Original Paper

Investigation of the Oxidative Stress and DIO1 Expression in CRF Patients Accompanied With and Without Euthyroid Sick Syndrome

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Key Words

Oxidative stress • Chronic renal failure • Euthyroid sick syndrome • DIO1

Abstract

Background/Aims: Chronic renal failure (CRF) is often accompanied by increased oxidative stress and euthyroid sick syndrome (ESS). The cause of ESS is unknown, and it is unknown whether there exists a link between oxidant stress and ESS in CRF patients. Therefore, we aim to investigate oxidative stress and type 1 deiodinase (DIO1) expression, which plays the key role in the ESS in CRF patients. **Methods:** In-patients with CRF were divided into the two group: Group 1 is ESS patients consisting of 60 patients with low free triiodothyronine (FT3) and Group 2 consisting of 60 patients with normal FT3. Group 3 consisted of 60 healthy volunteers recruited as controls. The baseline clinical parameters of patients were evaluated with standard routine methods in a clinical laboratory. Serum levels of 8-isoprostane and DIO1 were measured by enzyme-linked immunosorbent assay (ELISA). Multiple regression analysis was used to analyze the relationship between oxidative stress, DIO1 and FT3. **Results:** The concentrations of serum 8-Isoprostane in Group 1 and Group 2 were substantially higher than that of Group 3 ($p < 0.05$), however there was no significant difference between Group 1 and Group 2 ($p = 0.516$). The serum DIO1 level was higher in Group 2 than in Group 1 and Group 3 ($p < 0.001$). Multivariate linear regression analysis revealed that the DIO1 concentration and FT3 level were not associated with the concentration of serum 8-Isoprostane. **Conclusions:** CRF patients showed elevated oxidative stress. The CRF patients without ESS showed higher expression of DIO1 than patients with ESS and the control group. The concentration of serum 8-Isoprostane was not correlated with FT3 and DIO1 levels.

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Introduction

Renal failure is characterized by the loss of kidney function resulting in the accumulation of metabolites in blood. Chronic renal failure (CRF), specifically, is a severe clinical problem that has significant socioeconomic impact worldwide [1-4]. Numerous studies have demonstrated that impaired renal function is closely associated with the elevation of inflammatory markers leading to both increased markers of oxidative stress and decreased antioxidant defense [5-7]. In addition, CRF is often accompanied by thyroid function disorders [8, 9]. Despite the absence of thyroid disease, CRF patients with nonthyroidal illness (NTI) frequently show changes in serum thyroid hormones concentration, characterized by low FT3 levels and normal FT4 and TSH levels. This is also referred to as “euthyroid sick syndrome” (ESS) [10, 11]. However, there is less research that discusses the reasons for which ESS occurs. Some studies have reported that type I deiodinase (DIO1) is a key deiodinase responsible for catalyzing the production of FT3 from T4 and inhibits the production of rT3 [12]. Until now, no studies have investigated the expression of DIO1 in CRF patients with and without ESS.

In order to investigate the oxidative stress and DIO1 expression of CRF patients with or without ESS, we measured the concentrations of 8-isoprostane, an oxidative stress marker, and DIO1 expression by ELISA. We then analyzed the relationship of oxidative stress, DIO1 and FT3 by multiple regression analysis.

Materials and Methods

Patients

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval was obtained from the Institutional Ethics Committee of Third Affiliated Hospital, Nanchang University, China, before the commencement of the study (ref: 15/NC/0243). Informed consent for participation was obtained from all participants including the healthy volunteers and patients. In-patients at the Second Affiliated Hospital of Nanchang University with CRF were recruited from October 2015 to October 2016. The exclusion criteria were as follows: (1) Patients with tuberculosis or other systemic diseases, (2) patients taking glucocorticoids or immunosuppressant, (3) patients with injuries, clinical symptoms or abnormal function of the heart, lung, and liver. We divided the patients into two groups: Group 1 (CRF with ESS) consisted of 60 patients with low FT3 levels (<2.3pg/ml); Group 2 (CRF without ESS) consisted of 60 patients with normal FT3 levels (2.3-4.2 pg/ml). Group 3 (control group) consisted of 60 healthy volunteers (25 men, 35 women; mean age: 70.0 ± 6.8 years) and were recruited through a routine community medical examination.

Evaluation of demographic and clinical parameters

Prior to the measurement of clinical parameters, date of birth/age and sex of the participant was recorded. Heart rate, respiration, blood pressure, and body mass index (BMI) were measured and recorded. The concentration of serum lipids, albumin, creatinine, fasting blood-glucose, uric acid (UA), white blood cell, hemoglobin, FT3, free thyroxine (FT4) and thyroid stimulating hormone (TSH) were measured by standard routine methods in a clinical laboratory and recorded.

Evaluation of 8-isoprostane and DIO1 with ELISA

The concentration of serum 8-isoprostane (Cayman Chemical, USA) and DIO1 (USCN, USA) was measured with an ELISA kit according to manufacturer instructions. The serum was collected and added into each well of the micro-titer plate and incubated for 2h. Each well was washed with wash buffer and incubated with 100 µl of enzyme-linked polyclonal antibodies for 2h. Following washes, substrate solution was added to each well. After incubation for 30 minutes at room temperature, the enzyme reaction was

stopped, and the intensity of each well was measured at 450 nm. The mean value of 2 assays was used for statistical analysis.

Statistical Analysis

Variables were given as mean $\pm$ standard deviation unless otherwise indicated. The t-test was used. Multivariate linear regression analysis was employed to determine the predictive value of quantitative parameters. P values less than 0.05 were viewed as significant. All data analysis was performed using SPSS 17.0 (Microsoft USA).

Results

Baseline information of patients

The main demographic, somatometric, clinical, and biochemical characteristics of patients included in the study are detailed in Table 1. There was no obvious difference in age and sex between Group 1 and Group 2. Because of the “rolling” method of recruitment of Group 3, there was an unequal distribution of sex and age between the patients and control group. There was no significant difference between the three groups with regards to the parameters of basic vital signs, the concentrations of serum white blood cell number, FT4, and TSH (Table 1).

Concentration of 8-Isoprostane in Groups 1, 2, and 3

The concentrations of serum 8-Isoprostane in Groups 1 were substantially higher in Group 1 and 2 than in Group 3 (Group 1 vs Group 3: 17.0 ± 8.05 pg/ml vs 4.41 ± 2.47 pg/ml, $p < 0.01$; Group 2 vs Group 3: 15.22 ± 8.4 pg/ml vs 4.41 ± 2.47 pg/ml, $p < 0.05$). Although the expression of 8-Isoprostane in Group 1 was higher than that in Group 2, there was no significant difference between the two groups (17.0 ± 8.05 pg/ml vs 15.22 ± 8.4 pg/ml, $p = 0.516$), as shown in Fig. 1.

Concentration of DIO1 in Groups 1, 2, and 3

In our study, we found that the concentration of DIO1 in the Group 2 was higher than in the Group 1 (2.34 ± 0.18 ng/ml vs 1.37 ± 0.089 ng/ml, $p < 0.001$) and Group 3 (2.34 ± 0.18 ng/ml vs 1.27 ± 0.25 ng/ml, $p < 0.001$), as shown in Fig. 2. However, there was no obvious difference in the concentration of DIO1 between Group 1 and Group 3 (1.37 ± 0.089 ng/ml vs 1.27 ± 0.25 ng/ml, $p = 0.976$).

Table 1. Demographic and laboratory characteristics of the three groups. [Group 1: CRF patients with low free triiodothyronine; Group 2: CRF patients with normal triiodothyronine; Group 3: control group]

Variable	Group 1	Group 2	Group 3	p for Trend (Group1 vs Group3)
Age(yr)	62 $\pm$ 12.38	61 $\pm$ 11.95	70 $\pm$ 6.08	<0.001
Male gender (n %)	66.7	71.7	41.7	0.002
Systolic pressure (mmHg)	145.95 $\pm$ 22.52	143.38 $\pm$ 17.92	141.53 $\pm$ 17.56	0.461
Diastolic pressure (mmHg)	78.63 $\pm$ 12.41	80.7 $\pm$ 11.56	77.03 $\pm$ 10.58	0.221
Heart rate (beats/min)	79.43 $\pm$ 8.45	80.45 $\pm$ 7.38	79.31 $\pm$ 10.07	0.736
Respiratory (beats/min)	19.35 $\pm$ 0.75	19.33 $\pm$ 0.79	19.11 $\pm$ 1.18	0.311
Weight (Kg)	62.45 $\pm$ 8.49	65.5 $\pm$ 9.3	52.72 $\pm$ 10.37	<0.001
Height (meter)	165.56 $\pm$ 6.55	167.52 $\pm$ 6.9	157.85 $\pm$ 7.1	<0.001
BMI(kg/m ²)	22.74 $\pm$ 1.95	23.27 $\pm$ 2.09	23.05 $\pm$ 3.18	0.498
Cholesterol (mmol/L)	3.87 $\pm$ 1.3	4.12 $\pm$ 1.09	4.67 $\pm$ 0.99	0.001
Hemoglobin (g/L)	77.76 $\pm$ 20.16	87.41 $\pm$ 18.36	131.88 $\pm$ 9.8	<0.001
Albumin (g/L)	32.29 $\pm$ 4.5	34.92 $\pm$ 4.27	46.8 $\pm$ 3.39	<0.001
Fasting blood-glucose(mmol/L)	5.8 $\pm$ 2.43	5.65 $\pm$ 1.61	4.87 $\pm$ 1.06	0.01
Creatinine (mmol/L)	657.84 $\pm$ 221.31	643.79 $\pm$ 161.46	80.79 $\pm$ 10.5	<0.001
White blood cell (10 ⁹ /L)	6.56 $\pm$ 2.97	6.4 $\pm$ 2.42	6.37 $\pm$ 1.12	0.877
Urea nitrogen (mmol/L)	19.03 $\pm$ 7.89	18.92 $\pm$ 7.13	5.56 $\pm$ 1.25	<0.001
UA (μ mol/L)	443.18 $\pm$ 151.92	452.16 $\pm$ 146.3	288.63 $\pm$ 69.51	<0.001
FT3 (pg/ml)	1.81 $\pm$ 0.36	2.56 $\pm$ 0.58	3.4 $\pm$ 0.45	<0.001
FT4 (ng/dl)	1.04 $\pm$ 0.27	1.13 $\pm$ 0.25	1.12 $\pm$ 0.16	0.085
TSH (μ IU/L)	3.16 $\pm$ 1.99	3.82 $\pm$ 1.54	2.27 $\pm$ 0.98	0.202

Fig. 1. Serum 8-Isoprostane concentration in the three groups. The expressions of 8-Isoprostane in Group 1 and Group 2 were significantly higher than that in Group 3 (Group 1 vs Group 3: 17.0 ± 8.05 pg/ml vs 4.41 ± 2.47 pg/ml, $p < 0.01$; Group 2 vs Group 3: 15.22 ± 8.4 pg/ml vs 4.41 ± 2.47 pg/ml, $p < 0.05$). Although the expression of 8-Isoprostane in Group 1 was higher than that in Group 2, there was no significant difference (17.0 ± 8.05 pg/ml vs 15.22 ± 8.4 pg/ml, $p = 0.516$). [Group 1: CRF patients with low free triiodothyronine; Group 2: CRF patients with normal triiodothyronine; Group 3: control group]

Fig. 2. Serum DIO1 concentration in the three groups. The expression of DIO1 in Group 2 was significantly higher than those in Group 1 (2.03 ± 0.16 ng/ml vs 1.31 ± 0.08 ng/ml, $p < 0.001$) and Group 3 (2.03 ± 0.16 ng/ml vs 1.27 ± 0.24 ng/ml, $p < 0.001$). Although the expression of DIO1 in Group 1 was higher than that in Group 3, there was no significant difference (1.31 ± 0.08 ng/ml vs 1.27 ± 0.24 ng/ml, $p = 0.976$). [Group 1: CRF patients with low free triiodothyronine; Group 2: CRF patients with normal triiodothyronine; Group 3: control group]

Table 2. Multivariate linear regression analysis of factors associated with the concentration of serum 8-Isoprostane. Dependent variable: 8-Isoprostane, $R^2 = 0.410$

Model	Unstandardized coefficients		Standardized coefficients		t	Sig.
	B	Std. Error	Beta			
Constant	27.027	15.899			1.7	0.091
Age	-0.011	0.052	-0.016		-0.208	0.835
BMI	-0.416	0.228	-0.117		-1.827	0.07
Systolic pressure	0.03	0.035	0.067		0.86	0.391
Diastolic pressure	0.114	0.062	0.152		1.839	0.068
Height	-0.089	0.064	-0.088		-1.4	0.163
Respiratory	0.025	0.529	0.003		0.047	0.962
Cholesterol	-0.439	0.497	-0.061		-0.883	0.379
Hemoglobin	-0.025	0.032	-0.082		-0.781	0.436
Albumin	-0.203	0.128	-0.174		-1.586	0.115
Fasting blood-glucose	0.025	0.016	0.112		1.545	0.124
Creatinine	0.003	0.004	0.115		0.889	0.376
White blood cell	0.01	0.253	0.003		0.039	0.969
Urea nitrogen	0.003	0.005	0.049		0.573	0.568
UA	0.012	0.117	0.012		0.099	0.921
TSH	0.026	0.052	0.035		0.497	0.62
FT3	-2.121	1.256	-0.194		-1.689	0.093
FT4	2.869	2.731	0.077		1.051	0.295
DIO1	-0.448	0.495	-0.07		-0.905	0.367

Regression analysis of multiple factor on 8-Isoprostane

In multivariate linear regression analysis, The DIO1 concentration ($R = -0.448$, $p = 0.367$) and FT3 level ($R = -2.121$, $p = 0.093$) were not associated with the concentration of serum 8-Isoprostane (Table 2).

Discussion

Chronic kidney disease (CKD) affects 10–15% of the people in the US, Europe, and Asia [13] and is characterized by reduced glomerular filtration rate or kidney damage. Oxidative stress is viewed as a critical pathogenic factor in the initiation, development, and progression of most renal diseases [14–16].

Thyroid dysfunction occurs commonly in CRF patients [17, 18]. Whether the thyroid hormone abnormalities found in ESS patients are a physiologic adaptation or a pathologic change is a topic that is still debated. Until now, there has been little research that discusses the relationship of oxidative stress, plasma FT3 levels, and DIO1 level in CRF patients.

In this study, we measured the plasma levels of 8-Isoprostane in the CRF patients with or without ESS. 8-Isoprostane is chemically stable, specific for lipid peroxidation, and has been suggested to be a marker of oxidative activities and oxidative stress [19–21]. We found that the concentration of serum 8-Isoprostane in the CRF patients was significantly higher than that of the control group. Our results were consistent with the results reported by Modaresi et al. which showed that progression of CKD to advanced stages was associated with a significant increase in the generation of reactive oxygen species (ROS) [22]. Tiryaki et al. also determined the level of 8-isoprostane in the patients with different stages of chronic kidney disease (CKD) and showed that the level of 8-isoprostane significantly increased through the progress of CKD [23]. However, we found that there was no significant difference in the serum levels of 8-Isoprostane between the CRF patients with ESS and without ESS, which indicated that ESS was not correlated with the oxidative stress.

Our research was the first to measure the serum levels of DIO1 in CRF patients with and without ESS. In humans, peripheral thyroid hormone metabolism is mediated by the three D iodothyronine deiodinases, which were divided into type I deiodinase (DIO1), type II deiodinase (DIO2), and type III deiodinase (DIO3) [24, 25]. Both DIO1 and DIO2 catalyze T_4 conversion to T_3 through 5' deiodination, whereas DIO3 inactivates thyroid hormone (TH) through 5 deiodination, converting T_4 to rT_3 and T_3 to 3, 3'-diiodothyronine [26]. DIO1 is predominantly expressed in the liver, kidney, and thyroid and contributes to the plasma T_3 in euthyroid. On the other hand, DIO2 generates T_3 mostly for local use at sites such as the brain, pituitary, and brown adipose tissue which is the key enzyme that maintains brain TH homeostasis during hypothyroidism and participates in the feedback system at the hypothalamo-pituitary-thyroid (HPT) axis. In our study [27, 28], we found that the concentration of DIO1 in CRF patients without ESS was higher than in CRF patients with ESS and healthy subjects. According to the above result, we can conject that the reason why CRF patients without ESS can maintain normal concentration of FT3 is due to the increased serum DIO1 which can elevate serum FT3 production from T_4 [29]. Further research is necessary to investigate the mechanism which triggers DIO1 expression in CRF patients without ESS and study whether the thyroid hormone abnormalities found in ESS patients are the pathologic change in CRF patients.

By multivariate linear regression analysis, we found that the DIO1 concentration and FT3 level were not associated with the concentration of serum 8-Isoprostane, which indicated that thyroid function in CRF patients was not correlated with oxidative stress, and oxidative stress was not the reason for the elevated expression of DIO1.

Conclusion

This study shows that 8-Isoprostane levels increased progressively in CRF patients and that CRF patients without ESS showed higher expression of DIO1 than patients with ESS and the healthy control group, whereas the concentration of serum 8-Isoprostane was not correlated with the FT3 and DIO1 levels.

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Disclosure Statement

The authors declare that there are no conflicts of interest regarding the publication of this article.

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