Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 2, 161-171

The Variable Levels of IL-6 and Nitric Oxide in Hemodialysis Patients upon Exposure to Toxoplasmosis

Mustafa A. Abood, Entsar J. Saheb

Department of Biology, College of Science, University of Baghdad,

Received: 1/6/2022 Accepted: 21/8/2022 Published: December 20, 2022

Abstract: *Toxoplasma gondii* is the most prevalent protozoa infection, affecting a wide variety of hosts. Toxoplasmosis is normally asymptomatic in immunocompetent people, but it can cause substantial issues in immunocompromised people and can progress to a life-threatening infection. This study aimed to determine the effect of toxoplasmosis on the levels of IL-6 and nitric oxide in hemodialysis patients. Overall, 300 patients referred to the Medical City, Al-karama General Hospital, Baghdad, Iraq were enrolled from 2021–2022. All serum samples were tested for *T. gondii* immunoglobulins (IgG and IgM) antibodies, IL-6 and nitric oxide levels. In hemodialysis patients infected with *T. gondii*, the IL-6 and nitric oxide levels were higher than the hemodialysis patient without toxoplasmosis. The mean IL-6 levels were higher in hemodialysis patients infected with toxoplasmosis compare with hemodialysis patient without toxoplasmosis in different gender and age while the level of nitric oxide had no significant differences according to gender. It was concluded imply that the accidental occurrence of toxoplasmosis could be seen as a danger sign for hemodialysis patients.

Keywords: Toxoplasmosis, Hemodialysis, IL-6, Nitric oxide.

Corresponding author: (Email: mustafabva2@gmail.com)

Introduction

Toxoplasma gondii, the parasite that causes toxoplasmosis, is an obligate intracellular infection. The parasite is found all over the world, particularly in hot and humid climates, and infection rates are believed to be over 30% of the global population. Children's infection rates rise in regions where cats are plentiful, as do adult infection rates from eating undercooked or raw meat (1). *T. gondii* has an asexual and sexual life cycle; the asexual stage occurs in intermediate hosts (mammals or birds), and the sexual stage occurs in the gut of the final host (2). *T. gondii* initiates the infection and activate the innate immune response resulting in slight inflammation (3). Toxoplasmosis affects nearly one third of people worldwide, especially immunocompromised people like HIV/AIDS patients, cancer patients, and organ transplant recipients(4, 5). The first line of protection for the host is innate immunity, which reacts quickly and

detects pathogen invasion through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). Induced proinflammatory cytokines such as TNF-, interleukin-6 (IL-6), and IL12 are produced as a result of ligand identification by PRRs, which also contributes to the subsequent activation of immune responses (6). Due to a failure in cell-mediated immunity, the detection of opportunistic infectious diseases in hemodialysis patients with low immunity, such as toxoplasmosis, is extremely important (7). IL-6 is a multitasking cytokine that has both pro- and anti-inflammatory effects in people. Depending on their biological roles and sources, it has a variety of traditional names (8). IL-6 is produced by a large number of cells, including monocyte macrophages, endothelial cells, fibroblasts, myelomatous and neoplastic cells (9). Therapeutic hemodialysis and peritoneal dialysis further cause inflammation inflammatory responses and increase IL-6 production in patients with end-stage renal disease (ESRD). Therapeutic hemodialysis and peritoneal dialysis boost the inflammatory process and increase IL-6 production in patients with end-stage renal disease (ESRD) (10). The loss of kidney function, uremia itself (and its consequences, such as fluid overload, oxidative stress, and infection susceptibility), and factors connected to dialysis are all possible causes of elevated plasma IL-6 levels in ESRD patients (11). Nitric oxide (NO) is a radical gas molecule with one unpaired electron, resulting in strong reactivity with other radicals and the generation of more reactive and poisonous derivatives. Multiple physiological responses involving the complex regulatory network underpinning NO signaling are mediated by NO-based protein changes, such as nitrosylation and nitration (12). Additionally, recent research has shown that constitutive NOS enzymes may play a role in the immune system's control of T-helper cell proliferation and cytokine production (13). The release of renin, extracellular fluid volume, glomerular and modular hemodynamics, and other critical cellular functions in the kidneys are all impacted by NO. However, NO can function as an inflammatory mediator and oxidative stress factor at high levels (14).

Materials and methods

Subjects and blood collection

This study was permitted by the Ethical Committee of Iraqi Ministry of Health, in which 300 blood samples were enrolled in this study and their age was between (20–70 years old). One hundred samples were taken from outpatient clinics as control groups and two hundred samples hemodialysis patients from Al-Karama Hospital and Medical City in Baghdad, Iraq. Samples of 5 ml blood were taken from patients' vein. The samples were collected in sterilized Gel Clot activator vacuum tubes and left for 10 min at room temperature for clotting. Then, the samples were centrifuged at 3000 round per minute for 5 min then dispensed into Eppendorf- tubes and stored at –20ºC until the test day (Reference No. CSEC/1021/0101).

Serological tests

Specific IgG antibodies were measured using commercial *Toxoplasma* IgG and IgM EIA Test Kit (ACON Laboratories, Inc. USA) (I231-1101) (I231-1101) based on the principle of ELISA. As well as, samples were tested

for serum mean titer of IL-6 and Nitric oxide by using The SHANGHAI human IL-6(Cat. No: YHB1747Hu) and nitric oxide (YLA0822HU) kits.

Statistical analysis

The Statistical Analysis System-SAS (2012) program was used to show the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and least significant difference -LSD test was used to significant compare between means in the study with values of P < 0.05 and 0.01 considered statistically differences.

Results

The mean levels of IL-6 in control groups and H.D patients according to toxoplasmosis

The mean level of IL - 6 in H.D patients infected with *T. gondii* was 54.554 ng/L which is higher than mean level in control groups infected with toxoplasmosis (37.832 ng/L). The differences in the levels of IL- 6 among the H.D patients and control subjects showed significant differences (P≤ 0.01) Table (1).

Table (1): **The mean levels of IL-6 in control groups and H.D patients according to toxoplasmosis**

IL-6 (ng/L)			
Studying groups	**Toxo (-ve)**	**Toxo (+ve)**	**P-Value**
Control	19.781 ±0.89	25.17 ±1.65	0.037 *
H.D	70.755 ±3.28	86.31 ±4.07	0.0094 **
P-Value	0.0001 **	0.0001 **	---
** (P≤0.01)			

The mean levels of IL-6 in different age of H.D according to toxoplasmosis

The different age of H.D patients that may have effect on the level of IgG antibody to *T. gondii* in H.D patients was investigated. The result showed that the higher mean titer was in (61- 70) year that are seropositive to anti- *Toxoplasma* IgG which was 113.33 ng/L followed by (31–40) year that is seropositive to anti-*T. gondii* IgG mean titer 112.72 ng/L and then followed (20 – 30) year by that is seropositive to anti-*T. gondii* IgG which was 101.43 ng/L and then followed (51 – 60) year by that is seropositive to anti-*T. gondii* IgG which was 81.733 ng/L and finally the (41 – 50) year that is seropositive to anti-*T. gondii* IgG and the mean titer 21.402 ng/L with statically significant differences (P<0.01) (Table 2).

Table (2): The mean levels of IL-6 in different age of H.D according to toxoplasmosis

Age	Control		HD patients		P-value
	Toxo (-ve)	Toxo (+ve)	Toxo (-ve)	Toxo (+ve)	
21-30	20.45	9.82	68.12	101.43	0.0001 **
31-40	21.33	21.68	61.42	112.72	0.0001 **
41-50	15.07	19.70	70	21.40	0.0001 **
51-60	18.72	24.89	86.3	81.73	0.0001 **
61-70	23.32	25.69	67.85	113.33	0.0001 **
P-value	0.0071 **	0.0001 **	0.0073 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01)					

The IL-6 mean levels in different gender of studying groups according to toxoplasmosis

The mean levels of IL- 6 according to gender were highest in the females than males. The mean levels in females were 88.56 ±3.9ng/L in compare with the control group which was 22.78±1.2 ng/L. In contrast, the mean levels of IL-6 in males was 84.95 ±3.6 ng/L compare with the control group which was 25.29 ±1.6 ng/L (Table 3).

Table (3): The IL-6 mean levels in different gender of studying groups according to toxoplasmosis

Gender	Control		HD patients		P-value
	Toxo (-ve)	Toxo (+ve)	Toxo (-ve)	Toxo (+ve)	
Male	21.73 ±1.4	25.29 ±1.6	74.96 ±2.76	84.95 ±3.6	0.0001 **
Female	17.82 ±0.84	22.78 ±1.2	65.39 ±2.31	88.56 ±3.9	0.0001 **
P-value	0.043 *	0.071 NS	0.056 NS	0.77 NS	--
* (P≤0.05), ** (P≤0.01)					

The mean levels of nitric oxide in control groups and H.D patients according to toxoplasmosis

The mean level of nitric oxide in H.D patients infected with *T. gondii* was 76.03 ±2.66ng/L which is higher than mean level in control groups infected with toxoplasmosis (11.023 ±0.78µmol/L). The differences in the levels of IL- 6 among the H.D patients and control subjects showed significant differences (P≤ 0.01) (Table 4).

Table (4) The mean levels of nitric oxide in control groups and H.D patients according to toxoplasmosis.

Nitric oxide (µmol/L)			
Studying groups	**Toxo (-ve)**	**Toxo (+ve)**	**P-Value**
Control	9.179 ±0.67	11.023 ± 0.78	0.0001 **
H.D	52.06 ±1.59	76.03 ± 2.66	0.0042 **
P-Value	0.0001 **	0.0001 **	---
** (P≤0.01).			

The mean levels of nitric oxide in different age of H.D according to toxoplasmosis

The results revealed that the highest mean titer of the nitric oxide was restricted to ages between (21-30) years in H.D patients who are seropositive for anti- *Toxoplasma* IgG (108.06 ±4.6 µmol/L) compared with control (8.427 ±0.42 µmol/L) who are seropositive for anti- *Toxoplasma* IgG, and there were statistically significant differences (P<0.01) (Table 5).

Table (5): The mean levels of nitric oxide in different age of H.D according to toxoplasmosis.

Ge	Control		HD patients		P-value
	Toxo (-ve)	**Toxo (+ve)**	**Toxo (-ve)**	**Toxo (+ve)**	
21-30	12.09 ±0.54	8.427 ±0.42	17.67 ±0.85	108.06 ±4.6	0.0001 **
31-40	7.239 ±0.41	8.856 ±0.57	32.5 ±1.47	79.83 ±2.9	0.0001 **
41-50	8.121 ±0.64	16.145 ±0.83	77.7 ±2.75	60.75 ±2.6	0.0001 **
51-60	7.087 ±0.39	10.644 ±0.57	80.8 ±3.67	57.162 ±2.1	0.0001 **
61-70	8.850 ±0.57	11.609 ±0.64	60.97 ±2.51	46.80 ±1.98	0.0001 **
P-value	0.038 *	0.0063 **	0.0001 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01)					

The nitric oxide means levels in different gender of studying groups according to toxoplasmosis

The mean level of NO in *T. gondii*-infected H.D patients in different gender. The highest mean level of NO appears in the females with mean (80.31 ±3.7µmol/L) compared with males (59.08 ±2.5 µmol/L) (Table 6).

Table (6) :The nitric oxide means levels in different gender of studying groups according to toxoplasmosis

Gender	Control		HD patients		P-value
	Toxo (-ve)	Toxo (+ve)	Toxo (-ve)	Toxo (+ve)	
Male	10.54 ±0.62	10.89 ±0.64	70.02 ±2.6	59.08 ±2.5	0.0001 **
Female	7.81 ±0.45	11.10 ±0.69	35.20 ±1.8	80.31 ±3.7	0.0001 **
P-value	0.088 NS	0802 NS	0.0001 **	0.0056 NS	---
* (P≤0.05), ** (P≤0.01)					

The mean levels of IL-6 and nitric oxide in studying group.

The mean titer of IL-6 in H.D who is seropositive to anti -*Toxoplasma* IgG was (86.31 ±4.55 pg/mL), while the mean titer of IL-6 in H.D without *Toxoplasma* was (70.755 ±2.61 pg/mL). There were statistically significant differences (P<0.01) (Table 1). The higher mean titer of nitric oxide in H.D patient who are seropositive to anti- *Toxoplasma* IgG was (86.31 ±4.55 µmol/L), while the mean titer of NO in H.D patient without *Toxoplasma* was (70.755 ±2.61 µmol/L). There were statistically significant differences (P<0.01) Table (7).

Table (7): The mean levels of IL-6, urea, creatinine and NO in studying group

Studying groups	IL-6 (ng/L)	NO (µmol/L)
Control	19.781 ±0.94	9.179 ±0.61
Toxo.	25.172 ±1.37	11.023 ±0.74
H.D Toxo (-ve)	70.755 ±2.61	52.06 ±2.37
H.D Toxo (+ve)	86.31 ±4.55	76.03 ±2.85
P-value	0.0001 **	0.0001 **
** (P≤0.01)		

Discussion

One of the most critical and sometimes fatal problems that dialysis patients deal with is immune system malfunction. After cardiovascular disorders, it is the second most frequent cause of high rates of morbidity and mortality in individuals with chronic renal failure (15). Because hemodialysis patients' immune systems are compromised, reactivating *T. gondii* tissue cysts causes a high and fatal recurrence of the chronic form of

toxoplasmosis (16). In CKD patients, the increased plasma IL-6 level is commonly reported (11). A increase in production caused along by oxidative stress, chronic inflammation, and fluid overload is what causes it most. While this is happening, the accumulation is being more impacted by the decreased renal function-related IL-6 clearance. Therapeutic hemodialysis and peritoneal dialysis in patients with end-stage renal disease (ESRD) further elicit inflammatory responses and increase IL-6 production(10). A study indicated that the level of IL-6 was twice greater in the course of toxoplasmosis than in healthy controls, and with who established that IL-6 essential for resistance against *T. gondii*. Elevated IL-6 levels in serum and infected tissues following *T. gondii* infection appear to indicate the existence of an early and sensitive, though nonspecific, marker of inflammatory conditions (17) .and with (17) who showed that IL-6 is required for *T. gondii* resistance. Following *T. gondii* infection, elevated IL-6 levels in serum and infected tissues seem to suggest the presence of an early and sensitive, though particular, marker of inflammatory conditions. A multiple-regression analysis was used to eliminate the factors impacting the serum IL-6 level because it appeared that the length of hemodialysis, age, and the characteristics of the dialysis membrane were all related to serum IL-6 levels (18). Inflammation, which results from a disruption of the cytokine network and its homeostasis, is a frequent finding in ageing and age-related disorders. Pro-inflammatory cytokines are crucial in the remodeling of the immune system with ageing and function downstream of NF-B signaling(19). Older hemodialysis patients accompany a high burden of functional impairment, limited life expectancy, and healthcare utilisation. According to a review, mortality is positively connected with cognitive impairment in elder hemodialysis patients (20). Among the other cytokines, IL-6 was shown to have the highest association with mortality. Plasma levels of IL-6 are significantly higher in advanced CKD compared to the other three acute phase proteins (CRP, TNF-, and albumin), and they independently predict overall and cardiovascular mortality in a cohort of patients at different stages of CKD(21). In this study, increased serum NO was higher in H.D patients in compare with healthy control. *T. gondii* patients had a higher level of NO as compare to healthy subjects, which confirm the presence of an inflammatory state. The synergistic action of the gamma interferon (e.g. tumor necrosis factor) stimulates the product of NO (22, 23). Toxoplasmosis is linked to a number of diseases, including cardiac conditions, obsessive-compulsive disorder, and schizophrenia because of its pathogenicity, which is associated with an increase in inflammation and oxidative stress (24). In the CRF patients who were on maintenance hemodialysis, the NO levels were significantly elevated. The process of dialysis itself stimulated cytokine-induced NO synthase, which in turn caused the platelets to produce more NO as a result of uraemia. Due to its high reactivity as a free radical, NO is a cytotoxic molecule at high concentrations that causes the problems of dialysis and Nitrosative Stress in these patients (25). This study showed there is a decrease in the levels of nitric oxide with aging. When we are young and healthy, the production of NO by the endothelium via L-arginine is efficient and sufficient; but, as we get older, our capacity to synthesis NO from the endothelium decreases.

Aging lowers the bioavailability or synthesis of NOS-derived NO, according to the vast majority of investigations on the impact of NO in cells and tissues. It's possible for superoxide to scavenge NO and turn it into peroxynitrite, which would reduce the actual levels of NO in cells (26). (27) have demonstrated that endothelial function gradually declines with age, with the oldest age group studied seeing a higher than 50% reduction in endothelial function as evaluated by forearm blood flow measurements. (28) revealed more startling results in the coronary circulation of ageing people, showing that patients between the ages of 70 and 80 lost 75% of the endothelium-derived nitric oxide compared to youthful, healthy 20-year-olds. These findings demonstrate that whereas males' renal vasculature is more sensitive to NO synthase inhibition, females' renal eNOS mRNA and protein levels are higher. According to the research, males' renal vasculatures may be more dependent on NO than those of females(29). In line with the findings of earlier research, a drop in NO level was seen in HD patients receiving recombinant human erythropoietin therapy. The prooxidative effects of NO and the associated harmful influence may be reduced by the action of rhEpo in lowering NO levels in H.D. patients. As a result, rhEpo raises the amounts of nitrate and nitrite in the urine (30). It has been demonstrated that estradiol promotes the release of NO, which causes vasodilation; a lack of NO can speed up kidney damage by reducing vasodilation and endothelial dysfunction (31). Men's kidney function may decline more quickly than women's due to the damaging effects of testosterone and/or the protective effects of estrogens in women, as well as unhealthy lifestyle habits (32). Immune system dysfunction is one of the most serious and life-threatening issues that dialysis patients face. It is the second most frequent factor in high rates of morbidity and mortality in people with chronic renal failure, behind cardiovascular diseases(15). Nitric oxide and reactive oxygen species (ROS) are produced in greater quantities by infected cells as a result of toxoplasma infection, which causes oxidative stress (33). This oxidative stress causes an early inflammatory response that is mediated by proinflammatory mediators and is associated with renal failure(34). The combination of chronic inflammation and oxidative stress may both raise the risk of cardiovascular and all-cause mortality in this population. Endothelial dysfunction and elevated oxidative stress and inflammation, which are both frequent symptoms of end-stage kidney disease (ESKD), have been suggested to be related (35). In human vascular endothelial cells, IL-6 reduces eNOS activation and increases endothelial nitric oxide synthase binding to stabilised caveolin-1 (36). According to recent research, proinflammatory cytokine levels are eight to ten times greater in HD patients than in healthy controls. Numerous studies have associated poor outcomes in renal patients and high levels of pro-inflammatory cytokines (37).

Conclusion

The level of IL-6 and nitric oxide were significantly higher in the hemodialysis patients with toxoplasmosis compare with control groups. Dialysis patients are at risk for toxoplasmosis and should be screened on a regular basis to

prevent the disease from spreading during hemodialysis.

Acknowledgements

We greatly appreciate the staff members of hemodialysis center labs in the Medical City Hospital for their assistance in the collecting the study samples. We did not get financial source of the study.

References

1. Agrawal; S. R.; Singh; V.; Ingale; S.;and Jain; A. P. (2014). Toxoplasmosis of spinal cord in acquired immunodeficiency syndrome patient presenting as paraparesis: a rare entity. Journal of Global Infectious Diseases; 6(4); 178-183.
2. Babekir; A.; Mostafa; S.; and Obeng-Gyasi; E. (2022). The Association of Toxoplasma gondii IgG Antibody and Chronic Kidney Disease Biomarkers. Microorganisms; *10*(1); 115.
3. Beberashvili; I.; Sinuani; I.; Azar; A.; Kadoshi; H.; Shapiro; G.; Feldman; L.; Sandbank; J.; and Averbukh; Z. (2013). Increased basal nitric oxide amplifies the association of inflammation with all-cause and cardiovascular mortality in prevalent hemodialysis patients. International Urology and Nephrology; 45(6); 1703-1713.
4. Carrero; J. J.; Hecking; M.; Chesnaye; N. C.; and Jager; K. J. (2018). Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. Nature Reviews Nephrology; 14(3); 151-164.
5. Coleman; J. (2002). Nitric oxide: a regulator of mast cell activation and mast cell-mediated inflammation. Clinical and Experimental Immunology; 129(1); 4-10.
6. Desai; A.; Zhao; Y.; and Warren; J. S. (2008). Human recombinant erythropoietin augments serum asymmetric dimethylarginine concentrations but does not compromise nitric oxide generation in mice. Nephrology Dialysis Transplantation; *23*(5); 1513-1520.
7. Dincel; G. C.; and Atmaca; H. T. (2016). Role of oxidative stress in the pathophysiology of Toxoplasma gondii infection. International Journal of Immunopathology and Pharmacology; 29(2); 226-240.
8. Dubey; J.; Lindsay; D.; and Speer; C. (1998). Structures of *Toxoplasma* gondii tachyzoites; bradyzoites; and sporozoites and biology and development of tissue cysts. Clinical Microbiology Reviews; 11(2); 267-299.
9. Egashira; K.; Inou; T.; Hirooka; Y.; Kai; H.; Sugimachi; M.; Suzuki; S.; Kuga; T.; Urabe; Y.; and Takeshita; A. (1993). Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. Circulation; *88*(1); 77-81.
10. Farahmand; M.; Ramezani Tehrani; F.; Khalili; D.; Cheraghi; L.; and Azizi; F. (2021). Endogenous estrogen exposure and chronic kidney disease; a 15-year prospective cohort study. BMC Endocrine Disorders; 21(1); 1-8.
11. Filisetti; D.; and Candolfi; E. (2004). Immune response to Toxoplasma gondii. Ann Ist Super Sanita; 40(1); 71-80.
12. Garcia; H. H.; Tanowitz; H.; and Del Brutto; O. H. (2013). Neuroparasitology and tropical neurology. Newnes.
13. Giovannini; S.; Onder; G.; Liperoti; R.; Russo; A.; Carter; C.; Capoluongo; E.; Pahor; M.; Bernabei; R.; and Landi; F. (2011). Interleukin- 6; C- reactive protein; and tumor necrosis factor-alpha as predictors of mortality in frail; community-living elderly individuals. Journal of the American Geriatrics Society; 59(9); 1679-1685.
14. Green; S. J.; Nacy; C. A.; and Meltzer; M. S. (1991). Cytokine- induced synthesis of nitrogen oxides in macrophages: a protective host response to Leishmania and other intracellular pathogens. Journal of Leukocyte Biology; 50(1); 93-103.
15. Hung; M.-J.; Cherng; W.-J.; Hung; M.-Y.; Wu; H.-T.; and Pang; J.-H. S. (2010). Interleukin-6 inhibits endothelial nitric oxide synthase activation and increases endothelial nitric oxide synthase binding to stabilized caveolin-1 in human vascular endothelial cells. Journal of Hypertension; 28(5); 940-951.

16. Kaizu; Y.; Kimura; M.; Yoneyama; T.; Miyaji; K.; Hibi; I.; and Kumagai; H. (1998). Interleukin-6 may mediate malnutrition in chronic hemodialysis patients. American Journal of kidney Diseases; 31(1); 93-100.
17. Kaur; S.; Bansal; Y.; Kumar; R.; and Bansal; G. (2020). A panoramic review of IL-6: Structure; pathophysiological roles and inhibitors. Bioorganic and Medicinal Chemistry; 28(5); 115327.
18. Lang; C.; Groß; U.; and Lüder; C. G. (2007). Subversion of innate and adaptive immune responses by Toxoplasma gondii. Parasitology Research; 100(2); 191-203.
19. Lu; N.; Liu; C.; Wang; J.; Ding; Y.; and Ai; Q. (2015). Toxoplasmosis complicating lung cancer: a case report. International Medical Case Reports Journal; 8; 37.
20. Meenakshi; S.; and Agarwal; R. (2013). Nitric oxide levels in patients with chronic renal disease. Journal of Clinical and Diagnostic Research: JCDR; 7(7); 1288.
21. Moawad; H.; Etewa; S.; Mohammad; S.; Neemat-Allah; M.; Degheili; J.; and Sarhan; M. (2022). Seropositivity of toxoplasmosis among hemodialysis children patients at Zagazig University Pediatrics Hospital; Egypt. Parasitologists United Journal; 15(1); 53-59.
22. Pecoits-Filho; R.; Heimbürger; O.; Bárány; P.; Suliman; M.; Fehrman-Ekholm; I.; Lindholm; B.; and Stenvinkel; P. (2003). Associations between circulating inflammatory markers and residual renal function in CRF patients. American Journal of kidney Diseases; 41(6); 1212-1218.
23. Pizzino; G.; Irrera; N.; Cucinotta; M.; Pallio; G.; Mannino; F.; Arcoraci; V.; Squadrito; F.; Altavilla; D.; and Bitto; A. (2017). Oxidative stress: harms and benefits for human health. Oxidative Medicine and Cellular Longevity; 2017.
24. Poole; R. K. (2018). Nitric Oxide and Other Small Signalling Molecules. Academic Press.
25. Rea; I. M.; Gibson; D. S.; McGilligan; V.; McNerlan; S. E.; Alexander; H. D.; and Ross; O. A. (2018). Age and age-related diseases: role of inflammation triggers and cytokines. Frontiers in immunology; 586;1-7.
26. Reckelhoff; J. F.; Hennington; B. S.; Moore; A. G.; Blanchard; E. J.; and Cameron; J. (1998). Gender differences in the renal nitric oxide (NO) system. American Journal of Hypertension; 11(1); 97-104.
27. Rezaei; F.; and Mohhamadi; R. (2018). Comparison of saliva nitric oxide between chronic kidney disease before and after dialysis and with control group. The Open Dentistry Journal; 12; 213.
28. Roach; T.; Kiderlen; A. F.; and Blackwell; J. M. (1991). Role of inorganic nitrogen oxides and tumor necrosis factor alpha in killing Leishmania donovani amastigotes in gamma interferon-lipopolysaccharide-activated macrophages from Lshs and Lshr congenic mouse strains. Infection and Immunity; 59(11); 3935-3944.
29. Saki; J.; Khademvatan; S.; Soltani; S.; and Shahbazian; H. (2013). Detection of toxoplasmosis in patients with end-stage renal disease by enzyme-linked immunosorbent assay and polymerase chain reaction methods. Parasitology Research; 112(1); 163-168.
30. Sasai; M.; and Yamamoto; M. (2013). Pathogen recognition receptors: ligands and signaling pathways by Toll-like receptors. International Reviews of Immunology; 32(2); 116-133.
31. Song; Y.-H.; Cai; G.-Y.; Xiao; Y.-F.; and Chen; X.-M. (2020). Risk factors for mortality in elderly haemodialysis patients: a systematic review and meta-analysis. BMC Nephrology; 21(1); 1-10.
32. Su; H.; Lei; C.-T.; and Zhang; C. (2017). Interleukin-6 signaling pathway and its role in kidney disease: an update. Frontiers in Immunology; *8*; 405.
33. Taddei; S.; Virdis; A.; Ghiadoni; L.; Salvetti; G.; Bernini; G.; Magagna; A.; and Salvetti; A. (2001). Age-related reduction of NO availability and oxidative stress in humans. Hypertension; 38(2); 274-279.
34. Tarakçıoğlu; M.; Erbağci; A. B.; Usalan; C.; Deveci; R.; and Kocabaş; R. (2003). Acute effect of hemodialysis on serum levels of the proinflammatory cytokines. Mediators of Inflammation; 12(1); 15-19.
35. Tonelli; M.; Wiebe; N.; Culleton; B.; House; A.; Rabbat; C.; Fok; M.;

McAlister; F.; and Garg; A. X. (2006). Chronic kidney disease and mortality risk: a systematic review. Journal of the American Society of Nephrology; *17*(7); 2034-2047.

36. Van Der Loo; B.; Labugger; R.; Skepper; J. N.; Bachschmid; M.; Kilo; J.; Powell; J. M.; Palacios-Callender; M.; Erusalimsky; J. D.; Quaschning; T.; and Malinski; T. (2000). Enhanced peroxynitrite formation is associated with vascular aging. The Journal of Experimental Medicine; 192(12); 1731-1744.
37. Watanabe; P. d. S.; Trevizan; A. R.; Silva-Filho; S. E.; Gois; M. B.; Garcia; J. L.; Cuman; R. K. N.; Breithaupt-Faloppa; A. C.; SantAna; D. d. M. G.; and Nogueira de Melo; G. d. A. (2018). Immunocompetent host develops mild intestinal inflammation in acute infection with Toxoplasma gondii. PloS one; 13(1); e0190155.
38. Al-Mosawi, P. R. A. (2015). Diagnostic and epidemiological study of Toxoplasma gondii for students of Thi-Qar University by ELISA and Real-Time PCR techniques. Iraqi Journal of Biotechnology, 14(2), 295-311.