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Original Article

Expression of *Interleukin-33* Gene in Hemodialysis Patients with Chronic Toxoplasmosis in Baghdad, Iraq

Yousif, W. T[1 *]

1. Department of Medical Laboratories, Medical Technical College, Middle Technical University, Baghdad, Iraq

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Corresponding Author: walaa.t.yousif@mtu.edu.iq

Abstract

Toxoplasmosis is a protozoan parasite with high distribution, leading to different abnormalities in hosts. The present study aimed to determine the distribution of toxoplasmosis in hemodialysis patients and the expression of the *Interleukin (IL)-33* gene in chronic Toxoplasmosis. The present study evaluated 120 subjects, including 60 patients who were undergoing dialysis and 60 healthy samples as the control group, from the 1st February to 1st November 2021. Anti-*Toxoplasma gondii* IgG was detected by using the enzyme-linked immunosorbent assay (ELISA) technique and the real-time polymerase-chain-reaction (PCR) was used to perform IL-33. The results demonstrated that the highest anti-toxoplasmosis IgG antibody rate was observed in the age group 51-70 years who were undergoing dialysis, in comparison with that in the control group ($P<0.05$). The male patients who had anti-toxoplasmosis IgG antibodies outnumbered the healthy people ($P<0.05$), while the female patients did not significantly differ from the healthy group. Chronic toxoplasmosis showed a higher number according to residency (the urban and rural patients), compared to healthy people. The frequency of dialysis times per week in chronic Toxoplasmosis patients was significantly higher among the infected patients. The findings were displayed to be positive in dialysis at 2 weeks ($P<0.05$). The expression of the *IL-33* gene was investigated in patients who were undergoing hemodialysis and in healthy controls by using real-time PCR. The findings demonstrated that there was a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration. The high prevalence of toxoplasmosis in dialysis patients and the role of IL-33 in cellular immunity in these patients highlight the need to investigate the mechanisms limiting infection with intracellular protozoa.

Keywords: Chronic Toxoplasmosis, Expression, Hemodialysis, IL-33 gene

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite which can infect humans and a wide range of mammals. Toxoplasmosis is one of the infectious diseases that lead to different abnormalities due to the formation of cysts in different tissues. *Toxoplasma* infection in humans is latent (asymptomatic); nonetheless, clinical signs are developed in immunocompromised patients and congenitally infected fetuses (1). Patients who undergo dialysis are at increased risk of several diseases, including viruses, bacteria, fungi, and even parasites, which may cause deterioration in their health status during the dialysis procedure (2).

Toxoplasma gondii is one of the microscopic organisms that may be associated with dialysis patients (3). Interleukin (IL)-33 is a cytokine that is elevated in humans in bacterial and viral infections, as well as parasitic infections, including toxoplasmosis. The high levels of this cytokine indicate the levels of infection with different pathogens (4). Unlike the majority of cytokines

which are actively released from cells, the nuclear cytokine IL-33 is passively secreted during cellular necrosis or after tissue damage, suggesting that it can act as an alarm signal alerting the immune system following epithelial or endothelial cellular damages during infections, physical stresses, or traumas (5).

The IL-33 has an essential role to play in type-2 innate immunity through the activations of allergic inflammation-related basophils, eosinophils, mast cells, macrophages, and group 2 innate lymphoid cells (ILC2s) via its receptor ST2 (6). The present study evaluated the level of IgG of *Toxoplasma gondii* in dialysis patients of different ages, and for the determination of the IL-33 source, immunostaining was performed and it was revealed that IL-33 was expressed in the intestinal epithelium and the lamina propria of dialysis patients (7).

2. Materials and Methods

2.1. Sampling

In this study, a total of 60 samples were collected from patients who were undergoing dialysis and 60 samples from non-infected people as the control group from 1st February to 1st November 2021.

2.2. Enzyme-linked Immunosorbent Assay

Blood samples were collected from all 120 individuals, and the serum was separated and stored in aliquots at -20°C. Anti-*Toxoplasma gondii* IgG was detected by using the enzyme-linked immunosorbent assay (ELISA) technique. All samples were tested according to the manufacturer's instructions (NovaTec GmbH, Germany).

2.3. Real-time Polymerase-Chain-Reaction Analysis

DNA isolation from the blood was performed by QIAamp DNA mini kit according to the manufacturer's protocol. The serum IL-33 concentration was estimated using the human IL-33 ELISA in accordance with the guidelines of the manufacturer. The sensitivity of the assay was 1.7 pg/ml (range: 7.8–500 pg/ml). The real-time polymerase-chain-reaction (PCR) analysis was used to perform IL-*33* genotyping (TaqMan probe). The kit of TaqMan, SNP genotyping assay was from Applied Biosystems. Primer F: 5-GAAGTCATCATCAACTTGGAACC-3, and reverse, R: 5GGATTGG AATCCCA TGGTC-3. DNA amplification and purification for IL33 gene. The restriction fragment length polymorphism (RFLP) PCR mix was prepared by using the *SspI* restriction enzyme. After PCR cycles were finished, 1 µl unit of the enzyme was added to (1µl) of IL-33 PCR product with (5µl) of enzyme buffer, then incubated for 5-15 min at 37°C and 3 h at 70 V.

2.4. Statistical Analysis

The data were analyzed in SPSS software (version 20) using the t-test, and a p-value less than 0.05 was considered statistically significant.

3. Results

The comparison of IgG antibody *T. gondii* and the age of hemodialysis patients demonstrated that the age group 51-70 years recorded the highest group of patients infected with *T. gondii* with the control group (*P=0.0166*) with an odds ratio of 8.880 (Table 1).

Table 1. Distribution of anti- *Toxoplasma* IgG antibodies in patients undergoing dialysis according to age groups

Ages	*Toxoplasma* IgG						Dialysis Patients & Healthy Odds Ratio (95% CI)**	
	Patients			Healthy				
	No. Tested *(%)χ^2	No. Positive *(%)χ^2	*P-value*	NT(%)χ^2	NP (%)χ^2	*P-value*	Odds Ratio	*P-value*
10-30	15	5 (33.3)		35	1 (2.85)		8.880 (1.509-50.915)	6.133* (*0.0166*)
31-50	15	10 (66.6)	9.333**	5	0	0.888NS	15.50 (744-323.913)	3.199.NS ((*0.0785*)
51-70	25	15	(*0.0051*)	1	(0.00)	(*0.599*)	2.3868 (0.099363.7077)	0.444NS (*0.6111*)
<71	5	2 (40)		0	0		1.000 (0.0137-72-278)	0.00 NS (*1.000*)
Total	60	32	*1.623*					

NT= No. tested; NP= No. positive, 95% Confidence Interval

As illustrated in table 2, males reported more positive *T. gondii* IgG results, compared to healthy people, with a highly significant difference (*P<0.05*), while the females were not significantly different from the healthy group (*P<0.05*).

Based on table 3, the chronic Toxoplasmosis according to residency demonstrated that the urban and rural patients outnumbered the healthy people (*P<0.05*).

The number of dialysis times per week in chronic Toxoplasmosis patients in table 4 pointed to a significant difference in the number of infections among those patients. The findings were shown to be positive in dialysis twice a week (*P=0.00076*), with an odds ratio of 0.8195; nonetheless, the confidence interval was 0.016-46.23.

The IL-33 VP2 gene amplification by PCR produced a single amplified 480 bp DNA fragment, coding to a mature IL-33 protein. The DNA sequencing exhibited that cDNA was the recorded sequence. The final IL-33 construct is able to express the recombinant IL-33 protein in fusion with a hexahistidine tag and allows for metal affinity purifications.

The *IL-33* gene expression was investigated in patients who were undergoing hemodialysis, in comparison with that in healthy controls by using real-time PCR. The results suggested that there was a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration (Figure 1).

Table 2. Distribution of anti- *Toxoplasma* IgG antibodies in patients undergoing dialysis according to gender

Gender	Toxoplasma IgG						Dialysis Patients & Healthy Odds Ratio (95% CI)**	
	Patients			Healthy				
	No. Tested *(%)χ2	No. Positive *(%)χ2	P-value	NT(%)χ2	NP (%)χ2	*P-value*	Odds Ratio	*P-value*
Male	40	25 (62.5)	1.078NS	35	1 (2.85)	10.00NS (1.00)	16.44 (2.0133.1355.35)	9.89** (0.0099)
Female	20	17 (37.5)	(0.733)	25	1 (2.85)		15.50 (744-323.913)	9.95** (0.0785)
Total	60	32		60	2 (5.70)	9.66** (0.0006)	18.0091 (4.055-78.996)	10.45 (0.0001)

NT= No. tested; NP= No. positive, 95% Confidence Interval

Table 3. Distribution of anti- *Toxoplasma* IgG antibodies in patients undergoing dialysis according to residency

Residency	*Toxoplasma* IgG						Dialysis Patients & Healthy Odds Ratio (95% CI)**	
	Patients			Healthy				
	No. Tested *(%)χ^2	No. Positive *(%)χ^2	*P-value*	NT(%)χ^2	NP (%)χ^2	*P-value*	Odds Ratio	*P-value*
Urban	29	18 (62)	5.66*	30	1 (3.33)	0.777NS (*0.979*)	23.000 (2.787-184.087)	11.33** (*0.0054*)
Rural	31	14 (45.1)	(*0.0355*)	30	0 (0.00)		15.440 (1.799-121.118)	9.23** ((*0.0998*)
Total	60	32 (53.3)		60	1 (0.16)		17.898 (4.3335-79.110)	11.95** (*0.0001*)

NT= No. tested; NP= No. positive, 95% Confidence Interval

Table 4. The number of dialysis times **and** the number of infections among the patients

Time of Dialysis	*Toxoplasma* IgG			Dialysis Patients & Healthy Odds Ratio (95% CI)**
	Patients			
	NT*	NP *(%)	(*4*)	Odds Ratio
Once per week	4	1 (25%)		0.4396 (90.005.35.400)
Twice per week	16	8 (50%)	9.884** (0.00076)	0.8195 (0.016-46.23)
Three times per week	30	14 (46.6)		0.7904 (0.015-40.292)
Total	50	23 (46)		0.7771 (0.014-36.665)

NT= No. tested; NP= No. positive, 95% Confidence Interval

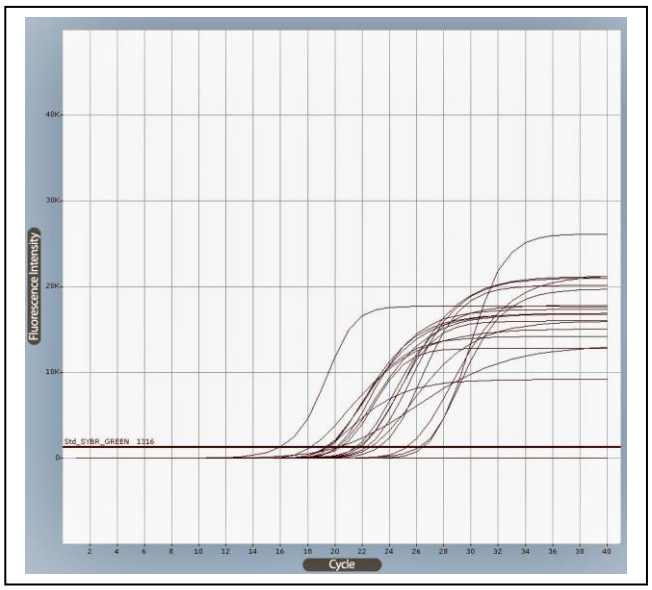

Figure 1. Expression of *IL-33* gene by using real-time with melting test analysis

4. Discussion

Chronic toxoplasmosis is a very common parasitic infection caused by *Toxoplasma gondii* with an estimated prevalence of 30%-70% among human populations across the globe. In the present study, the prevalence of *T .gondii* was assessed by measuring the IgG antibody at different ages. The results pointed out that the majority of Anti-***T***. *gondii* IgG antibodies were observed in the age group 51-70 years. This finding is in agreement with those reported by Soltani, Foroutan (8) who reported that hemodialysis patients in this age group are most affected by toxoplasmosis since they have low immunity; therefore, such diseases as toxoplasmosis can lead to mortality.

In terms of gender, males reported more positive *T. gondii* IgG results, compared to healthy people, with a highly significant difference, while the females were not significantly different from the healthy group**.** Achaw, Tesfa (9) reported that the prevalence of toxoplasmosis in male hemodialysis patients was higher than that in female ones. They attributed this finding to the fact that men are more likely to suffer from kidney failure than women (9). The chronic Toxoplasmosis according to residency illustrated that urban and rural patients outnumbered the healthy people. This result is in accordance with the finding reported by Mahdi (10) who stated that hemodialysis patients living in cities were less prone to Toxoplasmosis infection, in comparison with their counterparts who resided in rural areas.

The frequency of dialysis time per week in chronic Toxoplasmosis patients indicated that the number of infections among those patients was higher. The findings were shown to be positive in dialysis at 2 weeks. Foroutan, Rostami (11) established that *Toxoplasma gondii* infections of those undergoing hemodialysis may have an effect on the frequency of dialysis, leading to the spread of this disease by exposure to continuous dialysis. Zahoor, Abubakar (12) confirmed that the genotyping of interleukin-33 is determined by a group of VB genes with a size of 400bp-500bp. The expression of the *IL-33* gene was investigated to be higher in patients undergoing hemodialysis, compared to that in the healthy controls, by using real-time PCR. The findings pointed out that there was a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration. Interleukin-33 is one of the cytokines that gives high positive results.

There are several types of genes possessed by this cytokine, and they have a distinct gene expression through which we can determine Toxoplasma infection. For instance, this expression that was determined on the gene site in B2 in 60 samples was tested for infected people with toxoplasmosis and those undergoing hemodialysis, as well as a corresponding 60 healthy control group, revealed an increase in the levels of interleukin 33, yielding positive results at high concentrations in this diagnosis. These findings were consistent with those reported by Drosatos, Tsoporis (13) and Koca, Kara (14). The high seroprevalence of *T. gondii* dialysis patients supported the local stray cat population as a risk factor for human toxoplasmosis in İzmir. The high seroprevalence of *T. gondii* indicates that this protozoan is a risk factor for hemodialysis patients.

Authors' Contribution

Study concept and design: W. T. Y.
Acquisition of data: W. T. Y.
Analysis and interpretation of data: W. T. Y.
Drafting of the manuscript: W. T. Y.
Critical revision of the manuscript for important intellectual content: W. T. Y.
Statistical analysis: W. T. Y.
Administrative, technical, and material support: W. T. Y.

Ethics

This study was approved by the Ethics Committee of Middle Technical University, Baghdad, Iraq. A written consent form was obtained from all participants.

Conflict of Interest

The authors declare that they have no conflict of interest.

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