Toxoplasma Gondii In Association with TLR4 Among Regular Hemodialysis Patients

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KEYWORDS

Toxoplasma gondii, Hemodialysis patients, TLR4.

ABSTRACT

Background: Hemodialysis patients can be considered immunocompromised as they have an increased risk of infections.
The activity of Toll -like receptors in defense against infections was observed particularly for Toll like receptor4 molecule. Several single-nucleotide polymorphisms (SNPs) residing in genes encoding Toll -like receptors were reported as significant genetic modifications associated with infections.
Aim: To determine the association between Toll- like receptors 4 polymorphisms and the susceptibility to infection with Toxoplasma gondii in hemodialysis patients.
Materials and Methods: This case- control study was conducted on 150 patients with hemodialysis and 150 apparently healthy blood donors as control group, the samples were collected in the period from march 2021 to November 2021 from Al-Karama hospital and Al-Yarmouk Teaching Hospital. All patients were investigated for the presence of Toxoplasma gondii and Abs (IgG, IgM) by using ELISA technique and conformation by molecular technique (Real time-PCR). The selected SNPs in Toll- like receptors 4 were amplified by using conventional polymerase chain reaction PCR and then sequencing there polymorphism.
Results: In the current study the comparison of median age between patients and controls was with no significant difference, while the laboratory tests revealed significant difference in median level of urea, creatinin , calcium , potassium ,albumin and hemoglobin, and insignificant difference for body mass index and sodium between studied groups.
The ratio of females infected with Toxoplasma gondii was higher than infected males.
The seropositive result in patients for Toxoplasma gondii - IgG and IgM was 15 and 6 respectively while 19 patients were positive by PCR.
However in control group there were 8 patients seropositive for IgG and positive in PCR results while the results of IgM and PCR were negative for all control group.
The Polymerase chain reaction (PCR) products of Toll- like receptors 4 genes were subjected for direct sequencing by using Bio edit software. And the resultant sequences were compared with reference sequences in NCBI. The analysis of TLR-4 rs4986790 genotypes were 24(80%) AA and 6(20%) AG in patients while 6 (60%) AA and 4(40%) AG in controls with insignificant difference, while the analysis of TLR-4 rs4986791 genotypes were 24(80%) CC and 6 (20%) CT in patients while 6 (60%) CC and 4(40%) CT in controls with insignificant difference. Conclusion: There was no significant association of Toxoplasma gondii with TLR4 genotypes of rs4986790 and rs4986791 SNPs in hemodialysis patients.

1. Introduction

Hemodialysis is a process of purifying the blood of a person whose kidneys are not working normally. This type of dialysis achieves the extracorporeal removal of waste products such as creatinine and urea and free water from the blood when the kidneys are in a state of kidney failure. Hemodialysis is one of three renal replacement therapies, the other two being kidney transplant and peritoneal dialysis (1).Chronic hemodialysis patients are at high risk for infection because the process of hemodialysis requires vascular access for prolonged periods. In an environment where multiple patients receive dialysis concurrently, repeated opportunities exist for person to person transmission of infectious agents, directly or indirectly via contaminated devices, equipment and supplies, environmental surfaces, or hand of person. Hemodialysis patients are immunocompromised as a result of uremia and numerous comorbid conditions, disposing them to various bacterial and parasitic infections (2). Wang et al. (3) conducted a global meta-analysis to assess the prevalence and odds ratios (ORs) of T. gondii infection in immunocompromised individuals and found that the estimated pooled prevalence of T. gondii infection in immunocompromised patients was significantly higher than that in the control group and their study was demonstrate that the immunocompromised patients are associated with higher odds of T. gondii infection. Toll-like receptor (TLR)/MyD88 signaling has been reported as the key pathway in a non-specific antimicrobial response against T. gondii The glycosylphosphatidylinositol (GPI) of T. gondii was demonstrated to trigger TLR4 signaling pathways (4).

2. Methodology

This case-control study was conducted on 150 patients with hemodialysis and 150 apparently healthy blood donors as control, the samples were collected in the period from march 2021 to November 2021. The patients were recruited from Al-Karama hospital and Al-Yarmouk Teaching Hospital. Their age ≥ 18 years old. The inclusion criteria included patients on regular hemodialysis period with one year, with Age ≥ 18 year, while exclusion criteria were hemodialysis patients with other chronic illness.

A 2 ml of blood sample was collected from each patient and healthy individual and sera were obtained which were stored at -20°C. All patients were investigated for the presence of Toxoplasma gondii by molecular technique (Real time-PCR) and detection of Ab by using ELISA. T. gondii DNA was detected by qPCR targeting the 529bp repeat element. The primers (F: 5'-TCTGGCTGGTTTAGAAGTCCA-3', R: 5'- AATTGCCAGCCATTTTCAAG-3') (5) were used for TLR4 detection in present study are the TLR4 Asp299Gly (rs4986790) and Thr399Ile (rs4986791) primers that amplify the SNP region These primers are able to amplify wide spectrum of DNA of TLR4 genotypes. Polymerase chain reaction was used for molecular detection of TLR4 in blood samples. The polymerase chain reaction products of TLR4 were followed by Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email then analyzed using geneious software. A consent approval obtained from all patients and control. This study was approved by the institutional review board (IRB) of the College of Medicine -Al-Nahrain University (IRB/95-2021).

Statistical analysis

SPSS v19.0 software was used to analyze the data. Chi-square test was used to perform the bivariate analysis in order to determine the risk factors linked with the seropositivity of microorganisms. P-value ≤ 0.05 was considered statistically significant

3. Result and Discussion

In current study the median age of patients and controls were 41.35 and 42.50 years respectively with no significant difference, out of 150 hemodialysis patients, 79 (52.7%) were male and 71 (47.3%)were female ,while in control group there were 80 (53.3%) male and 70 (46.7%) were female , and in significant difference for body mass index .According Of the 150 hemodialysis patients 19 (12.7%) were positive for Toxoplasma gondii by RT-PCR , while 8(5.3) in controls , with significant difference (table-1). On the other hand there were 15(10%) of patients were positive for Toxoplasma gondii -IgG antibody by ELISA and 6 (4%) had Toxoplasma gondii -IgM antibody , while in healthy control 8(5.3%) were positive for Toxoplasma gondii IgG while IgM antibodies were negative for all samples (tables 2 and 3) . Concerning the sex there were 10(14.1%) females and 9 (11.4%) males positive for Toxoplasma gondii by PCR , while 8 (10.1%) females and 7(9.9%) males were positive for Toxoplasma gondii IgG , and 4 (5.6%) females versus 2(2.5%) males were positive for Toxoplasma gondii IgM (table -4) .The majority of hemodialysis patients seropositive for Toxoplasma gondii in the age group ≤ 30 years with insignificant difference (table-5) . The laboratories tests of patients and controls showed a significant difference between patients and controls regarding median level of urea , creatinin, calcium, potassium, albumin and heamoglobin, and not significant for sodium (table-6). Analysis of TLR-4 rs4986790 polymorphism revealed only two genotypes in both patients and controls(AA and AG) .The frequency of the heterozygous genotype (AG) were lower in patients than controls (37.5% versus 62.5%) with a insignificant difference (p=0.653). At allelic level, the frequency of mutant allele (allele G) was equal in patients and controls 50% in each them (table-7) . TLR-4 rs4986791 similar to TLR-4 rs4986790 also had only two genotypes CC and CT. However, the frequencies of these genotypes were incompatible in patients and controls without a significant difference (p=0.390) (table-8) . As well as there was no association between both SNPs (rs4986790 and rs4986791 genotypes and alleles) with seropositivity and molecular detection of Toxoplasma gondii (table 9 and 10). Pearson's correlation test revealed a significant correlation between Toxoplasma gondii positivity by PCR and both Toll-like receptors 4 SNPs [(rs4986790) P=0.013, (rs4986791) P= 0.011]. Also there was a significant correlation between

TLR-4 rs4986791 and seropositivity of Toxoplasma-IgG, P=0.011. On the other hand there was a significant correlation between both TLR-4 SNPs (P=0.027) (table-11).

Table (1) Detection of Toxoplasma gondii by (qRT-PCR)

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		Study groups	
		Patients	Control
Toxo PCR	Positive	19	8
		12.7%	5.3%
	Negative	131	142
		87.3%	94.7%
Total		150	150
		100.0%	100.0%
p value		**0.021**	
odd ratio (95%CI)		2.574	1.09-6.08

Table (2): Seroprevalence of IgG for anti toxoplasma Abs in studied groups**.**

		Study groups	
		Patients	Control
Anti-Toxoplasma IgG	Positive	15	8
		10.0%	5.3%
	Negative	135	142
		90.0%	94.7%
Total		150	150
		100.0%	100.0%
p value		**0.192** ^{NS}	

Table (3): Seroprevalence of IgM for anti-toxoplasma Abs

		Study groups	
		Patients	Control
Anti-Toxo IgM	Positive	6	0
		4.0%	0.0%
	Negative	144	150
		96.0%	100.0%
Total		150	150
		100.0%	100.0%

p value	**0.015**	

Table (4): Association of *Toxoplasma gondii* seropositivity and RT- PCR results with sex.

		Toxo PCR		**Anti-Toxo IgG**		**Anti-Toxo IgM**	
		Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Sex	Female	10	61	8	64	4	67
		14.10%	85.90%	10.10%	90.10%	5.60%	94.40%
	Male	9	70	7	71	2	77
		11.40%	88.60%	9.90%	89.90%	2.50%	97.50%
Total		19	131	15	135	6	144
		12.70%	87.30%	10.00%	90.00%	4.00%	96.00%
p value		0.621		0.587		0.291	

Table (5)**:** Association of *Toxoplasma gondii* seropositivity and PCR results with age groups

		Toxo PCR		**Anti-Toxo IgG**		**Anti-Toxo IgM**	
		Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Age groups	≤30 years	7	27	6	28	1	33
		20.60%	79.40%	17.60%	82.40%	2.90%	97.10%
	31-40 years	4	29	2	31	2	31
		12.10%	87.90%	6.10%	93.90%	6.10%	93.90%
	41-50 years	5	29	4	30	1	33
		14.70%	85.30%	11.80%	88.20%	2.90%	97.10%
	51-60 years	2	28	2	28	1	29
		6.70%	93.30%	6.70%	93.30%	3.30%	96.70%
	61-70 years	1	18	1	18	1	18
		5.30%	94.70%	5.30%	94.70%	5.30%	94.70%
Total		19	131	15	135	6	144
		12.70%	87.30%	10.00%	90.00%	4.00%	96.00%
p value		0.408		0.442		0.954	

Table (6): Laboratories tests of patients and controls

	Study groups						p value
	Patients			Control			
	Median	Percentile 05	Percentile 95	Median	Percentile 05	Percentile 95	
Urea	110.00	68.00	220.00	34.00	21.00	48.00	<0.001
Creatinine	9.15	3.50	13.70	0.7	0.5	1.30	<0.001
Ca	8.65	6.30	10.50	9.10	7.40	10.50	<0.001
Na	141.00	93.00	178.00	141.00	119.00	167.00	0.783^{NS}
K	4.40	2.10	6.30	3.90	2.60	5.30	0.016
Albumin	4.40	2.10	6.70	4.80	2.70	6.90	0.049
Hb	10.50	6.50	15.00	13.70	10.30	15.80	<0.001

Table (7): The frequency of genotypes and alleles of TLR-4 rs4986790 in study groups.

rs4986790		Study groups		P value
		Patients count	Controls count	
Genotype	AA	24	6	0.232^{NS}
		80.00%	60.00%	
	AG	6	4	
		20.00%	40.00%	
Allele	A allele	54	16	0.257^{NS}
		90.00%	80.00%	
	G allele	6	4	
		10.00%	20.00%	

Table (8): The frequency of genotypes and alleles of TLR-4 rs4986791 in study group

rs4986791		**Study groups**		**P value**
		Patients count	**Controls count**	
Genotype	CC	24	6	0.232^{NS}
		80.00%	60.00%	
	CT	6	4	
		20.00%	40.00%	

Allele	A allele	54	16	0.257^{NS}
		90.00%	80.00%	
	G allele	6	4	
		10.00%	20.00%	

Table (9): The association of genotypes and alleles of TLR-4 rs4986790 with *Toxoplasma gondii*

rs4986790	**Toxo PCR**		**Anti-Toxo IgG**		**Anti-Toxo IgM**		**Total**
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	
AA	9	15	7	17	3	21	24
	37.50%	62.50%	29.17%	70.83%	12.50%	87.50%	100.00%
AG	1	5	1	5	0	6	6
	16.67%	83.33%	16.67%	83.33%	0.00%	100.00%	100.00%
A	19	35	15	39	6	48	54
	35.19%	64.81%	27.78%	72.22%	11.11%	88.89%	100.00%
G	1	5	1	5	0	6	6
	16.67%	83.33%	16.67%	83.33%	0.00%	100.00%	100.00%
P value	0.653^{NS}		0.520		0.823		

Table (10): The association of genotypes and alleles of TLR-4 rs4986791 with *Toxoplasma gondii*.

rs4986791	**Toxo- PCR**		**Anti-Toxo IgG**		**Anti-Toxo IgM**		**Total**
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	
CC	9	15	7	17	3	21	24
	37.50%	62.50%	29.17%	70.83%	12.50%	87.50%	100.00%
CT	1	5	1	5	0	6	6
	16.67%	83.33%	16.67%	83.33%	0.00%	100.00%	100.00%
C	19	35	15	39	6	48	54
	35.19%	64.81%	27.78%	72.22%	11.11%	88.89%	100.00%
T	1	5	1	5	0	6	6
	16.67%	83.33%	16.67%	83.33%	0.00%	100.00%	100.00%
	0.653		0.870		0.990		

Table (11): Correlation between Toxoplasma gondii and TLR-4

		Toxo PCR	Anti-Toxo IgG	Anti-Toxo IgM	TLR4 rs4986790	TLR4 rs4986791
Toxo PCR	r	1.000	0.875**	0.434**	0.203**	0.341**
	p		0.000	0.000	0.013	0.011
Anti-Toxo IgG	r	0.875**	1.000	0.045		
	p	0.000		0.582	0.225**	0.011
Anti-Toxo IgM	r	0.434**	0.045	1.000	0.520	0.309**
	p	0.000	0.582			1.000
TLR4 rs4986790	r	0.203**	0.225**	0.015	0.000	
	p	0.013	0.520	0.823	0.823	0.027
TLR4 rs4986791	r	0.341**	0.309**			0.990
	p	0.011	0.027	0.990	0.155	

Disscution

This case control study revealed no significant difference in mean age between patients and controls, because the age of control group were selected according to patients group, and the majority of hemodialysis patients (53.1%) were in the age group ≤ 30 years. Most worldwide studies showed that older ages patients (≥ 50 years) were more likely to have infections by microorganisms (6).

However, some studies did not find such association (7). In contrast, a Brazilian study involving both sexes showed younger ages were more affected than older ages (8).

The most reasonable explanation for the higher involvement of older age is the greater exposure of older to the microorganisms during their lives, because these antibodies persist for a long time, and the increment of the seroprevalence with age is associated with life-time exposure. In hemodialysis patients there were 79 (52.7%) male and 71 (47.3%) female, while in control group there were 80 (53.3%) male and 70 (46.7%) female.

However the incidence of female infected with Toxoplasma gondii was higher than infected male , this study similar to many studies (9), this may be due to many factors such as women dealing with meats heavily , preparing salads, cooking and cleaning (10).In this study there are significant difference between patients and controls regarding median level of urea creatinin , calcium , potassium ,albumin and hemoglobin, and non-significant difference of Sodium .

Previous studies have found that Hb is mainly affected by the amount of fluid removed during hemodialysis (11). It was shown that most the patients on hemodialysis are suffering from chronic illness anemia. The studies suggest that lower Hb is related to insufficient intradialysis fluid removal and dilution during the HD session. The previous study has shown that lower Hb is related to age, higher serum levels of urea, creatinine and potassium. However, the serum albumin level showed an inverse correlation with Hb (12). Many patients with kidney disease and ESRD including those undergoing hemodialysis treatments suffer from protein-energy wasting (13). Protein-energy wasting and inflammation, which are hence referred to collectively as the Malnutrition-inflammation complex syndrome (MICS), are associated with poorer quality of life and higher morbidity and mortality in hemodialysis patients. There are few studies have been carried out in Iraq about the seroprevalence of toxoplasmosis in patients suffering from chronic kidney diseases under hemodialysis. In the presence study 10 % of patients were positive for anti-toxoplasma IgG, while 5.3 % of control group were

positive for anti -toxoplasma IgG antibodies, on the other hand 4 % of patients were positive for anti-toxoplasma IgM , and all control group were negative with significant difference . Abdul-Aziz and Zghair (14) studied the seroprevalence of toxoplasmosis in chronic renal failure patients in some of Baghdad hospitals he found that 32.25% of these patients were seropositive for IgG antibodies. Al-Saadawi and Alkhaled (15) investigated the prevalence of anti-T. gondii antibodies in hemodialysis patients with CRF attended delivery hospital in Al-Muthanna province (Iraq) and found that 13.04% and 1.09% of the patients were seropositive for IgG and IgM, respectively. In contrast, Al-Dulaimi et al. (16) reported that 80.9% of the patients with CRF who undergoing regular hemodialysis at Al-Kindy Hospital in Baghdad, Iraq were found seropositive for IgG while 44.1% of CRF patients who haven't any hemodialysis session showed seropositivity for the same antibody. The finding of the present study and the other previous studies conducted in Iraq indicate that the hemodialysis patients might at risk of getting toxoplasmosis and demonstrate the need for further studies to be done in other parts of Iraq. The observed differences between the results of the present study and the studies conducted in different countries may be related to various factors such as environmental and cultural differences. Furtado et al. (17) reported that the global differences regarding the prevalence of toxoplasmosis could be attributed to various factors such as country-specific environmental conditions, cultural differences regarding hygienic and feeding habits, climate, and host susceptibility. It has been found that as a parasite, T. gondii causes glomerular lesions and urinary abnormalities which lead to renal failure which can be detected by an increase in creatinine levels in the urine. Mahboub et al. (18) reported that the increase in the urea concentration in the serum of patients infected with T. gondii may be due to the serious kidney damage caused by this parasite (19). On the other hand, Eleftheriadis et al. (20) reported that CRF patients undergoing hemodialysis are prone to acquire various infections. As long as hemodialysis patients are immunocompromised and infection with T. gondii can cause serious clinical complications (21), it is possible to propose that toxoplasmosis can pave the way for CRF and vice versa and this depends on which one establishes first. This study, observed no association of TLR4 polymorphism with Toxoplasma gondii, infection. The current study showed no significant association between the AG genotype of TLR-4 rs4986790 and susceptibility to infections. The presence of G allele in The TLR-4 rs4986790 polymorphism is associated with a reduced interaction between TLR-4 and the of pathogen-associated molecular patterns of T. gondii, with eventual reduction in the signaling cascade which limit the immune response. This will lead to higher susceptibility of AG genotype carriers compared with AA carriers. How can rs4986790 SNP alter the structure and/or function of TLR4 is a question the exact answer of which is still a controversial issue. However, mutant allele can exploit one or more of three possible ways to influence TLR4 function; expression, signaling, or ligand binding. The majority of researches in this regard pointed out that expression of TLR4 is not affected by these SNPs (22). Wu, 2011 hypothesized a disruption in the interaction between mutant TLR4 and serum components such as CD14, LBP, or MD-2 which are part of the functional response of TLR4. This disruption results from conformational changes in the receptor. Henckaerts and co-workers proposed saddle-like surface of extracellular domain of mutant TLR4 with the Asp299Gly and Thr399Ile amino acids positioned at opposite ends of the saddle, and the concavity between the two amino acids suggests a possible docking site for either ligand or co receptor that may disrupt the normal function of the receptor .The current study showed a significant

correlation between TLR-4 genotypes (rs4986790 and rs4986791 SNPs) and susceptibility for toxoplasmosis, there are few studies which addressed this issue. Wujcicka et al. (2017), recruited 116 Polish pregnant women including 51 patients with toxoplasmosis and 65 age matched controls, to investigate the association of 4 polymorphisms in TLR-2, TLR-4 and TLR-9 with toxoplasmosis (13). The author did not find any significant association between TLR4 rs4986790 or rs3050791 polymorphisms and toxoplasmosis.

Some previous studies showed a dual function of TLR4 in the immune response after T. gondii infection (23). In addition to triggering TLR4 signaling, associated with activation of the immune response, the parasite was also reported to use this molecule to escape the immune responses (24). Hence, the modified TLR4 protein could be helpful for the host immune system to interrupt T. gondii

dissemination in the infected organism. Rallabhandi et al. (25) examined TLR4 mRNA and protein expression in HEK293T cell lines transiently cotransfected with wild type or mutant TLR4, MD-2 and CD14 and found no significant difference in TLR4 expression among the wild type or mutant TLR4 cell lines. While other studies detected differences in surface expression of TLR4 based on the genotype polymorphisim (26). For TLR4 rs4986790, a significant association has been found with Crohn disease and ulcerative colitis risk in Whites but not in Asians. While TLR4 rs4986791 was associated with inflammatory bowel disease susceptibility only in Caucasians (27). Ding et al revealed that the rs4986791 polymorphism decreased the risk of cancer in both Whites and Asians (28).

Other study found rs4986790 polymorphism of TLR4 gene can modulate the susceptibility towards P. vivax infection. AA genotype is found to be protective against the development of P. vivax infection in the local population of Pakistan (29).

4. Conclusion and future scope

Seroprevalence of Toxoplasma gondii in patients may consider as risk factor .There was a correlation between Toxoplasma gondii with TLR4 genotypes (rs4986790 and rs4986791 SNPs) in hemodialysis patients.

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