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CLINICAL STUDY

The association of oxidant–antioxidant status in patients with chronic renal failure

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Abstract

Oxidative stress has been linked to disease progression, including chronic renal failure (CRF). The aim of the present study was to determine malondialdehyde (MDA) as a sign of lipid peroxidation, and to investigate the association between antioxidant activities and three trace elements, in 49 patients with CRF. The erythrocyte and plasma trace elements [selenium (Se), zinc (Zn), and copper (Cu)] and antioxidant defense levels were determined: glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), vitamins E and C. The obtained values were compared with 42 age- and sex-matched healthy controls. There were significantly lower mean values of plasma Se, GPx, vitamins E and C, erythrocyte Se, SOD and CAT levels in the patient group compared to the control group ($p < 0.001$). Plasma MDA showed a significant increase in all CRF patients in comparison with controls. No significant difference was found in plasma Cu, Zn, and erythrocyte GPx, Cu and Zn levels between patient and control groups. These findings indicate oxidative stress is present in patients of CRF, and may serve to establish a simple protocol for evaluation of renal function.

Keywords

Antioxidants, oxidative stress, trace elements, lipid peroxidation, chronic renal failure

History

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Introduction

Chronic renal failure (CRF) is defined as progressive and irreversible loss of renal function over a period of months or years. Failure occurs through different stages characterized by an abnormally low and deteriorating glomerular filtration rate (GFR). An estimated GFR <60 mL/min/1.73 m^2 is diagnostic of CRF.[1,2] CRF is a common and serious problem that negatively affects human health, decreases life expectancy and increases healthcare costs. The increasing incidence cannot be fully explained by traditional risk factors. Therefore, nontraditional factors, such as oxidative stress, should be taken into account.[3,4]

Over the last decade, several experimental studies suggested that oxidative stress is common in CRF patients, and is a key process in the development of complications of chronic renal disease.[5,6] Oxidative stress is defined by the imbalance of free radicals and antioxidants. The potential consequence of increased radical production and/or decreased antioxidant levels can result in chemical alterations of biomolecules causing various structural and functional modifications in the plasma of CRF patients.[7–9]

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The degree of oxidative stress can be influenced by alterations in the levels of certain essential trace elements such as zinc (Zn), copper (Cu) and selenium (Se).[10] These trace elements are cofactors or structural components of antioxidant enzymes that catalyze the breakdown of free radicals.[11] Owing to their short-half lives and reactive nature, free radicals are difficult to quantify. Consequently, indirect methods of measuring products of lipid peroxidation such as malondialdehyde (MDA) concentration, and antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and nonenzymatic chain breaking antioxidants like vitamin E and vitamin C are used.[12,13] MDA, is a short-chain aldehyde that can be used as an indirect measure of polyunsaturated fatty acids oxidation.[8] SOD catalyzes the dismutation of superoxide free radicals into hydrogen peroxide. Hydrogen peroxide, in turn, is converted to water and molecular oxygen by CAT or GPx, which uses glutathione as a substrate.[14] The nonenzymatic antioxidant components activate oxygen species preventing the propagation of free radical chain reactions,[15] as well as protecting tissue from any oxidative damage.[16]

The present study was undertaken to examine MDA levels and determine the status of the essential trace elements Zn, Cu and Se in CRF patients. In addition, the relationship between antioxidant defense levels (in both erythrocyte and plasma) and individual trace element levels were investigated.

Materials and methods

Study subjects

Forty-nine patients with CRF were recruited into the study, none of which were taking lipid-lowering drugs or antioxidant supplements. The diagnosis of CRF was based on history, biochemical parameters and demonstration of small kidneys on ultrasound. All patients were treated at the Nephrology Department of Al Kadhmiya Teaching Hospital, Al-Nahrain University. A control group comprised 42 healthy volunteers. The study purpose was explained to each participant and investigation carried out following written consent.

Materials

NaCl (BDH Laboratory Supplies, Dorset, England), thiobarbituric acid (Merck KGaA, Darmstadt, Germany), trichloroacetic acid (Sigma, St. Louis, MO), sulfuric acid (BDH Laboratory Supplies, Dorset, England), sodium sulfate solution (BDH Laboratory Supplies, Dorset, UK), NADH (reduced adenine dinucleotide) (Roche, Boehringer-Mannheim, Germany), $MnCl_2 \cdot H_2O$ (BDH Laboratory Supplies, Dorset, UK), EDTA (ethylenedinitrilotetraacetic acid) (Gibco/BRL Life Technologies, Grand Island, NY), 2-mercaptoethanol (Merck KGaA, Darmstadt, Germany), NADPH (adenine dinucleotide phosphate) and tert-butylhydroperoxide solution obtained from Sigma (St. Louis, MO).

Demographic data

Demographic data were collected from each participant and included age, gender, height and weight. Body mass index (BMI) was calculated as weight (kg)/height^2 (m).

Blood sampling

Blood sample was collected in the morning after overnight fasting. Plastic disposable syringes were used for biochemical experiments. Venous blood was collected in plain vacutainer tubes containing lithium heparin BD (Becton, Dickinson and Company, Franklin Lakes, NJ) for hematology analysis. Plasma was immediately separated from blood cells by centrifugation at 1000 *g* for 15 min at 4 °C (Sorvall® 4K15 centrifuge); the resulting plasma samples were stored at −70 °C until usage. Erythrocyte pellets were washed three times in 5 mL of sterile 9 g/L NaCl solution and stored at −80 °C until use.

Biochemical analyses

GPx activities of erythrocyte and plasma were assayed by the coupled method of Paglia and Valentine,[17] with *t*-butylhydroperoxide as the substrate.[9] CAT activity was determined by the method described by Aebi,[18] which involved the detoxification of hydrogen peroxide (H_2O_2). CAT enzyme activity was determined by using the peroxidatic function of CAT at 540 nm with an assay kit from Merck Millipore Chemical, Bedford, MA (cat. no. 219265). The activity of SOD was based on the inhibition of NADH by generation of superoxide radical ions, as described previously.[19] One unit of SOD is defined as the amount of enzyme able to inhibit oxidation by 50%. Enzyme activity was expressed in units (U) per g Hb or per L of plasma. Zn and Cu levels were determined using Shimadzu model AA-670 Flame Atomic Absorption Spectrophotometer. Flameless atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) was used to determine Se level. Vitamin E was measured in plasma with an assay kit from Cayman Chemical (Ann Arbor, MI) (cat. no. 10010621). The absorbance of samples was measured between 405 and 420 nm by a plate reader. Vitamin C levels were estimated by the method of Tietz.[20] Plasma MDA was investigated according to the modified method of Satoh.[21]

Statistical analysis

Statistical analyses were performed using SPSS, version 21 (IBM Inc., Chicago, IL). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student *t*-test. The probability $p < 0.001$ was regarded as significant. Pearson's correlation analysis was used to test the linear relationship between parameters. ANOVA was used to determine differences between group variables.

Results

Demographic characteristics

The demographic characteristics of participants are listed in Table 1. There was no significant difference with regard to age, weight and BMI between patients and controls. The patient group had significantly lower levels of GFR compared with the control group, $p < 0.001$.

Plasma and erythrocyte concentration of GPx, CAT, SOD, Zn, Cu, Se, MDA, vitamins C and E

The biochemical characteristics of patients and controls are listed in Table 2. The patient groups had significantly lower levels ($p < 0.001$) of plasma GPx, Se, vitamins C and E, erythrocyte SOD, CAT and Se and significantly higher levels of MDA compared to controls with no differences found between male and female. Similarity, there were no significant gender differences in erythrocyte GPx, Zn and Cu and plasma Zn, and Cu levels in the patient group compared to the control group.

Correlation analysis

Pearson's correlation analysis showed a positive correlation between plasma Se and GPx ($r = 0.583$, $p = 0.0001$), between erythrocyte Se and GPx ($r = 0.778$, $p = 0.0001$) (Figures 1 and 2), between erythrocyte Cu and SOD ($r = 0.180$, $p = 0.217$) and between erythrocyte Zn and

Table 1. Demographic characteristics of the study populations.

	CRF ($n = 49$)	Control ($n = 42$)
Age (years)	56 ± 9	52 ± 12
Weight (kg)	63 ± 16	62 ± 23
BMI (kg/m^2)	24.5 ± 6.3	20 ± 12
Sex ratio (M/F)	25/24	19/23
GFR (mL/min)	43 ± 7*	93 ± 8

Notes: CRF, chronic renal failure; M, male; F, female; BMI, body mass index (weight kg/height m^2); GFR, glomerular filtration rate. Data are expressed as mean ± SD.

*$p < 0.001$ versus the control group.

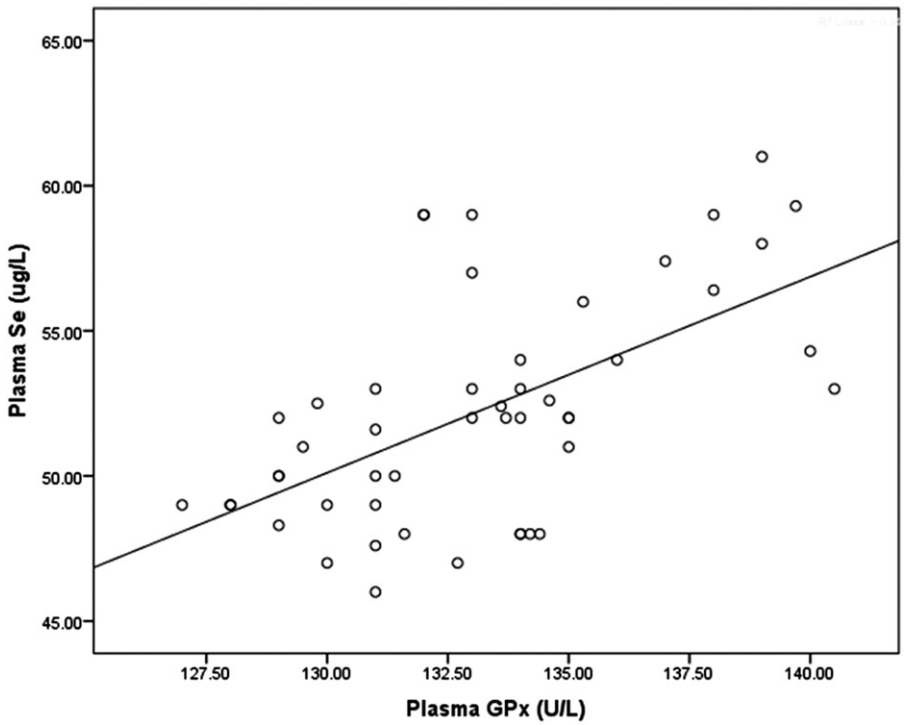

Figure 1. Correlation between plasma Se levels and plasma GPx activity. $r = 0.583$, $p = 0.0001$.

Table 2. Biochemical parameters of the study groups.

	CRF ($n = 49$)	Control ($n = 42$)
Plasma		
GPx (U/L)	132 ± 12*	178 ± 17
MDA (μmol/L)	2.67 ± 0.16	1.39 ± 0.23
Vit E (mg/dL)	0.65 ± 0.03*	1.21 ± 0.32
Vit C (mg/dL)	0.77 ± 0.11*	1.63 ± 0.22
Cu (mg/L)	0.86 ± 0.26	1.29 ± 0.19
Zn (mg/L)	0.83 ± 0.10	1.12 ± 0.19
Se (mg/L)	51.2 ± 15.9*	83.2 ± 12.9
Erythrocyte		
GPx (U/g Hb)	65 ± 15	74 ± 14
SOD (U/g Hb)	626 ± 110*	935 ± 229
CAT (k/g Hb)	183 ± 11*	276 ± 36
Cu (mg/L)	0.77 ± 0.12	0.84 ± 0.09
Zn (mg/L)	12.6 ± 2.1	13.1 ± 1.2
Se (μg/L)	73 ± 17*	119.21

Notes: CRF, chronic renal failure; GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase: CAT, catalase; Hb, hemoglobin; Cu, copper; Se, selenium; Zn, zinc; Vit E, vitamin E; Vit C, vitamin C.
*$p < 0.001$ versus the control group.

SOD ($r = 0.286$, $p = 0.047$) (data not shown). However, plasma MDA levels were negatively correlated with GPx ($r = -0.869$, $p = 0.0001$), vitamin E ($r = -0.906$, $p = 0.0001$) and vitamin C ($r = -0.730$, $p = 0.0001$) (Figures 3–5).

Discussion

Previous studies have demonstrated increased oxidative stress and reduction of antioxidant defenses in patients with CRF.[22–25] The current study assessed lipid peroxidation, antioxidant defenses and trace elements in CRF patients compared with healthy controls. Trace elements such as Se, Zn and Cu are required during several steps in the intermediary metabolism of mammalian cells. These elements are necessary components of several enzymes connected with metabolism. Deficiency of trace elements can lead to oxidative stress due to the increased production of free radicals.[26]

Decreased Se levels and GPx activity in blood components are common in CRF patients.[27] GPx is a selenium-containing tetrameric enzyme, which reduces H_2O_2, lipoperoxides and, other organic hydroperoxides to their corresponding hydroxyl compounds using glutathione as a hydrogen donor.[28] In the current study, results indicate that Se levels in plasma and erythrocyte of the CRF group are significantly reduced compared to the control group. Previous research did not find any difference between erythrocyte and plasma Se levels of CRF patients and controls[29,30]; however, lower levels were observed.[31–33] Although the reason for reduced erythrocyte and plasma Se levels in CRF patients is not completely explained, reduced dietary intake, impaired intestinal absorption, increased urinary loses and abnormal binding to Se transport proteins could be responsible for this phenomenon.[34,35] Decreased protein intake has been proposed as a significant cause of Se insufficiency, as protein foodstuffs contain the largest amount of Se.[9]

The current investigation showed a significant decrease in plasma GPx activity in CRF patients compared to controls. However, no significant differences were observed in erythrocyte GPx levels between patient and control groups. These results are in agreement with the findings of Sindhua et al. and Zachara et al.,[28,9] whereby plasma GPx activity in CRF patients was significantly reduced, while the enzyme activity in erythrocytes was comparable to that of the controls. The reason for reduced erythrocyte activity of GPx in CRF patients was unable to be explained. GPx in human plasma is synthesized by different tissues, yet renal proximal tubular epithelial cells are the main source of this enzyme, therefore presenting a challenge to the renal function that could result in lower enzyme production.[9] In addition, the enzyme may be used to counteract the effect of increased peroxides, which

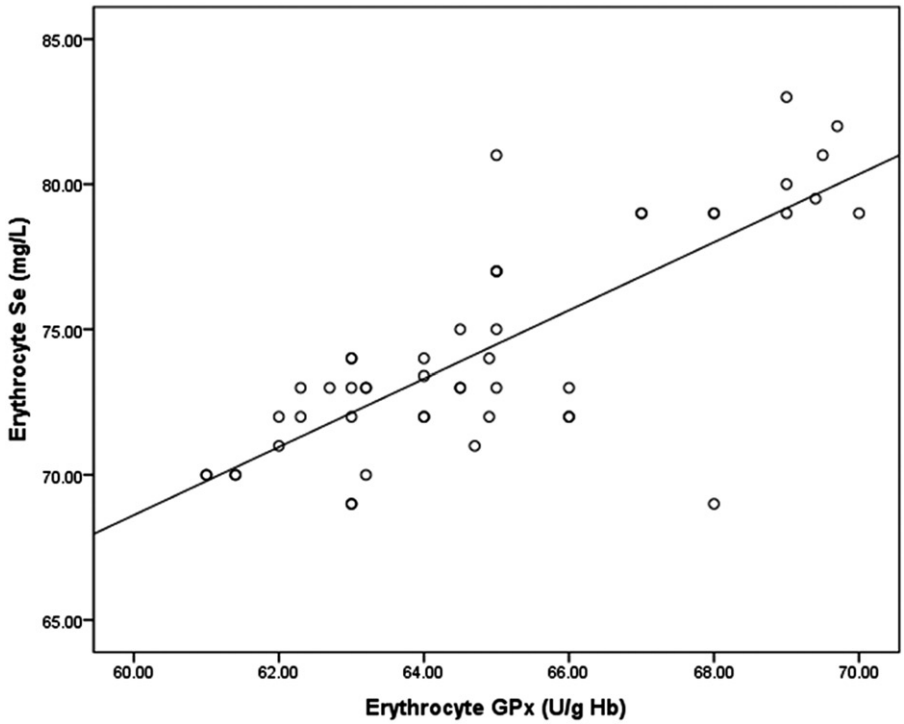

Figure 2. Correlation between erythrocyte Se levels and erythrocyte GPx activity. $r = 0.778$, $p = 0.0001$.

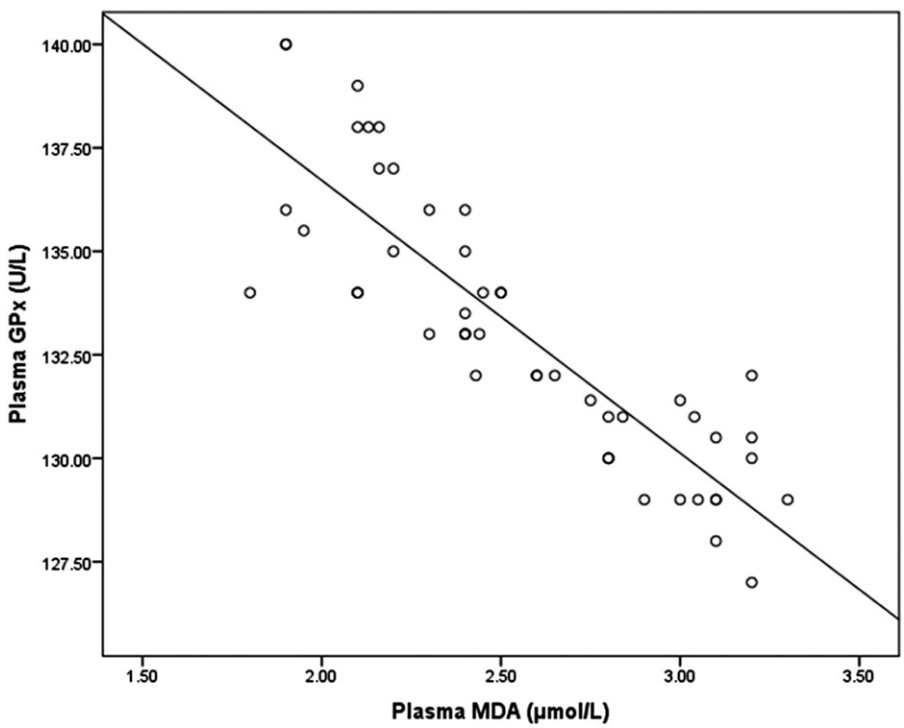

Figure 3. Correlation between GPx activity and MDA in plasma. $r = -0.869$, $p = 0.0001$.

accompanies renal damage. Moreover, the presence of toxin complexes in the blood of patients with CRF may inhibit the activity of the enzyme.[9,14] Additionally, the reduction of plasma GPx has been attributed to Se deficiency. It is well known that Se and GPx play an important role in protecting cell membranes from oxidative damage. Our study showed a direct relationship between GPx activity and Se level in plasma and erythrocytes of CRF, confirming the link between GPx and its cofactor Se. Therefore, evaluation of GPx activity can be a useful indicator of renal failure.[25,36,37]

The second antiradical enzyme activity is that of SOD, also diminished in CRF patients, while other studies have shown that erythrocyte SOD activity was the same as in the control group.[5,38] The effects of Zn and Cu on the structure of this enzyme have been described previously.[39] The present study found a similar link between reduction in SOD activity and Zn and Cu levels. The most probable explanation for decreased activity is a potential direct inactivation of the enzyme by its product hydrogen peroxide, or by the superoxide anion itself.[5] Pugalendhi et al.[12] demonstrated that the

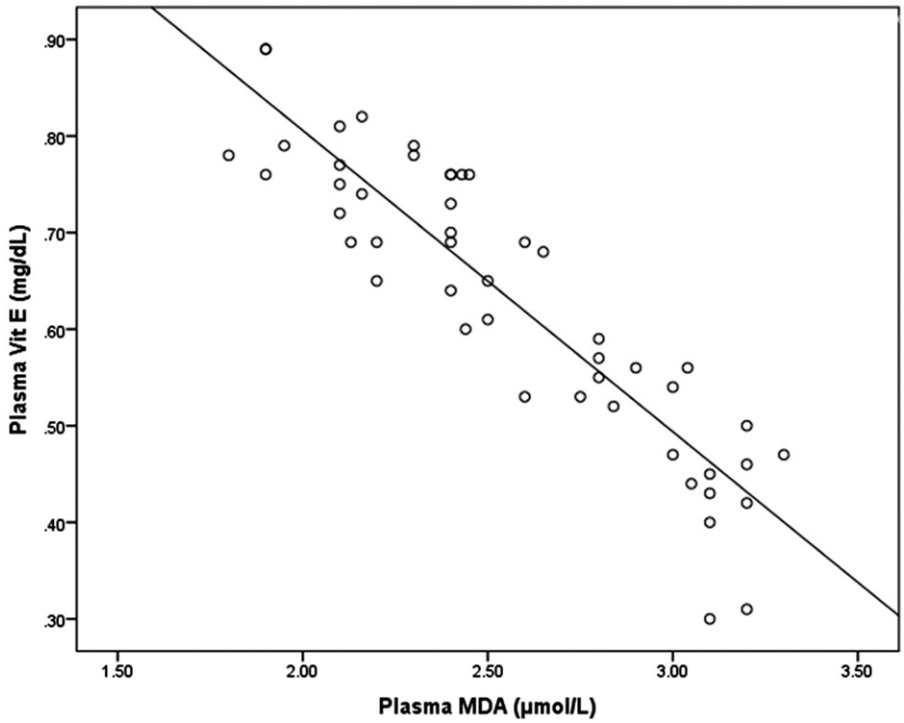

Figure 4. Correlation between vitamin E and MDA levels in plasma. $r = -0.906$, $p = 0.0001$.

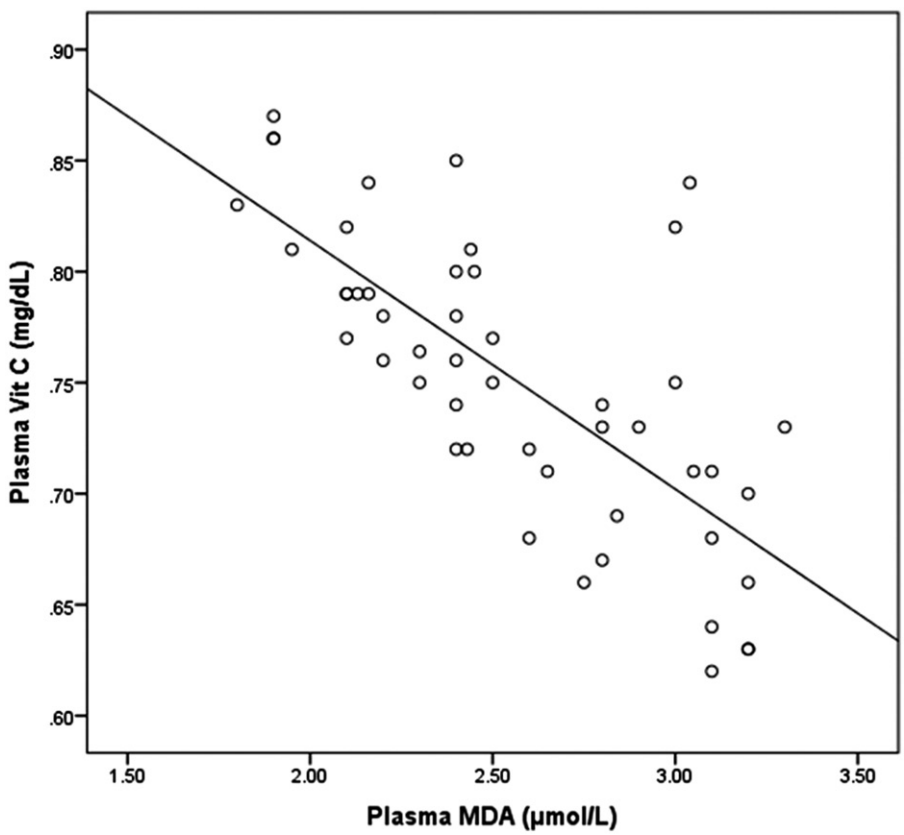

Figure 5. Correlation between vitamin C and MDA levels in plasma. $r = -0.730$, $p = 0.0001$.

decreased GPx activity in erythrocytes leads to accumulation of hydrogen peroxide that may cause inhibition of SOD activity. The positive correlation between the SOD activity and Cu and Zn levels proves that Cu and Zn have an important role in the function of antioxidant SOD enzyme.

CAT is a tetrameric hemin-enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa, each containing a ferriprotoporphyrin moiety.[40] According to Raes et al.,[41] the protective effects of GPx against oxygen derived free radicals are better than CAT and SOD. Though the catalytic characterization of CAT is similar to GPx, its role of detoxifying hydrogen peroxide is not as significant as GPx.[41,42] CAT activity in erythrocytes is 3600 times higher than in plasma.[14] In our study, CAT activity was measured in

erythrocytes only, and found to be significantly lower than in controls. Decreased CAT activity may be due to the depletion or inactivation of the enzyme by the production of free radicals, such as superoxide and H_2O_2. These, in turn, generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation.[43]

Of note, the level of plasma vitamins E and C in the present study was also diminished; indicating either a defect of the nonenzymatic antioxidant system or the possibility of enhanced lipid peroxidation. The protective effect of vitamins E and C appears to be mainly due to their free radical scavenger effects through break free radical chain reactions, decreasing consumption of endogen antioxidant enzymes, and preventing important biological macromolecules such as lipids, proteins and DNA from oxidative damage.[44,45] The decrease in these parameters is in line with the previous research.[46,47] MDA, another indicator of oxidative stress, is a product of lipid peroxidation. A significant increase in mean plasma MDA level in the patient group was observed. The MDA increase may reflect the presence of increased oxidative stress as a result of membrane lipid peroxidation. The weakly negative correlation between plasma GPx, vitamins E and C levels and MDA suggests that duration and severity of the disease may parallel increased oxidative stress. Therefore, to decrease MDA level and prevent occurrence of CRF complications, the CRF patients can be supplemented with antioxidant vitamins.[48]

Conclusion

The significantly decreased levels of erythrocyte SOD, CAT, and Se, and plasma GPx, Se, vitamins E and C of CRF patients compared to control participants indicate the existence of oxidative stress. In addition, increased MDA levels are a reliable marker and a product of lipid peroxidation in CRF patients and are, thus, an indirect indication of greater vulnerability of CRF patients to oxidative–antioxidative imbalance. The resulting permanent oxidative stress could act as a significant mediator contributing to the progression of renal failure and associated complications. Further investigation of oxidative stress markers in CRF patients is required.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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