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The prognostic value of oxidative stress and inflammation in Chinese hemodialysis patients

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ABSTRACT

Background: There is limited information about oxidative stress and inflammation on the mortality of Chinese hemodialysis (HD) patients.

Subjects and methods: A total of 177 HD patients and 35 healthy controls were enrolled. Their demographic information, clinical characteristics, oxidant and inflammation markers were compared. Multivariate Cox regression analysis was used to assess the risk factors for mortality.

Results: Twenty-seven (15.3%) HD patients died during the one-year follow-up. The mean age, age $\geq$70 years, serum level of cardiac troponin T (cTnT), malondialdehyde (MDA) $>$ 5 nmol/L, as well as CRP $>$10 mg/L and the level of interleukin (IL)-6 were significantly different between the nonsurvival and survival HD patients. Multivariate Cox's regression analysis identified age, age $\geq$70 years, cTnT, and IL-6 were independent predictors of mortality in HD patients.

Conclusions: Age, age $\geq$70 years, cTnT, and IL-6 were independent predictors of mortality in Chinese HD patients. Elevated IL-6 level, instead of MDA, was predictive of poor outcome in Chinese hemodialysis patients.

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KEYWORDS
End-stage renal disease; hemodialysis; oxidative stress; inflammation; mortality

Background

Despite continued improvement in dialysis technology and treatment, the mortality in HD patients remains unacceptably serious.[1] Numerous risk factors for this extravagant rate have been identified, including cardiovascular disease (CVD) and diabetes mellitus.[2] Our previous studies have suggested that oxidative stress was an important effector of tubule cell injury and podocyte injury.[3,4] Nonetheless, the role of oxidative stress in HD patients is often disparaged in clinical practice. Previous studies indicated that oxidative stress with chronic inflammation might be major risk factors for mortality in this population.[5] However, there is limited information about oxidative stress and inflammation on the mortality of Chinese HD patients.[6]

The aim of this prospective cohort study was to characterize the inflammation and oxidative stress in prevalent Chinese HD patients, and to correlate these findings with clinical outcomes in this population in terms of all-cause mortality. To address these issues, a total of 212 Chinese subjects (177 hemodialysis patients and 35 healthy controls) were recruited into this study.

Subjects and methods

Subjects

This prospective study was conducted between October 2014 and September 2015. The study protocol was approved by the Ethics Committee of Shanghai Tenth People's Hospital and performed in accordance with the ethical principles of the Declaration of Helsinki. Details of the study protocol were explained to all participants, and written informed consents were obtained before the study. Participants information, including age, gender, and history of diabetes was well recorded.

A total of 177 HD patients attending Shanghai Tenth People's Hospital were enrolled. To be eligible for the study, all patients had to be older than 18 years of age and had to have received thrice-weekly HD for at least three months. Patients with infection, cancer, AIDS and autoimmune disease, as well as smokers and patients using shunt or central catheters as blood access for HD, were excluded.

The patients studied were compared to 35 age- and sex-matched healthy controls, who were without any disease and not on any medication.

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Biochemical investigation

Blood samples were drawn from each subject between 8 and 11 AM, after overnight fasting and before the dialysis session (for HD patients) using a syringe containing ethylenediaminetetraacetic acid (EDTA, 1.0 mg/mL) as the anticoagulant. Plasma was separated and stored at −80 °C until subsequent batch assay.

Levels of cTnT were determined using commercially available Roche Cardiac T^{TM} immunoassay kit (Roche, Basel, Switzerland). MDA was evaluated using a lipid peroxidation colorimetric assay kit purchased from Oxis International (Beverly Hills, CA). The superoxide dismutase (SOD) activity was identified with the Superoxide Dismutase Assay kit (Cayman Chemical Company, Ann Arbor, MI). Interleukin (IL)-6 plasma levels were established by enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, Wiesbaden, Germany). Other blood chemistry was measured with an automatic biochemical analyzer (Roche, Mannheim, Germany).

Statistical analysis

Descriptive statistics for continuous variables were expressed as mean ± SD (standard deviation). Categorical variables were expressed as proportions (percentage). Binomial variables were compared between groups using χ^2 or Fisher's exact tests, and continuous variables were analyzed by the Student's *t*-test. A *p* values of <.05 was considered significant.

The independence of the association with hemodialysis patients was assessed using a logistic regression procedure. In this procedure, variables with a significant bivariate relationship (as defined by a *p* values of ≤.05) to the outcome were evaluated for inclusion in the model. For the assessment of independent predictors of survival, a multivariate Cox regression with forward stepwise likelihood ratio was performed. Statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL).

Results

General characteristics of hemodialysis patients

This study was performed on 177 HD patients and 35 controls. Detailed baseline clinical characteristics of all subjects are presented in Table 1. Mean age of HD patients and control groups were 62.40 ± 14.05 and 64.09 ± 9.59 years ($p = .497$), respectively. Mean dialysis vintage for HD patients was 35.79 ± 33.70 months.

Diabetes mellitus was observed in 29.38% of HD patients. Accordingly, HD patients had an increased glucose level than healthy controls (8.16 ± 3.90 vs. 4.56 ± 0.56, $p = .000$). Compared with healthy controls, the levels of serum creatinine, uric acid, phosphate, Ca × P, albumin <35 g/L, triglyceride (TG), and cardiac

Table 1. Demographic and clinical characteristics of the study population.

		Hemodialysis patients			
Parameters	Control group ($n = 35$)	Survival ($n = 150$)	Nonsurvival ($n = 27$)	Total ($n = 177$)	*p* Values (between groups)
Male	21 (60.00)	92 (61.33)	20 (74.07)	112 (63.28)	.423
Age (years)	64.09 ± 9.59	60.84 ± 14.12[a]	71.04 ± 10.05[a,b]	62.40 ± 14.05	.001
≥70 years	9 (25.71)	41 (27.33)	15 (55.56)[a,b]	56 (31.64)	.011
Dialysis months	NA	35.53 ± 34.92	37.22 ± 26.28	35.79 ± 33.70	NA
Diabetes mellitus	0 (0.00)	41 (27.33)	11 (40.74)	52 (29.38)	.000
Glu (mmol/L)	4.56 ± 0.56	7.73 ± 3.26[a]	10.53 ± 5.93[a]	8.16 ± 3.90[a]	.000
SCr (mg/dL)	0.65 ± 0.11	11.26 ± 3.40[a]	9.75 ± 3.15[a]	11.03 ± 3.40[a]	.000
UA (mg/dL)	3.69 ± 0.88	7.72 ± 2.11[a]	7.53 ± 2.03[a]	7.69 ± 2.10[a]	.000
WBC ($\times 10^3$ cells/μL)	6.59 ± 1.28	6.55 ± 2.13	6.40 ± 1.92	6.53 ± 2.09	.923
Hb (g/dL)	13.31 ± 1.07	10.89 ± 1.99[a]	10.49 ± 1.48[a]	10.82 ± 1.92[a]	.000
PLT ($\times 10^4$ cells/μL)	25.57 ± 4.63	17.49 ± 6.11[a]	20.13 ± 15.05	17.89 ± 8.14[a]	.000
Ca (mg/dL)	8.49 ± 0.81	9.40 ± 6.16	9.61 ± 1.31[a]	9.43 ± 5.69	.611
P (mg/dL)	4.05 ± 1.28	7.39 ± 2.44[a]	7.55 ± 1.79[a]	7.42 ± 2.35[a]	.000
Ca × P > 55 mg^2/dL^2	0 (0.00)	107 (71.33)[a]	20 (74.07)[a]	127 (71.75)[a]	.000
Alb (g/L)	46.17 ± 2.11	39.25 ± 4.87[a]	38.00 ± 3.43[a]	39.06 ± 4.69[a]	.000
<35 g/L	0 (0.00)	15 (10.00)	4 (14.81)[a]	19 (10.73)[a]	.092
Chol (mmol/L)	4.49 ± 0.78	3.91 ± 1.45[a]	4.25 ± 1.05	3.96 ± 1.40[a]	.048
TG (mmol/L)	1.00 ± 0.54	1.62 ± 1.42[a]	1.79 ± 1.33[a]	1.65 ± 1.40[a]	.025
HDL (mmol/L)	1.40 ± 0.46	0.87 ± 0.38[a]	0.94 ± 0.32[a]	0.88 ± 0.37[a]	.000
LDL (mmol/L)	2.66 ± 0.72	2.30 ± 1.04[a]	2.40 ± 0.81	2.31 ± 1.00	.138
GPT (U/L)	22.23 ± 11.96	11.99 ± 10.19[a]	10.19 ± 5.87[a]	11.72 ± 9.66[a]	.000
cTnT ($\times 10^{-3}$ ug/L)	5.92 ± 2.87	35.07 ± 9.23[a]	109.42 ± 31.29[a,b]	46.41 ± 30.59[a]	.000

Data are expressed as *n* (%) or means ± SD.
NA: not applicable.
[a]$p <$ .05 versus control group.
[b]$p <$ .05 versus survival.
WBC: leucocytes count; Hb: hemoglobin; PLT: platelet count; SCr: serum creatinine; BUN: blood urea nitrogen; UA: uric acid; Alb: albumin; Chol: cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; Ca: calcium; P: phosphate; GPT: glutamic pyruvic transaminase; γGT: γ glutamyl transferase; cTnT: cardiac troponin T.

troponin T (cTnT) were much higher in HD patients. Inversely, the levels of hemoglobin, platelet count, albumin, cholesterol (Chol), high-density lipoprotein (HDL), and glutamic pyruvic transaminase (GPT) decreased significantly in HD patients.

Characteristics of nonsurvivors undergoing hemodialysis

Altogether, 27 (15.3%) HD patients died during the one-year follow-up (Table 1). Compared with survivors of HD patients, the mean age was older in the nonsurvivors (71.04 ± 10.05 vs. 60.84 ± 14.12, $p = .000$). More nonsurvival HD patients were ≥70 years than survivors (55.56% vs. 27.33%, $p = .004$). The serum level of cTnT was elevated significantly in non-survivors than in survivors (109.42 ± 31.29 vs. 35.07 ± 9.23, $p = .000$). Additional parameters listed in Table 1 showed no significant differences between nonsurvivors and survivors.

Oxidative stress and inflammation in HD patients

Compared with healthy controls, both MDA and SOD levels were found to be significantly increased in HD patients (Table 2). Though the mean MDA and SOD levels were not significantly different between HD survivors and HD nonsurvivors, the MDA >5 nmol/L was more commonly observed in no-survivors than in survivors (33.33% vs. 15.33%, $p = .025$).

As shown in Table 2, the levels of CRP, CRP >10 mg/L, and IL-6 were increased notably in HD patients than in health controls. Moreover, the prevalence of CRP >10 mg/L was more commonly recorded in nonsurvivors than in survivors (55.56% vs. 24.67%, $p = .001$). The level of IL-6 was significantly higher in nonsurvivors than in survivors (5.22 ± 1.42 vs. 4.24 ± 0.98, $p = .005$).

Risk factors for mortality in HD patients

As shown in Table 3, multivariate Cox's regression analysis identified age (OR, 1.187; 95%CI: 1.081, 1.303; $p = .000$), age ≥70 year s(OR, 13.670; 95%CI: 1.783, 104.8; $p = .012$), cTnT (OR, 1.046; 95%CI: 1.034, 1.058; $p = .000$), and IL-6 (OR, 1.495; 95%CI: 1.025, 2.180; $p = .037$) were independent predictors of mortality in HD patients. However, MDA >5 nmol/ml (OR, 1.401; 95%CI: 0.593, 3.308; $p = .442$) and CRP >10 mg/L (OR, 1.254; 95%CI: 0.487, 3.231; $p = .639$) were not independent predictors in the multivariate models.

Discussion

The mortality in HD patients is much higher than in the general population.[1,7] In the present study, 15.3% of HD patients died during the one-year follow-up. Several risk

Table 2. Oxidant and inflammation markers of the study population.

		Hemodialysis patients			
Parameters	Control group ($n = 35$)	Survival ($n = 150$)	Nonsurvival ($n = 27$)	Total ($n = 177$)	p Values (between groups)
MDA (nmol/mL)	3.46 ± 0.96	4.24 ± 1.19[a]	4.43 ± 1.11[a]	4.27 ± 1.18[a]	.001
>5 nmol/mL	2 (5.71)	23 (15.33)	9 (33.33)[a,b]	32 (18.08)[a]	.012
SOD (U/mL)	59.29 ± 12.83	119.97 ± 32.01[a]	129.86 ± 30.90[a]	121.48 ± 31.95[a]	.000
CRP (mg/L)	3.33 ± 2.23	9.66 ± 17.59[a]	14.83 ± 4.61[a]	10.42 ± 17.22[a]	.016
>10 mg/L	0 (0.00)	37 (24.67)[a]	15 (55.56)[a,b]	52 (29.38)[a]	.000
IL-6 (pg/mL)	1.35 ± 0.25	4.24 ± 0.98[a]	5.22 ± 1.42[a,b]	4.39 ± 1.11[a]	.000

Data are expressed as n (%) or means ± SD.
NA: not applicable.
[a]$p < .05$ versus control group.
[b]$p < .05$ versus survival.
MDA: malondialdehyde; CRP: C reactive protein; SOD: superoxide dismutase; IL-6: interleukin-6.

Table 3. Multivariate cox regression analysis of risk factors for mortality in HD patients.

							95.0% CI for Exp(B)	
Variables	B	SE	Wald	df	p Values	OR	Lower	Upper
Age	0.171	0.048	12.905	1	.000	1.187	1.081	1.303
Age ≥70 years	2.615	1.039	6.333	1	.012	13.670	1.783	104.800
cTnT	0.045	0.006	61.589	1	.000	1.046	1.034	1.058
MDA >5 nmol/mL	0.337	0.438	0.591	1	.442	1.401	0.593	3.308
CRP >10 mg/L	0.226	0.483	0.220	1	.639	1.254	0.487	3.231
IL-6	0.402	0.193	4.350	1	.037	1.495	1.025	2.180

cTnT: cardiac troponin T; MDA: malondialdehyde; CRP: C-reactive protein; IL-6: interleukin-6.

factors for this high rate have been identified, including comorbid conditions such as diabetes mellitus and cardiovascular disease, malnutrition and inadequate dialysis.[8–10] To the best of our knowledge, there are limited researches about oxidative stress and inflammation on the mortality of Chinese HD patients.[6] To address these issues, we compared the clinical and biochemical variables of nonsurvival HD patients with those of survivors. Finally, multivariate Cox's regression analysis showed that age, age ≥70 years, cTnT, and IL-6 were independent predictors of mortality in HD patients.

Consistent with previous reports, our data showed that more death occurred in the elderly HD patients (especially whose age ≥70 years).[11] Atherosclerosis and vascular calcification are major problems in patients on chronic hemodialysis.[12] Cardiac, infectious, and withdrawals were the most common causes of death in the elder HD patients.[13] HD patient with the increased cTnT level was at increased risk of all-cause mortality.[14] In our study, the mean level of cTnT was significantly elevated in the nonsurvival HD patients.

More risk factors, including oxidative stress and inflammation, were associated with decreased survival among HD patients.[5,15–17] Oxidative stress is excessively activated in many pathological conditions, and it also occurs as an important part of host defense mechanisms.[18] In our study, it is worth noting that the mean levels of MDA and SOD were significantly observed in the HD patients than the healthy controls. Raised SOD might be a compensatory adaptive response in a state of increased oxidative stress.[19,20] Though HD patients with MDA >5 nmol/ml was more frequently observed in the nonsurvivors than in the survivors, the mean levels of MDA and SOD were not significantly different between the two group.

Increased systemic inflammation is well established as a nontraditional key player in the progression of chronic kidney disease (CKD), and atherogenic vascular complications.[4,15] In the present study, the elevated inflammation markers, CRP and IL-6, were observed in HD patients. Moreover, IL-6 were independent predictors of mortality in HD patients. Increased inflammation in HD patients is probably due to exposure to endotoxin and other pollutants, biocompatible dialysis solution, and dialysis membrane.[21,22]

In the present study, no significant difference was observed between the HD survivors and the non-survivors in terms of anemia, calcium and phosphate metabolism, dyslipidemia, diabetes mellitus, hypoalbuminemia, vintage of hemodialysis, or liver function. The mortality difference may also be a result of differences in patient characteristics, region, arteriovenous fistulae, physician contact, or facility characteristics.[7]

Actually, a number of risk factors were reported to be associated with the mortality in HD patients.[23,24] In our study, the following factors were not studied, such as the smoking habits, blood pressure, body mass index, parathyroid hormone, and so on. In addition, multicenter studies with long-term follow-up periods are needed to deepen the knowledge of the inflammation and oxidative stress in prevalent Chinese HD patients.

In summary, we have shown here that age, age ≥70 years, cTnT, and IL-6 were independent predictors of mortality in Chinese HD patients. Elevated IL-6 level, instead of MDA, was predictive of poor outcome in Chinese HD patients.

Compliance with ethical standards

Conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Ethical approval

All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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