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Oxidative stress biomarkers and chromogranin A in uremic patients: Effects of dialytic treatment

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ABSTRACT

Objective: To evaluate oxidative stress in uremia and dialysis and chromogranin A, a stress hormone that could be related to oxidative processes.

Methods: Plasma oxidative stress biomarkers (–SH, 8-OHdG, and ox-LDL) and chromogranin A were measured in 89 outpatients (21 uremic patients, 17 in peritoneal dialysis, and 51 in haemodialysis), and in 18 subjects with normal renal function.

Results: –SH groups were significantly reduced in heamodialysis, peritoneal, and uremic patients as compared with the control group ($p=0.01$), while 8-OHdG was increased ($p<0.01$). No differences were observed for ox-LDL. Chromogranin A was increased in uremic, peritoneal and haemodialysis patients ($p<0.01$), showing a positive correlation to 8-OHdG ($p<0.01$).

Conclusion: Oxidative stress biomarkers and chromogranin A levels differ between control subjects when compared to both uremic and dialysis patients. No differences were observed between uremic and dialysis patients, suggesting that uremia is the major source of the increase in oxidative stress and CgA levels in patients with end stage renal disease.

Introduction

Traditional cardiovascular risk factors, including aging, diabetes mellitus, hypertension and dyslipidemia have an important role in the progression of cardiovascular disease also in patients with chronic renal disease [1]. In uremic patients, nontraditional or 'novel' risk factors, including inflammation, oxidative stress, anemia, and vascular calcifications confer additional cardiovascular risk to this population. The presence of these uremia-related risk factors may also promote the progression of kidney disease and could justify the high incidence of cardiovascular disease found in uremic and dialyzed patients [2].

Several markers of oxidative stress have been investigated in the last few years in dialysis patients. In particular, in these patients vitamins A and E are decreased in plasma [3] and erythrocytes [4], as well as plasmatic and erythrocyte superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx) activity [5,6]. Altogether these findings suggest that the plasma environment of uremic patients has a strong tendency toward a pro-oxidant status.

It is also generally assumed that dialysis treatment is a cause of generalized stress and increases the oxidative stress finally causing a multi-organ stress [7–12]. Oxidative stress depends on the misbalance between the synthesis of oxidation derived products on the one hand, and the anti-oxidant mechanisms on the other. In clinical practice oxidative stress is usually evaluated by measuring the oxidation of specific macromolecules, including proteins, DNA and lipids. Chromogranin A (CgA) is a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins and is located in secretory vesicles of neurons and endocrine cells [13,14]. Among cells producing CgA are chromaffin cells of the adrenal medulla, enterochromaffin-like cells and beta cells of the pancreas. CgA is considered a neuroendocrine stress hormone [13–15] and is the precursor to several functional peptides including vasostatin, pancreastatin, catestatin and parastatin [13,15], which may have different roles in inflammatory and oxidative processes. It is not known whether there is a relationship between CgA and oxidative stress in uremic patients.

In uremic patients different molecules, commonly considered as biomarkers, are retained and accumulated in plasma, potentially causing a confounding effect on their biological interpretations.

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To evaluate whether dialysis treatment is an additional cause of stress besides uremia, we have measured plasma levels of thiol groups, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and oxidized-low density lipoprotein (ox-LDL) as oxidative stress biomarkers, and plasma levels of CgA, as a 'generalized stress' marker, in uremic patients treated or not with replacement therapy (peritoneal dialysis and bicarbonate haemodialysis). The acute effect of haemodialysis on these parameters was also evaluated.

Methods

Study subjects

Eighty-nine outpatients admitted to the Clinica Nefrologica, Azienda Ospedaliera San Gerardo, Monza, Italy, were studied. The study was approved by the local Ethics Committee. All patients gave written informed consent. A total of 21 predialysis uremic patients (class IV), 17 outpatients in peritoneal dialysis, and 51 patients in haemodialysis (standard bicarbonate) were involved in the study. Mean peritoneal dialytic age was 28 months (min 2–max 64 months), while mean haemodialysis dialytic age was 58 months (min 3–max 356 months). Haemodialysis patients were dialyzed three times a week for 4–5 h and adequacy parameters were kept constant (Kt/V>1.0). Among haemodialysis patients, blood samples were taken 15 min before and after the midweek dialysis. Patients with known recent (<6 months) inflammatory diseases, malignancy or myocardial infarction were excluded from the study. A control group ($n=18$) with normal renal function was identified from the general population and matched for age. Patients underwent clinical examination and blood pressure was measured using a mercury sphygmomanometer, after a 5 min rest. Blood pressure was measured 3 times at 2 min intervals and expressed as the mean of the 3 readings. Systolic blood pressure was indicated by the first Korotkoff sound and diastolic blood pressure was indicated by the disappearance of Korotkoff sound. In all patients peripheral whole blood samples were collected from an antecubital vein to measure total cholesterol and triglycerides, HDL and LDL cholesterol, creatinine, albumin, urea, and iron using routinely laboratory tests. The Cockcroft–Gault formula was used for calculation of estimated glomerular filtration rate (eGFR). 8-OHdG levels, ox-LDL levels and chromogranin A, were measured using specific ELISA, and sulfhydryl groups (–SH) were evaluated by Ellman test [16].

Plasma total thiol assay

Basal values of sulfhydryl groups were measured in Na-citrate whole blood samples. The blood was centrifuged at 2000 rpm for 15 min and stored at −20 °C. Plasma total thiols (–SH) were measured using the spectrophotometric method based on the colorimetric reaction of sulfhydryls, mainly cysteine residues in albumin, with Ellman's reagent, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), according to the method of Ellman [16], with minor modifications [17]. Briefly 1.25 mL of RB buffer (0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA) and 125 μL of plasma or standard, used as positive control, were added to a tube, followed by 25 μL of 10 mM DTNB in RB buffer. Blanks were run for each sample, prepared as described previously, with the exception that there was no DTNB. Following incubation for 15 min at room temperature, absorbance was read at 412 nm. The absorbance of blank was subtracted from the absorbance of correspondent sample. For each experiment, a GSH standard curve was constructed using varying concentrations of GSH (0–1.25 mM), and a curve-fitting software program (Curve Expert 1.3) was used to quantify thiol group concentration in plasma samples. All data were corrected for plasma protein content, measured by Bradford assay (BioRad), and expressed as μmol/gram total protein.

Serum 8-OHdG assay

Serum 8-OHdG levels were measured with a commercial competitive enzyme-linked immunosorbent assay kit (High Sensitivity 8-OHdG Check; Japan Institute for the Control of Aging, Shizuoka, Japan), as previously described [18]. Following the manufacturer's instruction, serum samples of patients and control group were separated by centrifugal ultrafiltration at 14,000 g for 45 min at 25 °C using Microcon YM-10 (Millipore Corp., Bedford, MA, USA). Blank sample (without primary antibody) has been considered as negative control, and standards as positive control. Briefly, 50 μL of standards or sample and the 8-OHdG monoclonal antibody were added to the microtiter plate which has been precoated with 8-OHdG and incubated at 4 °C overnight. After the wells were washed three times, horseradish peroxidase conjugated antibody was added, followed by incubation for 60 min. The wells were again washed three times, the enzyme substrate solution was added and the wells were incubated for 15 min. The reaction was stopped by 1 M phosphoric acid. The absorbance was read at a wavelength of 450 nm. For each experiment, a 8-OHdG standard curve was constructed (0–10 ng/mL) and a curve-fitting software program (Curve Expert 1.3) was used to quantify 8-OHdG in serum samples. Data were expressed as ng/mL.

Ox-LDL assay

Ox-LDL levels in plasma sample were measured using a commercial sandwich ELISA (Mercodia Oxidized LDL ELISA, Mercodia, Uppsala, Sweden) [19]. Calibration curve and internal low and high controls were processed following the manufacturer's instruction. Calibrator 0 (0 mU/l) has been considered as negative control. 'Low' and 'high' positive controls included in the commercial kit have been used.

As suggested by the manufacturer, the plasma samples of control and patient groups were diluted at 1:6561 and 25 μL of diluted plasma samples was added to plate and incubated for 2 h at room temperature. After six washes, a peroxidase conjugated anti-human apolipoprotein B antibody was added to plate and incubated for 1 h at room temperature. After six washes, the TMB substrate was added and incubated for 15 min. The reaction was stopped by Stop solution and the absorbance was read at a wavelength of 450 nm. For each experiment, an ox-LDL standard curve was constructed (0–25 mU/L) and a curve-fitting software program (Curve Expert 1.3) was used to quantify ox-LDL in plasma samples. Data were expressed as ox-LDL/LDL ratio.

Chromogranin A assay

CgA assay was performed by sandwich enzyme-linked immunosorbent assay (ELISA) based on an anti-CgA monoclonal antibody (B4E11) and a rabbit-polyclonal anti-CgA antiserum. Monoclonal antibody (MoAb) B4E11 is a mouse immunoglobulin (Ig) G_1 that recognizes an epitope of CgA corresponding to residues 68–70 of human CgA (A). Pools of samples from healthy subjects and from heart failure patients with low and high levels of CgA, respectively, were used as controls. The assay was performed as previously described [20].

Statistical analysis

Descriptive statistics were computed for demographic variables and haematochemical evaluations in the four groups of analysis. Categorical variables were described by a fraction of subjects falling in each specific category and distributions were compared by Chi-square test. Continuous variables were described by mean and standard deviation and distribution were compared by non parametric analysis of variance.

The three oxidative stress variables and chromogranin A were analyzed according to each study group. One-way non parametric