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Evaluation of some immunological and physiological parameters in patients with chronic kidney disease infected with Toxoplasma gondii

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Abstract **Background -** Chronic kidney disease (CKD) is defined as a decrease in glomerular filtration rate, increased urinary albumin excretion, and, ultimately, complete loss of kidney function or kidney damage. *Toxoplasma gondii* is a protozoan parasite responsible for toxoplasmosis a significant disease that affects over a billion people worldwide in humans, toxoplasmosis spreads through two primary routes: horizontal transmission, which occurs through accidental ingestion of *Toxoplasma gondii* oocysts present in contaminated food or water and vertical transmission where the infection is passed from mother to baby via the placenta. **The aim** of the current study was to detect the effect of toxoplasmosis on kidney function and determining some immunological and physiological parameters in immunosuppressed patients (kidney failure patients) compared to healthy people **Methods** The study involved the analysis of 90 blood samples, comprising 80 samples from patients diagnosed with Chronic Kidney Disease (CKD) and 10 samples from healthy individuals who served as control subjects **Results** Results revealed decrease in the level of both $CD4^+$ and $CD8^+$ in infected CKD compared with the control group, The $CD4^+/CD8^+$ concentration ratio was increased in infected CKD As for biochemical tests, the results showed an increase in the concentration of urea, creatinine, phosphorus, and the liver enzyme ALT, and no significant differences were recorded in the concentration of the liver enzyme AST and Alk.phosphatase **Conclusions**: The study revealed a decrease in immunological parameters and an increase in physiological parameters in kidney failure patients .

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Keywords: *Toxoplasma gondi: CKD chronic kidney disease; $CD4^+$; $CD8^+$; biochemical test*

1. INTRODUCTION

Toxoplasmosis, a parasitic infection caused by the intracellular protozoan *T. gondii* is among the most prevalent infections affecting humans and other warm-blooded animals (Montoya and Liesenfeld , 2004) . In immunocompromised individuals, it can lead to congenital toxoplasmosis, resulting in significant ocular and neurological complications, as well as causing abortion or zoonosis (Tenter *et al* ., 2000). In contrast, healthy individuals infected with Toxoplasma often remain asymptomatic since their immune systems can effectively suppress the parasite's activity. If symptoms do occur, they are typically mild and resemble flu-like conditions (Liao *et al* ., 2009). Most infants who are infected while in the uterus have no symptoms at birth, but the symptoms may be developed through his Spontaneous abortion is the fate of about 15% of all clinically recognized pregnancies(Raza *et al* ,.2019) . *T. gondii* can be isolated from the eyes, where it causes ocular toxoplasmosis (Hasan *et al* .,2023) *T. gondii* undergoes three distinct stages: the oocyst phase, the tachyzoite phase, and the tissue cyst phase. Infection in intermediate hosts typically occurs through the ingestion of oocysts via contaminated water, fruits, or vegetables. It can also be transmitted by consuming raw or undercooked meat containing tissue cysts. Additionally, the infection may spread from mother to fetus through the placenta or via blood transfusions and organ transplants. The definitive hosts are members of the cat family, which become infected by consuming the meat of mammals or birds that harbor *T. gondii* tissue cysts. Cats serve as the primary source of infection as they represent the parasite's final host.(B. Adugna *et al* ., 2021)

Chronic kidney disease (CKD) has emerged as a significant global public health challenge, imposing a substantial burden on countries worldwide. Over time, patients with CKD inevitably progress to end-stage kidney disease (ESKD) due to the persistent and irreversible decline in kidney function, necessitating lifelong replacement therapy. Impaired immune function, particularly cellular immunodeficiency, plays a crucial role in advancing the progression of CKD. (Vaziri , 2004) This phenomenon not only contributes to the development of chronic kidney disease (CKD) but also worsens metabolic disorders, oxidative stress, and toxin buildup within the CKD environment. Alongside cellular immune deficiency and immune system imbalance, humoral immunity is further compromised. (Sallustio , 2016) . The two primary causes of

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chronic kidney disease (CKD), diabetes and hypertension, are widely treated; yet, the prevalence of CKD is still rising quickly (globally *et al*.,2018). End-stage renal disease, a risk for cardiovascular disease, and an early death are the results of chronic kidney disease . (Kim *et al*., 2019). Consequently, the growing number of CKD patients is seen as a serious public health issue (Xie et al ., 2018).. Patients undergoing dialysis are at increased risk of several diseases, including viruses, bacteria, fungi and even parasites, which can cause their health to deteriorate during dialysis. (Prasad and Patel , 2018). *T. gondii* is one of the microscopic organisms that may be associated with dialysis patients(Soltani *et al* ., 2020)

2. Materials and methods

2.1. Blood Sample Collection

This research was conducted in the city of Kirkuk visiting the Amal Dialysis Center during the period that began in October 2023 until the end of March 2024. Blood samples were drawn from 80 patients with chronic kidney failure after they were diagnosed by specialized doctors, , and 10 blood samples from healthy individuals who did not suffer from any chronic diseases, . These samples were collected by drawing 5 ml of venous blood from the connection of dialysis patients using a 10 ml medical syringe. The blood was separated by a centrifuge at a speed of 3000 rpm for 10 minutes in order to ensure the separation of serum. The serum was then transferred to Eppendorf tubes and stored at a low temperature of -20°C until immunological and physiological tests were performed on it. The immunological parameters, $CD4^+$ and $CD8^+$were evaluated using an ELISA kit manufactured by the Chinese company Sunlong Biotech , by (Abdulla , 2015)The physiological parameters, include urea, creatinine, by (Hani and Zainal ,2024) phosphorus, liver enzymes ALT, AST, and the ALK phosphatase, were evaluated using optical absorption spectroscopy.

2.2. Statistical analysis

The program SPSS (2019) was used in the statistical analysis of the data to study the effect of various factors on the variables and characteristics studied, and the significant differences between the means were compared with the least significant difference (LSD) test according to the analysis of variance (ANOVA) .

3. Results and Discussion

The current study focused on studying some immune and physiological parameters in patients with immunocompromised individual (kidney failure patients) and determining their immune status compared to healthy individuals. The results of Table (1) showed decreased in the concentration of $CD4^+$ and $CD8^+$ lymphocytes in CKD patients with *toxoplasmosis* and those without *toxoplasmosis* compared with the healthy individuals at a probability level of $P\leq0.05$. as well as an increase in the $CD4^+/CD8^+$ ratio in CKD patients with toxoplasmosis . The current study agrees with the results of (Salem *et al*, 2022), (Xiong *et al*., 2021) (Amro *et al*., 2021)., Also agrees with (Mukherjee and Kumar , 2017) , (Hassan *et al* ,. 2017) and (Hamad *et al* ,.2019)

The reason may be attributed to a decrease in the percentage of CD4+ and $CD8^+$ cells, which exacerbated the infection because T cells are the main mediator in most resistance mechanisms among the reasons leading to a decrease in lymphocytes in patients with kidney failure is the accumulation of toxins in the body, repeated washing operations, and changing their lifestyle due to. Commitment to the number of sessions and taking the treatment continuously, which leads to a defect in the immune system (Kaszubowska *et al*., 2018) .

Table (1): *Concentration of $CD4^+$ and $CD8^+$ in chronic kidney disease (CKD) patients infected with toxoplasmosis and the control group .*

		CKD patients Infected with *Toxoplasmosis*	CKD patients without *toxoplasmosis*	control	Statistical analysis
$CD4^+$	Minimum	0.26	0.38	1.01	L.S.D. = 0.636 * P-value = 0.0137
	Max	2.41	3.60	4.84	
	Mean	0.879 b	1.289 ab	1.896 a	
	S.D	0.574	0.770	1.156	
$CD8^+$	Minimum	-0.475	-0.462	1.320	L.S.D. = 2.084 * P-value = 0.0498
	Max	5.105	8.991	9.131	
	Mean	1.036 b	2.097 ab	3.399 a	
	S.D	2.074	2.954	2.903	
$CD4^+/CD8^+$		0.848	0.614	0.557	

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The results of the current study, presented in Table (2), indicate a significant increase ($p \leq 0.001$) in blood serum urea concentration among patients with renal failure infected with toxoplasmosis (136.19 ± 34.03 mg/dL) and those with renal failure but not infected (125.93 ± 16.83 mg/dL), compared to the control group (19.36 ± 6.78 mg/dL). The creatinine concentration demonstrated a significant increase ($p \leq 0.001$) in patients with renal failure, both those infected with toxoplasmosis (6.67±2.03 mg/dL) and those not infected (6.66±2.68 mg/dL), when compared to the control group (0.473±0.22 mg/dL) this is consistent with the result (Hani and Zainal , 2024). in addition to the presence of a significant increase in the concentration of phosphorus (5.73±1.46 mg/dL) (5.08±0.86 mg/dL) in the blood serum of the two groups of patients compared with the control group (2.70 ±1.24 mg/dL) and the results were consistent with the findings of (Abd and Ahmed, 2024). It also agrees with the results of a study conducted in Libya (Qaliwan, 2021). Another study was conducted in Dohuk (Mikaeel, 2020)

As for the results of liver enzymes, the current results showed a significant increase ($p \leq 0.05$) in the concentration of ALT in the two groups of patients (13.41±10.69 U/L) and (15.30±10.63 U/L) compared to the healthy control group (22.05±9.82). U/L) while not show any significant difference in the concentration of AST and the activity of the enzyme alkaline phosphatase (ALP) in the two groups of patients compared with the control group. The current results were consistent with the findings of (Mohieddin, 2023), (Qalwan, 2021) and (Karunakaran *et al*., 2024), while they do not agree with (Mikaeel , 2020).

This study showed a significant increase in the levels of physiological parameters in kidney failure patients with toxoplasmosis. This indicates that *T. gondii* causes necrosis in vital organs such as the kidneys and liver, which leads to abnormal secretion of liver function enzymes and in the levels of both urea and creatinine.

Table (2): *Physiological variables in renal failure patients infected and*

Physiological variables	CKD patients infected with *Toxoplasmosis*	CKD patients without *toxoplasmosis*	Control group	L.S.D (P-value)
	S.D ± M	S.D ± M	S.D ± M	
S. Urea	136.19 ±34.03 A	125.93 ±16.83 A	19.36 ±6.78 B	23.897 ** (0.0001)
S. Creatinine	6.67 ±2.03 A	6.66 ±2.68 A	0.473 ±0.22 B	1.542 ** (0.0001)
S. Phosphorus	5.73 ±1.46 A	5.08 ±0.86 A	2.70 ±1.24 B	1.078 ** (0.0001)
ALT	13.41 ±10.69 B	15.30 ±10.63 ab	22.05 ±9.82 A	8.168 * (0.050)
AST	18.84 ±20.29	21.79 ±14.36	18.47 ±9.01	14.590 NS (0.9022)
S. Alk. Phosphatase	248.2 ±167.1	255.72 ±66.49	145.11±51.73	117.23 NS (0.1307)

4. Conclusions

This study shows a significant decrease in both CD4+ and CD8+ lymphocytes among renal failure patients infected with toxoplasmosis compared to healthy individuals as analyzed using ELISA technology. Additionally, it revealed a significant elevation in biochemical parameters in these patients. This is attributed to the parasite inducing necrosis in vital organs like the liver and kidneys, resulting in abnormal secretion of liver enzymes such as AST and ALT, along with increased levels of urea, creatinine, and phosphorus .

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