGT activity was considerably higher in the sick group compared to the control group. This enzyme is found in hepatocytes and is released by intra- and extra-ductal cells [28].

The fact that this enzyme is increasing more than serum ALP activity suggests that toxoplasmosis is affecting both the hepatocytes and the bile ducts. When diagnosing obstructive liver disease, GGT is more sensitive than ALP, indicating that patients with infection have evident liver involvement [42]. Multiple studies have shown that changes in serum protein fractions and cellular metabolism are commonly associated with infection and the entry of parasites into the body, occurring during the various stages of the parasite's life cycle and migration within the host [43]. Research conducted through experiments has demonstrated that parasite infections can alter the amount of aminotransferase. Alpha-ketoacid is given an amine group by these enzymes [44, 45]. The liver has a high level of the enzyme alanine aminotransferase (ALT). High concentrations of aspartate aminotransferase (AST) are seen in the heart, liver, and skeletal muscle [46, 47]. Any decrease in (AST/ALT) levels may be brought on by extrahepatic cholestasis or acute viral hepatitis, while any increase may be the result of cirrhosis, cholestasis, hepatocellular cancer, or chronic hepatitis. The damaging effects of parasitic infections on liver function could be one explanation for this shift in enzyme levels. Some parasites select the liver as a site for permanent or temporary implantation, and their visceral migration also harms the cells [46, 47].

Prothrombin time (PT), International normalized ratio (INR) and serum albumin

The CLD seropositive and CLD negative groups were found to have significantly different INR values, despite the fact that the PT values were detected to be lower in the Control-parasites negative group. Most hospitalized patients with liver cirrhosis or other chronic liver disorders have coagulation problems, which are linked to significantly extended PT and high international normalized ratio (INR) values [10]. The results of the study suggest a possible link between elevated INR levels and low TSH. This outcome was in line with that of [48], who found that PT and INR levels were higher in hypothyroidism patients. It also agreed with the conclusions of [49]. The prothrombin time (PT) and the INR test were not the same. PT did not show any discernible differences between the groups. Patients with positive and negative CLD parasites showed a minor difference, but it was still slightly longer. The elevated INR seen in CLD patients who tested positive for parasites was mostly caused by a longer prothrombin time (PT). The notion that prolonged PT and thrombocytopenia are frequent test results that indicate the likelihood of developing cirrhosis highlights the importance of the data gathered. The liver is acknowledged to be a tolerogenic environment and is the location of deterioration in CLD patients. These findings corroborated those of [50], who demonstrated that (PT) value differed from INR and found that (PT) did differ significantly between patients with CLD-*parasites* positive and negative status. The investigation's albumin value results were not substantially affected by the presence of parasites. In other words, the presence of parasites did not significantly affect the level of albumin in the serum. Serum albumin concentrations can only be shown to decline during the acute stage of *T. gondii* infection. Researchers have shown that liver damage in experimentally infected mice results in lower serum albumin levels. This shows that a variety of conditions, such as infectious diseases and parasite infections, can influence the variation of blood proteins, especially albumin. This result was in line with the results of other study [50]. Conversely, individuals with intestinal infections may be malnourished. One of the main indicators of chronic liver disease and protein-calorie deficiency is hypoalbuminemia. Serum albumin has also recently been suggested as a crucial indicator of how severely sick patients may respond to nutritional therapy and their tolerance to enteral feeding. When essential nutrients are not consumed in sufficient amounts or when medical problems impair the body's ability to absorb nutrition, people may develop hypoalbuminemia. A few receiving chemotherapies could not be eating enough. One of the main indicators of CLD and malnutrition is hypoalbuminemia. Subsequently, serum albumin has recently been suggested as a crucial predictor of how critically sick patients may respond to nutritional therapy and tolerate enteral feeding [51, 52].

Interleukin 13 (IL-13) and Transforming Growth Factor (TGF)

The findings demonstrated that IL-13 varied significantly between groups. Patients with CLD whose parasites were positive or negative had the highest values for IL-13 levels. The lowest IL-13 levels were found in the control-*parasites* positive and control-*parasites* negative groups. The increase in IL-13 is linked to growing fibrosis and/or inflammation in the livers of persons with chronic liver disease [11]. TGF and IL-13 are both necessary for organ fibrosis, hence there is an ongoing debate about the synergic signalling relationship between these two important pro-fibrotic cytokines during fibrogenesis. Under specific circumstances, TGF and IL-13 may work together to cause fibrosis. The wound-healing response brought on by liver damage-induced levels of active TGF- includes the production of myofibroblasts and the deposition of extracellular matrix, which also enhance hepatocyte mortality and activate hepatic stellate cells and fibroblasts [12]. High non-significant levels of IL-13 were linked to toxoplasma positivity. Natural killer (NK) cells are produced in greater quantities as a result of toxoplasmosis. Focusing on intracellular parasites enables the early control of parasite infection. NK cells can make IL-13, control innate defences, and even create other cytokines. As memory-like cells, NK cells can play a role in adaptive immunity and may be crucial in the case of subsequent infections. The rise in this cytokine in CLD- parasites positive compared to parasite negative can be explained by the reasons given above. TGF is a crucial cytokine for the generation of immune responses against *T. gondii, Cryptosporidium, Giardia and Entamoeba* because it plays a crucial role in the formation of adequate mucosal immune responses. The ability of *T. gondii* to suppress immunological responses against itself, however, is also supported by some evidence. It appears that in this case, TGF- suppresses immune responses by inducing immune tolerance to *T. gondii* antigens. TGF therefore seems to have two functions in toxoplasmosis [53]. As a result of the significant increase in biochemical parameters in the patient group, this study concludes that toxoplasmosis affects liver and kidney functions. This has the potential to impact certain enzyme systems, which may then lead to serious pathologies such as hepatitis, pneumonia, blindness, and severe neurological disorders [54].

Conclusion

The positive status of protozoan parasites is more expected to be occurred in patients with chronic liver diseases.The positive status of parasites had no influence on cortisol, testosterone, insulin, prolactin or TSH. Liver enzymes (ALT, AST and ALP) were strongly correlated with parasite positivity in CLD patients. The other biochemical and immunological indicators (IL-13 and TGF), as well as PT, INR, and albumin, did not correlate with parasites positivity.

References

[1] M. S. Bugdaci, C. Karaca, A. R. Koksal, S. Boga, C. Alkim, and M. Sokmen, "The association with histopathological findings and predictive significance of transforming growth factor beta 1 (TGF β1) in patients with chronic viral hepatitis", *Archives of Hepatitis Research,* vol. 9, pp. 005-010, 2023.

[2] B. L. Ungar, R. Soave, R. Fayer and T. E. Nash, "Enzyme immunoassay detection of immunoglobulin M and G antibodies to Cryptosporidium in immunocompetent and immunocompromised persons", *Journal of Infectious Diseases*, vol.153, pp.570-578, 1986.

[3] K. S. Warren, "Immunology and molecular biology of parasitic infections" ,1993.

[4] E. Dereb, M. Negash, T. Teklu, , D. Damtie, , A. Abere, , F. Kebede and E. Kasa, "Intestinal parasitosis and its association with CD4+ T cell count and viral load among people living with HIV in parasite endemic settings of Northwest Ethiopia", *HIV/AIDS-Research and Palliative Care*, vol. 33, pp.1055-1065, 2021.

[5] M. Hegab, S. M. Zamzam, N. M. Khater, D. M. Tawfeek, and H. M. Abdel-Rahman, "Opportunistic intestinal parasites among children with chronic liver disease", *Journal of the Egyptian Society of Parasitology,* vol. 33, pp. 969-977, 2003.

[6] Y. Karoli, R. Karoli, J. Fatima, and M. Manhar,"Study of hepatic osteodystrophy in patients with chronic liver disease", *Journal of Clinical and Diagnostic Research: JCDR*, vol. 10, pp. 31, 2016.

[7] L. J. Al-Ani and H. S. Al-Warid, "Nutritional Status and Lipid Profile Among Children Infected with Giardia lamblia and Cryptosporidium", *Iraqi Journal of Science,* vol. 64, pp. 2717-2725, 2023.

[8] L. J. Al-Ani and H. S. Al-Warid, "Evaluation of Adipokines Among Children Infected with Some Protozoan Intestinal Parasites", *The Egyptian Journal of Hospital* Medicine, vol. 88, pp. 3906-3910, 2022.

[9] S. K. Jha and S. Kannan, "Serum prolactin in patients with liver disease in comparison with healthy adults: A preliminary cross-sectional study", *International Journal of Applied and Basic Medical Research,* vol. 6, pp. 8, 2016.

[10] A. A. E.-R. El-Henawy, A. Abdel-Razik, S. Zakaria, D. Elhammady, N. Saudy, and M. S. Azab, "Is toxoplasmosis a potential risk factor for liver cirrhosis?", *Asian Pacific journal of tropical medicine,* vol. 8, pp. 784-791, 2015.

[11] J. R. Carvalho and M. V. Machado, "New insights about albumin and liver disease", *Annals of hepatology,* vol. 17, pp. 547-560, 2018.

[12] S. Dooley and P. Ten Dijke, "TGF-β in progression of liver disease", *Cell and tissue research,* vol. 347, pp. 245-256, 2012.

[13] M. Ghanam, M. Shataat, M. Mel-S, A. Hassan, and A. Younis, "Evaluation of the role of some parasitic infections as a cause of acute and chronic hepatic diseases", *Journal of the Egyptian Society of Parasitology*, vol. 31, pp. 37-42, 2001.

[14] C. Alvarado-Esquivel, J. L. Torres-Berumen, S. Estrada-Martínez, O. Liesenfeld, and M. F. Mercado-Suarez, "Toxoplasma gondii infection and liver disease: a case-control study in a northern Mexican population", *Parasites & vectors*, vol. 4, pp. 1-7, 2011.

[15] T. A. Younes, M. M. Hussein, S. M. Kamal, D. M. Mahmoud and S. F. Metwaly, "Parasitological and bacteriological studies in recurrent diarrhoea in patients with chronic liver diseases", *Journal of the Egyptian Society of Parasitology*, vol. 26, pp. 697-708, 1996.

[16] S. Shrestha, S. Larsson and J. Serchand, "Bacterial and cryptosporidial infection as the cause of chronic diarrhoea in patients with liver disease in Nepal", *Tropical Gastroenterology: Official Journal of the Digestive Diseases Foundation*, vol. 14, pp. 55-58, 1993.

[17] S. Benamrouz, V. Conseil, C. Creusy, E. Calderon, E. Dei-Cas, and G. Certad, "Parasites and malignancies, a review, with emphasis on digestive cancer induced by Cryptosporidium parvum (Alveolata: Apicomplexa)", *Parasite: journal de la Société Française de Parasitologie,* vol. 19, pp. 101, 2012.

[18] M. Sarkar *et al.*, "Sex Hormone–Binding Globulin Levels in Young Men Are Associated With Nonalcoholic Fatty Liver Disease in Midlife", *Official journal of the American College of Gastroenterology| ACG,* vol. 114, pp. 758-763, 2019.

[19] H. R. Mahbodfar, E. Yousefi-Razin, J. Saki, A. Rafiei, and S. Khademvatan, "Study of latent Toxoplasma gondii role in level of testosterone, DHEA, cortisol and prolactin hormones of young persons", *Asian Journal of Epidemiology,* vol. 8, pp. 64, 2015.

[20] A. L. Sawaya, "Malnutrition: longterm consequences and nutritional recovery effects", *estudos avançados,* vol. 20, pp. 147-158, 2006.

[21] C. Gluud, "Serum testosterone concentrations in men with alcoholic cirrhosis: background for variation", *Metabolism,* vol. 36, pp. 373-378, 1987.

[22] D. H. Van Thiel, S. Kumar, J. S. Gavaler and R. E. Tarter, "Effect of liver transplantation on the hypothalamic-pituitary-gonadal axis of chronic alcoholic men with advanced liver disease", *Alcoholism: Clinical and Experimental Research*, vol. 14, pp. 478-481,1990.

[23] M. Sinclair, M. Grossmann, , R. Hoermann, , P. W. Angus and P. J. Gow, "Testosterone therapy increases muscle mass in men with cirrhosis and low testosterone: a randomised controlled trial", *Journal of hepatology*, vol. 65, pp. 906-913, 2016.

[24] D. Schwinge, A. Carambia, A. Quaas, T. Krech, C. Wegscheid, G. Tiegs and C. Schramm, "Testosterone suppresses hepatic inflammation by the downregulation of IL-17, CXCL-9, and

CXCL-10 in a mouse model of experimental acute cholangitis", *The Journal of Immunology*, vol. 194, pp. 2522-2530, 2015.

[25] J. Flegr, M. HRŮSKOVÁ, Z. Hodný, M. Novotna, and J. Hanušová, "Body height, body mass index, waist-hip ratio, fluctuating asymmetry and second to fourth digit ratio in subjects with latent toxoplasmosis", *Parasitology,* vol. 130, pp. 621-628, 2005.

[26] F. M. R. Lopes, D. D. Gonçalves, R. Mitsuka-Breganó, R. L. Freire, and I. T. Navarro, "Toxoplasma gondii infection in pregnanc", *Brazilian Journal of Infectious Diseases,* vol., pp. 496-506, 2007.

[27] A. M. de Souza Santos, M. Ferraz, C. Teixeira, F. Sampaio, and C. da Fonte Ramos, "Effects of undernutrition on serum and testicular testosterone levels and sexual function in adult rats", *Hormone and metabolic research,* vol. 36, pp. 27-33, 2004.

[28] B. Als-Nielsen, W. Chen, C. Gluud, and L. L. Kjaergard, "Association of funding and conclusions in randomized drug trials: a reflection of treatment effect or adverse events?", *Jama,* vol. 290, pp. 921-928, 2003.

[29] E. Karagianni, C. Papazachos, D. Panagiotopoulos, P. Suhadolc, A. Vuan, and G. Panza, "Shear velocity structure in the Aegean area obtained by inversion of Rayleigh waves", *Geophysical Journal International,* vol. 160, pp. 127-143, 2005.

[30] M. Legorreta-Herrera, "The influence of prolactin on the immune response to parasitic diseases", *Advances in Neuroimmune Biology,* vol. 7, pp. 107-114, 2018.

[31] R. D. Hussein and A. S. Mohammed, "Relationship Between Toxoplasma gondii and Sex Hormone", *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology,* vol. 14, pp. 229-236, 2022.

[32] M. Kawaguchi, M. Takahashi, T. Hata, Y. Kashima, F. Usui, H. Morimoto and U. Ikeda, "Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury", *Circulation*, vol. 123, pp. 594-604, 2011.

[33] S. Shirbazou, A. Delpisheh, R. Mokhetari, and G. Tavakoli, "Serologic detection of anti Toxoplasma gondii infection in diabetic patients", *Iranian Red Crescent Medical Journal,* vol. 15, pp. 701, 2013.

[34] H. S. Oz, "Toxoplasmosis, pancreatitis, obesity and drug discovery", *Pancreatic disorders & therapy,* vol. 4, pp. 9, 2014.

[35] J. H. Hussien, A. Mohammed, and R. H. Aljorani, "Study the effect of acute toxoplasmosis infection on some hormones and the phagocytic activity of neutrophils in pregnant and non-pregnant women before and after treatment", *International Journal of Current Microbiology and Applied Sciences,* vol. 4, pp. 459-65, 2015.

[36] Y. Wu *et al.*, "Usefulness of serum thyroid-stimulation hormone (TSH) as a prognostic indicator for acute-on-chronic liver failure", *Annals of Hepatology,* vol. 14, pp. 218-224, 2015.

[37] S. Kharb, M. Garg, P. Puri, K. S. Brar, A. Pandit, and S. Srivastava, "Assessment of thyroid and gonadal function in liver diseases", *Indian journal of endocrinology and metabolism,* vol. 19, pp. 89, 2015.

[38] Š. Kaňková, L. Prochazkova, J. Flegr, P. Calda, D. Springer and E. Potlukova, "Effects of latent toxoplasmosis on autoimmune thyroid diseases in pregnancy", *PloS one*, vol. 9, e110878, 2014.

[39] A. AlQahtani *et al.*, "Thyroid dysfunction in patients admitted in cardiac care unit: Prevalence, characteristic and hospitalization outcomes", *International Journal of General Medicine,* vol. 4, pp. 505-514, 2021.

[40] X.-c. Liu, G.-d. He, K. Lo, Y.-q. Huang, and Y.-q. Feng, "The triglyceride-glucose index, an insulin resistance marker, was non-linear associated with all-cause and cardiovascular mortality in the general population", *Frontiers in cardiovascular medicine,* vol. 7, pp. 628-109, 2021.

[41] P. Punekar, A. K. Sharma, and A. Jain, "A study of thyroid dysfunction in cirrhosis of liver and correlation with severity of liver disease", *Indian journal of endocrinology and metabolism,* vol. 22, pp. 645, 2018.

[42] A. P. Delitala, G. Fanciulli, M. Maioli and G. Delitala, "Subclinical hypothyroidism, lipid metabolism and cardiovascular disease", *European journal of internal medicine*, vol. 38, pp. 17-24, 2017.

[43] A. Barragan, F. Brossier, and L. D. Sibley, "Transepithelial migration of Toxoplasma gondii involves an interaction of intercellular adhesion molecule 1 (ICAM-1) with the parasite adhesin MIC2", *Cellular microbiology,* vol. 7, pp. 561-568, 2005.

[44] S. H. Garba, N. Sambo and U. Bala, "The effect of the aqueous extract of Kohautia grandiflora on paracetamol induced liver damage in albino rats", Nigerian Journal of Physiological Sciences, vol. 7, pp.24, 2009.

[45] I. Onyesom and N. Onyemakonor, "Levels of parasitaemia and changes in some liver enzymes among malarial infected patients in Edo-Delta Region of Nigeria", *Current Research Journal of Biological Sciences*, vol. 3, pp. 78-81, 2011.

[46] A. Pohl, C. Behling, D. Oliver, M. Kilani, P. Monson, and T. Hassanein, "Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection", *The American journal of gastroenterology,* vol. 96, pp. 3142-3146, 2001.

[47] R. H. G. L Hultcrantz., H. Glaumann, G. Lindberga and L., H: son Nilsson, "Liver investigation in 149 asymptomatic patients with moderately elevated activities of serum: aminotransferases", *Scandinavian journal of gastroenterology*, vol. 21, pp. 109-113, 1986.

[48] R. S. Mani, M. A. Amin, , Li, X. Kalyana-Sundaram, S., B. A Veeneman,., L. Wang and A. M Chinnaiyan, "Inflammation-induced oxidative stress mediates gene fusion formation in prostate cancer", *Cell reports*, vol. 17, pp. 2620-2631, 2016.

[49] S. Gullu, H. Sav, and N. Kamel, "Effects of levothyroxine treatment on biochemical and hemostasis parameters in patients with hypothyroidism", *European journal of endocrinology,* vol. 152, pp. 355-361, 2005.

[50] R. H. Nafal, H. S. Al-Warid, and H. J. Al-Sultan, "Seroprevalence of Toxoplasmosis in patients with chronic liver disease in Baghdad", *Iraqi Journal of Science,* vol. 96, pp. 1667-1672, 2019.

[51] S. Mobarhan, "The role of albumin in nutritional support", *Journal of the American College of Nutrition,* vol. 7, pp. 445-452, 1988.

[52] Keller, "UNutritional laboratory markers in malnutrition", *Journal of clinical medicine*, vol. 8, pp. 775, 2019

[53] M. Zare-Bidaki *et al.*, "TGF-β in Toxoplasmosis: Friend or foe?", *Cytokine,* vol. 86, pp. 29-35, 2016.

[54] F. Robert-Gangneux and M. L. Dardé, "Epidemiology of and diagnostic strategies for toxoplasmosis", *Clinical microbiology reviews*, vol. *25*, pp. 264-296, 2012.