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Clinica Chimica Acta 355 (2005) 91–96

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Reduction of urinary thiols in nephrotic syndrome—a possible effect of free iron

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Received 15 November 2004; received in revised form 15 December 2004; accepted 15 December 2004

Abstract

Background: Albumin is a potent antioxidant as it chelates transitional metals and contains antioxidants like thiol and bilirubin. In neprotic syndrome, the defining parameter is proteinuria with hypoalbuminemia. Therefore albuminuria in nephrotic syndrome may increase toxic transitional metal ions and also can cause loss of albumin associated antioxidants causing oxidative stress to the individual.
Methods: We investigated this possibility and estimated some markers of oxidative stress in 20 nephrotic syndrome patients and healthy controls along with urinary thiols, urinary bilirubin and plasma free iron in both cases and in the controls.
Result: We found oxidative stress in 20 nephrotic syndrome patients and the markers of oxidative stress correlated significantly with proteinuria, but the urine of nephrotic syndrome patients (28.33 ± 4.2 μmol/g creatinine)contained significantly less thiols compared to the healthy controls (88.45 ± 10.6 μmol/g creatinine) and no biliribin. The patients plasma also showed free iron (0.7 ± 0.05 μmol/l), a parameter undetectable in the healthy controls.
Conclusion: We suggest that oxidative stress and presence of free iron in the patients were responsible for less thioluria and no bilirubinuria. A detailed study of oxidative biology in a large cohort of nephrotic syndrome patients is necessary to confirm the presence of free iron as appropriate chelation of free iron may benefit the long-term prognosis of the disease.

Keywords: Oxidative stress; Nephrotic syndrome; Thiol; Free iron

1. Introduction

Albumin, the plasma protein which maintains the oncotic pressure, is an important extra cellular antioxidant. It binds heme and copper ions. Albumin also contains an exposed cysteine–SH (thiol) group and provide the bulk of total plasma thiol, a well

Abbreviations: GSH, glutathione; SOD, superoxide dismutase; FOX, ferrous ion oxidation xylenol orange assays; H_2O_2, hydrogen peroxide; DTNB, 5,5′dithio-bis-nitrobenzoic acid; BPS, bathophenanthroline disulphonate; ROS, reactive oxygen species.

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doi:10.1016/j.cccn.2004.12.011

known antioxidant. The putative antioxidant bilirubin is also transported in albumin bound form [1].

Nephrotic syndrome, a disease characterized by massive albuminuria, is basically the result of glomerulonephritis of any form [2]. Glomerulonephritis is independently known to produce oxidative stress [3]. Therefore, nephrotic syndrome should be associated with oxidative stress. Recently it is proved that plasma of nephrotic syndrome patients exhibit less reactive oxygen species (ROS) scavenging property which correlates with hypoalbuminemia [4]. Albuminuria can cause oxidative stress by increase in redox active transitional metal ions in the plasma or due to loss of albumin associated antioxidant like thiol groups and bilirubin. To our knowledge, this aspect of nephrotic syndrome has not been previously examined.

2. Materials and methods

2.1. Patients selection

Confirmed cases of nephrotic syndrome with anasarca, hypercholesterolemia (serum cholesterol≥250 mg/dl), proteinuria (urine protein≥3.5 g/day) and hypoalbuminemia (serum albumin<2.5 g/dl) were selected. Smokers, any evidence of uremia [blood urea>40 mg/dl, serum creatinine>2 mg/dl, or patient on hemodialysis), hematuria, hemolysis and patients on herbal drugs or angiotensin converting enzyme inhibitor were excluded from the study. We detected hematuria by the benzidine test and detected hemolysis by visual observation of the plasma after centrifugation. Only the newly diagnosed cases were included and any previous history of steroids or immunosuppressants use were excluded. Informed consents were obtained from human subjects included in the study. Further details of the human subjects enrolled are shown in Table 1.

2.2. Sample preparation

Urine and blood samples with heparin as anticoagulant were collected from normal individuals and nephrotic syndrome patients. Urinary hydrogen peroxide (H_2O_2) and thiols were estimated within 30 min of sample collection. Other markers were estimated

Table 1
Markers of oxidative stress in nephrotic syndrome patients and healthy controls

	Plasma GST activity IU/L	Plasma hydro-peroxide µmol/l	Plasma Bilirubin mg/dl	Plasma total thiol µmol/l	Plasma Fe^{2+} iron µmol/l	Plasma albumin g/dl	RBC MDA nmol/ml of packed cell suspension	RBC catalse activity S^{-1} per ml of packed cell suspension	RBC SOD activity U/ml of packed cell suspension	RBC GSH mg/g of Hb	Urine total thiol µmol/g creatinine	Urine H_2O_2 mmol/g creatinine	Urine total protein g/day	Urine bilirubin mg/dl
Controls n=20	6.9±2.1	1.5±0.53	0.5±0.2	547±80.2	Undetectable	4.5±1.2	0.5±0.08	94.5±30.5	170±26.5	3.57±0.46	88.45±10.6	252.64±80.7	Undetectable	Undetectable
Cases n=20	11.5±3.2 r=0.39 p<0.05	4.2±1.2 r=0.52 p<0.01	0.2±0.09 r=0.65 p<0.001	234±40.5 r=0.54 p<0.01	0.7±0.05 r=0.41 p<0.05	1.5±0.9 r=0.56 p<0.01	1.6±0.53 r=0.59 p<0.01	142.8±17.6 r=0.37 p<0.05	150±15.2 r=0.43 p<0.05	1.2±0.09 r=0.85 p<0.001	28.33±4.2 r=0.38 p<0.05	397.12±50.5 r=0.38 p<0.05	6.2±2.5	Undetectable

The control group (n=20) comprises of 8 individuals (6 males, 2 females) between (age 4–12 years) and 12 individuals (8 males, 4 females) between age 13–45 years. The case group (n=20) comprises of 8 individuals in the age range 4–12 years. Out of which 5 are males. The remainder of the patients, i.e., 12 individuals (7 males and 5 females) are between 13 and 45 years. The nephrotic syndrome patients had proteinuria >3.5 g/day, serum cholestrol ≥250 mg/dl and generalized edema. Values are expressed as mean±SD.