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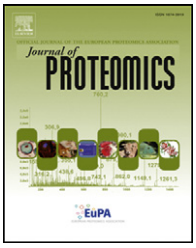

Blood modifications associated with end stage renal disease duration, progression and cardiovascular mortality: a 3-year follow-up pilot study

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ABSTRACT

Chronic kidney disease is a risk factor for cardiovascular mortality. This study uncovers pieces of hematological and erythrocyte protein variability observed in end stage renal disease (ESRD) in relation to disease progression/duration and mortality. Using a variety of experimental approaches, erythropoietin/dialysis-treated patients were compared to healthy individuals and had been followed for 36 months. During that period, half of the patients died from cardiovascular diseases. The high levels of uremic toxins in those patients were associated with damaged erythrocytes, bad tolerance and poor response to hemodialysis therapy. The postmortem study revealed significant variation in alkaline phosphatase, duration of dialysis, erythrocyte transformation and intracellular hemoglobin concentration compared to the survived patients. The erythrocyte proteins showed substantial remodeling characteristic of pathologic regulation of cell hydration and susceptibility to the dialysis-induced oxidation defects. According to the follow-up study, duration of hemodialysis was associated with a trend towards increased intracellular hemoglobin concentration, membrane expression of glucose transporter-1 and stomatin as well as lower levels of circulating stomatocytes. The uremic index variation in long survived patients is accurately reflected in plasma and erythrocyte oxidative stress modifications.

Abbreviations: ALP, alkaline phosphatase; aquaporin 1, Aqp1; DHA, dehydroascorbic acid; Epo, erythropoietin; ESRD, end stage renal disease; FRAP, ferric reducing ability of plasma (or antioxidant power); Hb, hemoglobin; HCT, hematocrit; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; γGT, gamma-glutamyltransferase; GLUT1, glucose transporter 1; MCH, mean cell Hb; MCHC, mean cell Hb concentration; MCV, mean cell volume; PCI, Proteome Carbonylation Index; Prx2, peroxiredoxin 2; RBCs, red blood cells; RDW, red cell distribution width; SGOT, serum glutamate-oxaloacetate transaminase; SGPT, serum glutamate-pyruvate transaminase; TAC, Total Antioxidant Capacity.

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The ESRD patients exhibit impressive compensatory responses to the chronic challenges of the uremic milieu.

Biological significance

This study demonstrates novel blood modifications probably associated with the duration of erythropoietin/hemodialysis treatment, disease progression and cardiovascular mortality in end stage renal disease. The observed variability adds new pieces to the erythrocyte pathophysiology puzzle in end stage renal disease and suggests novel hematologic and proteomic factors for consideration in future large scale studies on cardiovascular morbidity and mortality candidate biomarkers in uremic patients.

1. Introduction

The uremic syndrome is related to a complex set of pathophysiological disturbances resulting in increased morbidity and mortality rate. The major cause of death in all forms of chronic kidney disease including the end stage renal disease (ESRD) on hemodialysis (HD) [1] is cardiovascular diseases. The high risk of cardiovascular morbidity and mortality in these patients is attributed to a complex interplay of traditional (e.g. age, dyslipidemia, hypertension, and diabetes mellitus) and novel or uremia-specific cardiovascular risk factors, including uremic toxins, uremic bone disease, disturbed calcium-phosphate metabolism [2], inflammation [3], endothelium dysfunction [4], oxidative stress [5] and anemia [6]. At the advanced stage of the disease, retention of uremic toxins, metabolic alterations and HD may further contribute to the high risk of mortality.

Anemia in ESRD is the consequence of reduced red blood cell survival and functional erythropoietin (Epo) deficiency. Erythrocytes in hemodialysis patients undergo shear stress generated during blood flow through the dialyzer and peristaltic pumps and metabolic stress caused by the unfavorable plasma environment that is characterized by metabolite accumulation and loss of glucose [7]. The ESRD further represents a high pro-oxidant activity disease due to contributing factors like advanced age, chronic inflammation and dialysis material biocompatibility issues [6]. More specifically, erythrocytes are subjected to enhanced oxidative stress as a result of reduced cellular and plasma anti-oxidant factors and inadequate glutathione-defense system. Although hemodialysis partially improves the endogenous ROS levels, the glutathione antioxidant system as well as the RBC membrane protein defects [8], it has been associated with oxidation of plasma ascorbic to dehydroascorbic acid [9] and aggravation of protein carbonylation [10,11].

Prognosis, risk stratification and monitoring the effects of treatment are fundamental elements in the clinical handling and therapy guidance of uremic patients. A variety of blood biochemical risk markers have been consistently linked to cardiovascular disease and reduced survival in patients on dialysis [12,13]. However, biomarker identification in this group of patients has been proven to be a difficult task in that well-known associations between established risk factors in the general population do not exist or appeared reversed in ESRD [13], while some of the novel risk factors for cardiovascular disease seem to play a more important role for morbidity and mortality in uremic patients than in the general population [13–15]. Based on these peculiarities, a multi-marker approach reinforced by additional proteomic tools have been strongly proposed in renal disease biomarker area, as a safe way to refine prognosis in patients on HD, after their full-scale evaluation in large longitudinal studies and clinical trials [15].

It has recently been shown that the RBCs of non-diabetic ESRD patients on HD show substantial membrane remodeling and overexpression of cellular stress and senescence markers [8]. In the present follow-up study, we re-examined the same group of patients three years later in order to retrace blood modifications probably associated with the duration of HD and the progression of the disease, compared to healthy controls studied for the same period. Furthermore, and since half of the patients passed away in the meanwhile by cardiovascular diseases, we retrospectively assess a series of blood and erythrocyte factors as novel candidate markers of increased cardiovascular mortality in ESRD patients.

2. Materials and methods

2.1. *Material supplies*

Antibodies against band 3, actin, spectrin and human IgGs as well as HRP-conjugated secondary antibodies and all chemicals (unless otherwise stated) were obtained from Sigma-Aldrich (Munich, Germany). Electron microscopy grade glutaraldehyde solution was from Serva (Heidelberg, Germany). Antibodies against hemoglobin (Hb) and flotillin-2 were from Europa Bioproducts (UK) and BD Transduction Laboratories (CA, USA), respectively. Primary antibodies against CD47, HSP70, calpain-1 (μ-calpain), clusterin-α (secretory Apolipoprotein J) and band 3 were from Santa Cruz Biotechnology (CA, USA). Antibodies against peroxiredoxin 2 (Prx2), adducin alpha and glucose transporter 1 (GLUT1) were from Acris GmbH (Herford, Germany). The Oxyblot® detection kit was obtained from Millipore (Temecula, CA) and 5-(and-6) chloromethyl-2′,7′-dichloro-dihydro-fluorescein diacetate, acetyl ester (CMH_2DCFDA) was from Invitrogen, Molecular Probes (Eugene, OR). HRP-conjugated antibodies to rabbit IgGs and ECL Western blot detection kit were from GE Healthcare (Buckinghamshire, UK). HRP-conjugated antibodies to mouse IgGs were from DakoCytomation (Glostrup, Denmark).