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ORIGINAL ARTICLE

Polymorphism of Toll-like Receptor 4 is Correlated with an Increased Risk of Mortality of Hemodialysis Patients: A Retrospective Cohort Study in Taiwanese

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Background: Hemodialysis (HD) is a public health issue, and HD-induced infection is a major risk factor for mortality. Toll-like receptor 4 (TLR4) plays a key role in the pathogenesis of infection. However, polymorphisms of TLR4 contribute to infection are still unknown. **Aim:** This study aimed to examine the correlation between polymorphisms of TLR4 and mortality among HD patients. **Methods:** This retrospective cohort study included 585 participants from Tri-Service General Hospital in Taipei from 2011 to 2015. All participants were genotyped for TLR4 rs10116253T/C and rs7873784G/C by the iPLEX Gold single-nucleotide polymorphisms assay. Analysis of the conditional Cox proportional hazard model was used to test the association between TLR4 polymorphisms and mortality. **Results:** Our results show that adjustment with age and gender, TLR4 rs10116253T/C demonstrates an association with mortality (hazard ratio [HR] =1.38, 95% confidence interval [CI] =1.00–1.89) in a dominant model, especially in infection-related mortality (HR = 2.14, 95% CI = 1.04–4.43) of HD patients. **Conclusion:** The results demonstrate that TLR4 rs10116253T/C may play a role in the etiology of infection among HD patients.

Key words: Hemodialysis, mortality, toll-like receptor 4, polymorphism

INTRODUCTION

End-stage renal disease (ESRD) prevails in Taiwan. The highest incidence and prevalence rates around the world not only burden health-care resources but also majorly impact patients and their families.[1] ESRD is associated with immune activation, marked by systemic inflammation, and immune deficiency, leading to a higher risk of mortality compared to healthy controls.[2]

Toll-like receptor 4 (TLR4) is responsible for the immediate response to Gram-negative bacteria and is recruited to initiate a signaling cascade leading to the production of pro–inflammatory cytokines.[3] Studies have proved the stimulation of lipopolysaccharide (LPS) induces the expression of TLR4, suggesting a role of TLR4 in infection.[4] Polymorphisms of TLR4 play a role in the concentration of cytokines in the body, affecting the over-activation of the immune system or immune deficiency, and resulting in systemic inflammation and higher mortality in hemodialysis (HD) patients.[5-7]

However, studies on the correlation between TLR polymorphisms and ESRD are scarce, among which there are two studies about TLR9 and the others is about TLR3.[6,8,9] For example, polymorphisms of TLR9 are associated with a higher risk of developing chronic kidney disease (CKD).[8,9] Plus, polymorphisms of TLR3-7C/A are also associated with susceptibility to ESRD.[6] A recent study by Dessing *et al*. found that the genetic polymorphism of the TLR family is associated with primary kidney disease. The paper mentioned that the specific TLR transmission pathway may be involved in the process of kidney injury.[5] However, whether the polymorphisms of TLR4 are associated with ESRD is unknown. Since the

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mortality is a major issue in ESRD care, the association between polymorphisms of TLR4 and mortality is needed to elucidate.

MATERIALS AND METHODS

Ethical approval

Participants were recruited from the Tri-Service General Hospital (TSGH) in Taipei, Taiwan. The project was reviewed and approved by the Institutional Ethical Committee of the TSGH (TSGH-1-104-05-006). All participants enrolled in the study provided written informed consent.

Study design

This study design was based on a retrospective cohort study. The participants were recruited from TSGH. All cases were undergoing dialysis and were diagnosed by nephrologists from 2011 to 2015. The exclusion criteria were as follows: (1) a dialysis period <3 months; (2) cancer diagnosis; and (3) insufficient blood samples. Finally, a total of 585 participants were included in the study analysis. Demographic data contained age, gender, education level, diabetes, history of hypertension, duration of dialysis, and biochemistry examination were collected from questionnaires and medical records. The results of death records were from the death registration files of 2011–2015.

Genomic DNA extraction and genotyping

Five mL of intravenous blood samples were collected from each participant. Genomic DNA from peripheral blood samples was processed using standard procedures for proteinase K (Invitrogen, Carlsbad, CA, USA) digestion and the phenol/chloroform method. *TLR4* (rs10116253T/C and rs7873784G/C) was genotyped by iPLEX Gold single nucleotide polymorphisms (SNP) genotyping. Genotyping was conducted based on blind conditions. At least 10% of the samples were randomly chosen for repeated genotyping to assess the results, and the concordance rate was 99%.

Statistical analysis

Continuous variables were displayed as means ± standard deviation and evaluated using Student's *t*-test. Genotypes and allelic frequencies were compared between patients with dialysis and healthy controls using the Chi-square test or

Table 1: Basic demographics and results of biochemistry examination of participants

	Mean±SD			*P*
	All patients (*n*=585)	Survival (*n*=400)	Deceased (*n*=185)	
Demographics				
Male (%)	49.2	47.5	53.0	0.218
Age (years)	64.79±14.69	61.42±14.52	72.04±12.26	<0.001*
Survival days	1050.34±408.66	1200.42±324.51	725.84±383.21	<0.001*
Dialysis period (years)	6.14±5.60	6.48±5.94	5.44±4.75	0.040*
Primary cause of dialysis (%)				
Glomerular nephritis	28.2	34.8	14.1	<0.001*
Hypertensive nephropathy	13.0	12.2	14.6	
Diabetic nephropathy	42.4	35.5	57.3	
Others	16.4	17.5	14.1	
Biochemistry examination				
Cholesterol (mg/dL)	157.64±39.12	161.82±38.62	148.59±38.77	<0.001*
Triacylglycerol (mg/dL)	141.09±90.86	148.90±99.89	124.20±64.40	0.002*
Calcium (mg/dL)	9.85±0.91	9.94±0.92	9.66±0.84	0.001*
Phosphorous (mg/dL)	4.88±2.86	5.01±3.18	4.61±1.97	0.115
Calcium-phosphate product	48.05±29.73	49.81±33.51	44.25±18.67	0.035*
Albumin (g/dL)	3.76±0.49	3.88±0.43	3.49±0.50	<0.001*
Parathyroid hormone (pg/dL)	366.42±481.03	387.66±489.66	320.99±460.08	0.124
Hemoglobin (g/dL)	10.26±2.03	10.36±1.53	9.99±2.97	0.063
Sodium (mEq/L)	137.62±6.25	137.75±7.08	137.33±3.90	0.440
Potassium (mEq/L)	4.72±0.84	4.74±0.79	4.67±0.94	0.339

*P<0.05. SD=Standard deviation

Fisher's exact test if appropriate. The Cox proportional hazard model was adopted to compute the hazard ratio (HR) and 95% confidence interval (CI) for the risk of mortality. Allele type, genotype, and dominant/recessive model were used to judge the risk between genetic polymorphism and mortality. This study set a $P < 0.05$ as significant for all analyses. Statistical analyses were executed using SPSS version 28.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of participants

Basic demographics and results of the biochemistry examination of participants were shown in Table 1. This study retrospectively analyzed 585 HD patients, 49.3% were males, the mean age was 64.79 ± 14.69 years, and the dialysis period was 6.14 ± 5.60 years. Diabetic nephropathy (42.3%) was the main cause of HD. After the follow-up of 5 years, 400 patients survive and 185 died. Those HD patients who survive were significantly younger and had higher values of cholesterol, triacylglycerol, calcium, calcium-phosphate product, and albumin than those in the deceased. In addition, basic demographics and results of biochemistry examination in patients with differential death causes were shown in Table 2. There were three main death causes, 47.6% cardiovascular disease (CVD), 22.7% infection-related, and 29.7% others. The group of infection-related causes had higher age than the other two groups. Neither significance was observed among three groups.

Association with toll-like receptor 4 gene polymorphisms and all-cause mortality

The relationships between TLR4 gene polymorphisms in HD patients and the risk of all-cause mortality are shown in Table 3. With adjustment of age and gender, TLR4 rs10116253T/C demonstrated risky association with all-cause mortality in CC genotype (HR = 1.70, 95% CI: 1.11–2.62), C allele (HR = 1.30, 95% CI: 1.05–1.61), dominant model (HR = 1.38, 95% CI = 1.00–1.89), and recessive model (HR = 1.46, 95% CI = 1.00–2.013). However, TLR4 rs7873784G/C had no association with all-cause mortality in HD patients.

Table 2: Basic demographics and results of biochemistry examination in patients with differential death causes

	Mean±SD			*P*
	CVD (*n*=88)	Infection (*n*=42)	Others (*n*=55)	
Demographics				
Male (%)	53.4	42.9	60.0	0.244
Age (years)	71.66±12.61	75.98±10.29	69.65±12.55	0.038*
Survival days	717.68±369.43	789.98±433.00	689.93±365.39	0.430
Dialysis period (years)	5.94±4.63	4.49±3.51	5.35±5.64	0.268
Primary cause of dialysis (%)				
Glomerular nephritis	11.4	11.9	20.0	0.785
Hypertensive nephropathy	15.9	16.7	10.9	
Diabetic nephropathy	56.8	59.5	56.4	
Others	15.9	11.9	12.7	
Biochemistry examination				
Cholesterol (mg/dL)	146.92±36.76	152.62±43.93	148.16±38.22	0.735
Triacylglycerol (mg/dL)	118.87±54.14	134.21±62.28	124.96±79.48	0.448
Calcium (mg/dL)	9.58±0.67	9.75±0.96	9.73±0.98	0.435
Phosphorous (mg/dL)	4.61±1.84	4.45±1.87	4.71±2.26	0.814
Calcium-phosphate product	44.09±17.55	42.76±17.56	45.64±21.30	0.751
Albumin (g/dL)	3.49±0.48	3.37±0.55	3.59±0.48	0.105
Parathyroid hormone (pg/dL)	315.74±471.57	292.17±400.06	350.60±489.42	0.820
Hemoglobin (g/dL)	10.40±4.46	9.80±1.27	9.69±1.45	0.425
Sodium (mEq/L)	137.20±4.40	137.77±3.82	137.18±3.04	0.708
Potassium (mEq/L)	4.70±0.98	4.42±0.91	4.81±0.89	0.123

*P<0.05. SD=Standard deviation; CVD=Cardiovascular disease

Table 3: Association with toll-like receptor 4 gene polymorphisms and all-cause mortality

TLR-4	Deceased	Survival	Crude-HR (95% CI)	*P*	Adjusted-HR (95% CI)[#]	*P*
rs10116253						
Genotype						
TT	57	152	Reference		Reference	
TC	95	197	1.26 (0.90–1.75)	0.172	1.29 (0.93–1.80)	0.133
CC	33	51	1.67 (1.09–2.56)	0.020*	1.70 (1.11–2.62)	0.016*
Allele model						
T allele	209	501	Reference		Reference	
C allele	161	299	1.29 (1.04–1.59)	0.020*	1.30 (1.05–1.61)	0.015*
Dominant model						
TT	57	152	Reference		Reference	
TC+CC	128	248	1.34 (0.98–1.84)	0.065	1.38 (1.00–1.89)	0.047*
Recessive model						
TT+TC	152	349	Reference		Reference	
CC	33	51	1.45 (1.00–2.12)	0.052	1.46 (1.00–2.13)	0.049*
rs7873784						
Genotype						
GG	144	320	Reference		Reference	
GC	41	74	1.25 (0.89–1.77)	0.202	1.19 (0.84–1.69)	0.318
CC	0	6	0.00 (0.00–Infinitive)	0.993	0.00 (0.00–Infinitive)	0.993
Allele model						
G allele	329	714	Reference		Reference	
C allele	41	86	1.06 (0.77–1.46)	0.710	1.03 (0.75–1.43)	0.844
Dominant model						
GG	144	320	Reference		Reference	
GC+CC	41	80	1.17 (0.82–1.65)	0.383	1.12 (0.79–1.59)	0.525
Recessive model						
GG+GC	185	394	Reference		Reference	
CC	0	6	0.00 (0.00–Infinitive)	0.993	0.00 (0.00–Infinitive)	0.993

*P<0.05; [#]Adjust for gender and age. TLR-4=Toll-like receptor 4; CI=Confidence interval; HR=Hazard ratio

Association with toll-like receptor 4 gene polymorphisms and cardiovascular disease -related mortality

The relationships between TLR4 gene polymorphisms in HD patients and the risk of CVD-related mortality are shown in Table 4. Both TLR4 rs10116253T/C and TLR4 rs7873784G/C had no association with CVD-related mortality in HD patients.

Association with toll-like receptor 4 gene polymorphisms and infection-related mortality

The relationships between TLR4 gene polymorphisms in HD patients and the risk of infection-related mortality are shown in Table 5. With adjustment of age and gender, TLR4 rs10116253T/C demonstrated a risky association with infection-related mortality in C allele (HR = 1.59, 95% CI: 1.01–2.49) and dominant model (HR = 2.14, 95% CI = 1.04–4.43).

Association with toll-like receptor 4 gene polymorphisms and others mortality

The relationships between TLR4 gene polymorphisms in HD patients and the risk of other mortality are shown in Table 6. Both TLR4 rs10116253T/C and TLR4 rs7873784G/C had no association with other mortality in HD patients.

DISCUSSION

Our results show that adjustment with age and gender, HD patients' polymorphism of TLR4 rs10116253T/C with the C allele demonstrates a 1.38-fold (95% CI = 1.00–1.89) increased risk of all-cause mortality, especially 2.14-fold (95% CI = 1.04–4.43) increased risk of infection-related mortality.

In this study, the risk factors for survival in HD patients were

Table 4: Association with toll-like receptor 4 gene polymorphisms and cardiovascular disease-related mortality

TLR-4	Deceased	Survival	Crude-HR (95% CI)	*P*	Adjusted-HR (95% CI)[#]	*P*
rs10116253						
Genotype						
TT	31	152	Reference		Reference	
TC	40	197	0.97 (0.60–1.55)	0.885	0.98 (0.61–1.57)	0.928
CC	17	51	1.56 (0.86–2.83)	0.140	1.59 (0.88–2.87)	0.128
Allele model						
T allele	102	501	Reference		Reference	
C allele	74	299	1.20 (0.88–1.63)	0.247	1.21 (0.89–1.65)	0.223
Dominant model						
TT	31	152	Reference		Reference	
TC+CC	57	248	1.09 (0.70–1.69)	0.701	1.11 (0.71–1.72)	0.652
Recessive model						
TT+TC	71	349	Reference		Reference	
CC	17	51	1.59 (0.94–2.71)	0.085	1.61 (0.94–2.73)	0.080
rs7873784						
Genotype						
GG	71	320	Reference		Reference	
GC	17	74	1.05 (0.62–1.79)	0.849	1.00 (0.59–1.70)	0.994
CC	0	6	0.00 (0.00–Infinitive)	0.995	0.00 (0.00–Infinitive)	0.995
Allele model						
G allele	159	714	Reference		Reference	
C allele	17	86	0.91 (0.56–1.50)	0.724	0.89 (0.54–1.46)	0.638
Dominant model						
GG	71	320	Reference		Reference	
GC+CC	17	80	0.98 (0.58–1.66)	0.942	0.94 (0.55–1.60)	0.820
Recessive model						
GG+GC	88	394	Reference		Reference	
CC	0	6	0.00 (0.00–Infinitive)	0.995	0.00 (0.00–Infinitive)	0.995

*$P<0.05$; [#]Adjust for gender and age. TLR-4=Toll-like receptor 4; CI=Confidence interval; HR=Hazard ratio

investigated. In the univariate analysis, it was found that the risk of death increased with age, as aging leads to a gradual decline in physical and physiological functions, resulting in an increased risk of death. This is consistent with other literature studies that have identified old-old age as one of the factors contributing to mortality in HD patients, especially subjects with death cause of infection.[10,11] This could be due to the aging process leading to immunosenescence that escalates morbidity and mortality due to infections, such as disturbance of regulation of pattern recognition receptor (PRR), a case in point, TLR.[12]

TLRs have been reported as one of the vital classes of PRRs, engaged in the host defense system against bacteria, viruses, fungi, and protozoa.[13] One of the well-documented TLRs is TLR4, the key receptor for the LPS element of Gram-negative bacteria.[14-16] Polymorphisms of TLRs are associated with infectious diseases such as Gram-negative infections.[17-20] Besides, TLR4 plays a vital role in the inflammatory response of the kidney disease.[21] It was found that TLR4 promotes renal fibrosis and the progression of CKD in mouse experiments, and regulates inflammatory factors involved in the immune system activation of renal tubular epithelial cells and inflammatory responses in diabetic nephropathy.[22,23]

TLR4 gene polymorphism rs10116253 may be associated with a decreased risk of gastric cancer and hepatocellular carcinoma.[24,25] However, there is little known on the association of these two SNPs with the survival of HD patients. We found that the rs10116253 C allele was positively associated with increasing risk of death in HD patients. The reason may be that TLR4 is one of the vital PPRs engaged in the recognition of Gram-negative bacteria LPS and is initiated

Table 5: Association with toll-like receptor 4 gene polymorphisms and infection-related mortality

TLR-4	Deceased	Survival	Crude-HR (95% CI)	*P*	Adjusted-HR (95% CI)[#]	*P*
rs10116253						
Genotype						
TT	10	152	Reference		Reference	
TC	25	197	1.93 (0.93–4.03)	0.079	2.08 (0.98–4.41)	0.056
CC	7	51	2.09 (0.79–5.50)	0.136	2.40 (0.90–6.39)	0.081
Allele model						
T allele	45	501	Reference		Reference	
C allele	39	299	1.48 (0.95–2.31)	0.080	1.59 (1.01–2.49)	0.045*
Dominant model						
TT	10	152	Reference		Reference	
TC+CC	32	248	1.96 (0.96–4.00)	0.063	2.14 (1.04–4.43)	0.040*
Recessive model						
TT+TC	35	349	Reference		Reference	
CC	7	51	1.37 (0.61–3.08)	0.453	1.50 (0.66–3.40)	0.334
rs7873784						
Genotype						
GG	32	320	Reference		Reference	
GC	10	74	1.38 (0.68–2.80)	0.376	1.37 (0.67–2.82)	0.384
CC	0	6	0.00 (0.00–Infinitive)	0.997	0.00 (0.00–Infinitive)	0.997
Allele model						
G allele	74	714	Reference		Reference	
C allele	10	86	1.15 (0.60–2.19)	0.681	1.16 (0.60–2.25)	0.650
Dominant model						
GG	32	320	Reference		Reference	
GC+CC	10	80	1.28 (0.63–2.60)	0.496	1.29 (0.63–2.63)	0.489
Recessive model						
GG+GC	42	394	Reference		Reference	
CC	0	6	0.00 (0.00–Infinitive)	0.997	0.00 (0.00–Infinitive)	0.997

*P<0.05; [#]Adjust for gender and age. TLR-4=Toll-like receptor 4; CI=Confidence interval; HR=Hazard ratio

to induce the secretion of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α.[26] Genetic variation, such as SNPs, may dominate its activity, and function.[27] Therefore, it is speculated that the rs10116253 C allele may increase the susceptibility of TLR4 to pathogens, thereby promoting the activity of nuclear factor κB, regulating downstream cytokines, and increasing the pro-inflammatory responses, leading to an increased risk of death in HD patients.[28-30] With the advancements in precision medicine, genomic factors that exhibit strong associations with diseases have emerged as valuable biomarkers for predicting disease risk and enabling early diagnosis. Hence, the results of the present study may contribute to the establishment of precision medicine or prevention for CKD.

Our study has two limitations. First, limitation is that our patients with unknown causes of death may be misclassified which could potentially impact the results. Second, the variables used in this study lack other biochemical values that could reflect the infection status, such as C-reactive protein and IL-6. Therefore, it is recommended that functional analysis is needed to validate the role of rs10116253 C allele in the pro-inflammatory responses.

CONCLUSION

The results demonstrate that TLR4 rs10116253T/C may play a role in the etiology of infection among HD patients. It could provide a glimpse into the role of TLR4 in HD patients to identify individuals at risk.

Data availability statement

The data that support the findings of this study are available

Table 6: Association with toll-like receptor 4 gene polymorphisms and others mortality

TLR-4	Deceased	Survival	Crude-HR (95% CI)	*P*	Adjusted-HR (95% CI)[#]	*P*
rs10116253						
Genotype						
TT	16	152	Reference		Reference	
TC	30	197	1.34 (0.73–2.47)	0.346	1.27 (0.69–2.35)	0.438
CC	9	51	1.73 (0.76–3.93)	0.189	1.66 (0.73–3.78)	0.225
Allele model						
T allele	62	501	Reference		Reference	
C allele	48	299	0.69 (0.34–1.42)	0.312	0.70 (0.34–1.43)	0.328
Dominant model						
TT	16	152	Reference		Reference	
TC+CC	39	248	1.42 (0.79–2.54)	0.243	1.35 (0.75–2.43)	0.317
Recessive model						
TT+TC	46	349	Reference		Reference	
CC	9	51	1.45 (0.71–2.97)	0.312	1.43 (0.70–2.94)	0.328
rs7873784						
Genotype						
GG	41	320	Reference		Reference	
GC	14	74	1.60 (0.87–2.96)	0.131	1.51 (0.82–2.79)	0.190
CC	0	6	0.00 (0.00–Infinitive)	0.996	0.00 (0.00–Infinitive)	0.996
Allele model						
G allele	96	714	Reference		Reference	
C allele	14	86	9103611.62 (0.00–Infinitive)	0.996	8613161.30 (0.00–Infinitive)	0.996
Dominant model						
GG	41	320	Reference		Reference	
GC+CC	14	80	1.48 (0.80–2.74)	0.208	1.41 (0.76–2.60)	0.276
Recessive model						
GG+GC	55	394	Reference		Reference	
CC	0	6	0.00 (0.00–Infinitive)	0.996	0.00 (0.00–Infinitive)	0.996

*$P<0.05$; [#]Adjust for gender and age. TLR-4=Toll-like receptor 4; CI=Confidence interval; HR=Hazard ratio

from the corresponding author, Sui-Lung Su, upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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