Original Paper

Medical Principles and Practice

Med Princ Pract 2004;13:84–87
DOI: 10.1159/000075634

Received: July 23, 2002
Accepted after revision: April 20, 2003

Oxidative Stress in Patients with Chronic Renal Failure: Effects of Hemodialysis

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Key Words
Chronic renal failure · Hemodialysis · Oxidative stress

Abstract
Objective: To investigate blood oxidative status of patients with chronic renal failure (CRF) and possible effects of hemodialysis on the development of oxidative stress in blood. ***Materials and Methods:*** The levels of malondialdehyde (MDA) and oxidation resistance (OR) values were measured in blood plasma, erythrocyte hemolysate and erythrocyte membrane fractions of 33 patients with CRF and of 12 healthy controls. Of the 33 patients, 17 subjects were under hemodialysis treatment. ***Results:*** MDA levels were found to be increased in all blood fractions of the patients. OR values were unchanged in erythrocyte hemolysates but decreased in plasma and erythrocyte membrane fractions of the CRF patients. Moreover, erythrocyte MDA levels were determined to be higher in hemodialyzed patients compared with both controls and non-hemodialyzed patients. OR values were lower in all blood fractions of the hemodialyzed patients relative to controls and non-hemodialyzed patients. ***Conclusion:*** Results suggest that there is a significant oxidative stress (expressed as peroxidation) in blood samples from patients with CRF, which is further exacerbated by hemodialysis.

Introduction

Cardiovascular disease is the major cause of mortality and morbidity in patients with chronic renal failure (CRF) [1] and anemia is a common feature of CRF, the severity of which is directly related to the extent of renal insufficiency [2]. Recent evidence suggests that oxidative stress increases in patients with CRF [3, 4]. It has been reported that the enzymatic antioxidant system is impaired in erythrocytes from patients with CRF [5], leading to oxidative stress. This may play a role in the development of some complications of CRF [6, 7]. It has been reported that oxidative damage due to free radical production is increased in uremic patients which might be a possible factor contributing to anemia and atherosclerosis [8]. Another study showed that protein oxidation increases in erythrocyte membranes of hemodialyzed patients [9].

The present study aimed to investigate blood oxidative status of patients with CRF and to elucidate possible effects of hemodialysis on the development of oxidative stress in the blood.

Subjects and Methods

Fasting blood samples were obtained from 33 volunteer patients with CRF (21 male, 12 female) and 12 healthy controls (7 male, 5 female). Seventeen of the patients were under regular hemodialysis for 1–4 years (mean ± SD: 2.6 ± 1.2). Hemodialysis was indicated

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Table 1. Mean ± SD values of MDA levels and OR values in plasma, erythrocyte hemolysate and erythrocyte membrane fractions of controls and patients

Groups	n	E-MDA nmol/ml	E-OR ml·h/μmol	P-MDA nmol/ml	P-OR ml·h/nmol	M-MDA nmol/mg	M-OR mg·h/nmol
I	12	328.9 ± 30.4	4.90 ± 0.36	1.15 ± 0.72	0.31 ± 0.10	1.79 ± 0.43	1.18 ± 0.30
II	16	367.9 ± 29.9	4.98 ± 1.42	1.68 ± 0.59	0.26 ± 0.06	2.43 ± 0.60	0.81 ± 0.38
III	17	412.8 ± 22.2	2.78 ± 0.37	1.66 ± 0.58	0.21 ± 0.04	2.36 ± 0.47	0.48 ± 0.10
Statistical evaluations							
I–II		$p < 0.005$	n.s.	$p < 0.025$	$p < 0.05$	$p < 0.005$	$p < 0.05$
I–III		$p < 0.0005$	$p < 0.0005$	$p < 0.025$	$p < 0.0005$	$p < 0.005$	$p < 0.0005$
II–III		$p < 0.0005$	$p < 0.0005$	n.s.	$p < 0.01$	n.s.	$p < 0.0005$

I = Control group; II = CRF group; III = CRF with hemodialysis group.
E = Erythrocyte hemolysate; P = plasma; M = erythrocyte membrane fraction.
n.s. = Not significant ($p > 0.05$).

in this group with end-stage CRF according to the initial pH and creatinine clearance values, which were significantly different from the other 16 who were managed without hemodialysis. Mean ± SD pH values were 7.1 ± 0.03 and 7.32 ± 0.05 ($p < 0.0005$), creatinine clearance values were 49.66 ± 5.09 and 67.36 ± 14.01 ($p < 0.0005$) in patients with and without hemodialysis indications, respectively.

The ages of the patients ranged from 38 to 70 years (mean ± SD: 51.4 ± 8.5) and those of the controls from 35 to 56 years (mean ± SD: 47.8 ± 5.6). The duration of complaints from renal failure ranged from 1 to 10 years (mean ± SD: 4.6 ± 2.7). Patients with infections, diabetes mellitus and vasculitis were not included in the study.

Blood samples were collected in tubes containing anticoagulant. Plasma, erythrocyte and membrane fractions were prepared from the blood as described previously [10]. Malondialdehyde (MDA) level, taken as an expression of oxidative stress, was measured as described previously [11], using spectrophotometric absorbance measurements of the pink-colored product of the thiobarbituric acid-MDA complex. MDA levels were expressed as nmol MDA/ml erythrocyte sediment, nmol MDA/ml plasma and nmol MDA/mg membrane protein. Oxidation resistance (OR) was established by measuring MDA levels before and after incubation with copper sulfate ($CuSO_4$,1 m*M*) as an oxidant for 1 h [12]. OR values were expressed as 1/μmol/h·ml sediment, 1/nmol/h·ml plasma and 1/nmol/h·mg membrane protein, with increased OR values demonstrating decreased oxidation sensitivity and vice versa. Protein was measured by Lowry's method [13].

Statistical analyses were performed by using Student's t test and Mann-Whitney U test. This study was performed in accordance with the ethical standards of the Helsinki Declaration.

Table 2. Correlation coefficient values of plasma, erythrocyte hemolysate and erythrocyte membrane fraction parameters of the groups

	CRF group	CRF with hemodialysis
Plasma		
MDA/OR	–0.5	–0.6
MDA/BUN	0.8	0.4
MDA/CRE	n.c.	n.c.
OR/BUN	–0.9	–0.6
OR/CRE	–0.6	n.c.
Erythrocyte hemolysate		
MDA/OR	–0.4	–0.8
MDA/BUN	n.c.	n.c.
MDA/CRE	n.c.	n.c.
OR/BUN	0.4	–0.4
OR/CRE	n.c.	n.c.
Erythrocyte membrane fraction		
MDA/OR	–0.8	–0.7
MDA/BUN	n.c.	–0.4
MDA/CRE	n.c.	–0.4
OR/BUN	n.c.	n.c.
OR/CRE	n.c.	n.c.

n.c. = No correlation.

Results

MDA levels were higher in all blood fractions of the patients compared to controls (table 1). Erythrocyte MDA levels were higher in hemodialyzed patients than in non-hemodialyzed patients, and OR values were found to be lower in all blood fractions of hemodialyzed patients than in non-hemodialyzed patients. Plasma creatinine and blood urea nitrogen (BUN) levels of the hemodialyzed group were 5.27 ± 0.58 and 59.8 ± 9.0 mg/dl, respectively. The plasma creatinine and BUN levels of the non-hemodialyzed CRF patients were 5.98 ± 0.57 and 58.1 ±

9.2 mg/dl, respectively. BUN levels did not exhibit a statistically significant difference between these groups while plasma creatinine levels were different ($p < 0.005$).

In the correlation analysis, negative correlations were established between MDA and OR values of the patients (table 2). The correlation analysis between renal function tests and MDA or OR values also yielded some significant relationships, especially between plasma MDA/BUN, plasma OR/BUN and plasma OR/creatinine values.

Discussion

The increased oxidative stress expressed as peroxidation (increased MDA levels) and the reduced OR capacity in the erythrocytes from patients with CRF may be due to several factors. Among these factors are uremia [8], damaged endogenous antioxidant systems [6, 14–16], reduced concentrations of some antioxidant substances such as vitamin C and glutathione [7, 17, 18]. Further potential sources of oxidative stress in dialysis patients are activation of leukocytes [19] and iron overload [20]. It has been established that hemodialysis treatment produces increased lipid peroxidation in blood samples [21] and it has been suggested that reactive oxygen species may be involved in a broad pattern of tissue injury in patients on regular hemodialysis treatment [14]. Ross et al. [17] reported that hemodialyzed patients are at increased risk from oxidative stress because of glutathione deficiency in whole blood and erythrocytes. Chen et al. [15] reported abnormalities of trace elements and a significant decrease in enzymatic antioxidant capacity in hemodialyzed patients. However, Jackson et al. [7] suggested that although the total antioxidant system is increased in hemodialyzed patients, depletion of some antioxidants leads to accelerated atherogenesis.

Our results are in agreement with these reports, which were generally carried out in one of the blood fractions. Increased MDA levels and decreased OR values in plasma and erythrocyte fractions might be indicators of oxidative stress accompanied by a poor defense in CRF patients. OR values were significantly decreased in patients undergoing hemodialysis compared to the non-hemodialyzed group while MDA levels were significantly increased only in the erythrocyte hemolysate. Therefore, it seems that hemodialysis impairs the oxidative resistance capacity more than exacerbating oxidative stress. Negative correlation established between MDA levels and OR values in the blood fractions also demonstrate that oxidative stress, which causes significant peroxidation in some cellular components, leads to impairment in the OR capacity in the blood samples of CRF patients. This is further supported by the strong positive correlation found between plasma MDA/BUN and negative correlations between plasma OR/BUN and plasma OR/creatinine in patients with CRF. These are all indicators of decreased renal functions accompanied by an increase in oxidative stress (expressed as peroxidation) and decrease in OR. Our results show that impaired renal function is accompanied with increased oxidation sensitivity and accelerated peroxidation reactions.

Conclusion

Our results suggest that there is significant oxidative stress and impaired OR capacity in blood samples of CRF patients, the latter being further exacerbated by hemodialysis. Therefore, it is likely that antioxidant therapy may be beneficial for these patients to cope with the oxidative stress and to prevent peroxidation reactions and thus improve the patient's health overall.

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