ABSTRACT

The appropriate biomarkers for oxidative stress (OS) in patients with end stage renal disease (ESRD) are important in renal pathology. Patients (56) with ESRD were investigated (35 men and 21 women). Patients, with mean age of 45±17 years, defined education, specific HD duration and calculated body mass index (BMI), were exposed to a polysulphone type HD membrane for approximately 4 hours per HD session, 3 times per week. The control group was composed of 31 healthy volunteers. The total antioxidative capacity (TAC) and the antioxidative (AO) enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx), were assessed. Analyses included Randox Crumlin GB; lipid peroxidation (LP) using its end product, malonyldialdehyde (MDA) (fluorimetric); and a LDL-ox immunoassay (Biomedica gruppe, Vienna, Austria). The TAS was higher in ESRD patients before HD (1.63±0.1 mmol/L) compared to the control group (1.23±0.03 mmol/L) (p

INTRODUCTION

Oxidative stress (OS) has been reported in end stage renal disease (ESRD) patients undergoing haemodialysis (HD). It may be a principal risk factor for cardiovascular morbidity and mortality of these patients, and it demands appropriate treatment (1, 2). Inflammation and fibrosis in ESRD patients are related to OS. ESRD is also linked to tubulointerstitial inflammation and fibrosis, tubular atrophy, glomerulosclerosis, renal vasculopathy, and fibrous tissue. The pathogenesis suggests a possible unifying mechanism with cardiovascular disease and its progression (3, 4).

OS may be caused by the activation of polymorphonuclear cells, which may be activated by the HD membrane and/or the type of anticoagulant. Anticoagulants function via complement system activation (5). Direct contact between neutrophils and the HD membrane initiates degranulation, which stimulates their oxidative metabolism. HD and uremic factors may influence monocyte activation. Interleukin-1 (IL-1) and tumour-necrotizing factor α (TNF-α) increase free radicals (FR) and induce OS. An imbalance exists between FR production and antioxidant defence. Raddeke HH et al, 1990 established that human mesangial cells can generate FR, as a response to IL-1 and TNF-α. An amount comparable to monocyte production (6). Uremic toxin retention may trigger a lymphocyte response. This response results in the production of IL-1β and IL-8, which provokes innate immunity through CD8+ cells. Uric toxins may also produce a superoxide ion FR via xanthine oxidoreductase activity, and can no longer bind NADH. Reduced L-arginine production leads to decreased NO activity, which is vital to endothelial function. This cascade is further affected by the atherosclerosis risk factor ox-LDL. The largest source of FR may be the mitochondria within the respiratory chain. Oth-
er factors, such as obesity, metabolic disease, genetic factors and lifestyle, may cause OS (Fig. 1).

The antioxidant system (enzymatic and non-enzymatic) scavenges FR and prevents OS. The first defence of the antioxidant system is superoxide dismutase (SOD), which accelerates the dismutation of the superoxide anion to hydrogen peroxide. Glutathione peroxidase (GPx) reduces organic lipid peroxide, which requires glutathione as a hydrogen donor. The total antioxidant system contains vitamins, uric acid, bilirubin, transport proteins, etc. The level of OS is determined by lipid peroxidation through its end products malonyldialdehyde and LDL-ox (3).

Previous research has attempted to identify the appropriate biomarkers for OS because of the short half-life of FR. Quantification of the redox-sensitive proteins of signalling microdomains is not straightforward. Our approach measured the stable end products in circulation during OS (7). We investigated appropriate markers of OS in ESRD patients.

MATERIAL AND METHODS

Patients (56) with ESRD, and undergoing HD, were investigated. The study contained 35 men and 21 women with an average age of 45±17 years. Patients had a low (n=37), intermediate (n=46) or high (n=17) level of education. Patients underwent HD (polysulphone type membrane) for a period of 2-5 years (n=12), 6-10 years (n=35) or 11+ years (n=9). Patients exhibited a body mass index (BMI) that was low (n=15), normal (n=25) or high (n=16) (Fig. 2). HD was performed for approximately 4 hours per session, 3 times per week. For inclusion in the study, patients were required to be more than 25 years old and to have been receiving stable HD for more than 2 years. A polysulphone type membrane was used for HD.

Fig 1. Description of material used, related to: gender (%), educational level (%), HD duration (in years), body max index - BMI (kg/m²)

Fig 2. TAC in ESRD patients (before and after HD) and in control group
Patients with other chronic diseases, such as diabetes mellitus or heart failure, and those using supplement therapy, such as antioxidants, vitamins, iron and erythropoietin, were excluded from the study. The control group was age- and sex-matched with 31 healthy volunteers. The enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx), and the combination kits by Randox, Crumlin, Great Britain were used to determine the total antioxidative capacity (TAC). The lipid peroxidation (LP) was determined by a fluorimetric method (Yagi et al, 1967) using the end product malonyldialdehyde (MDA) as a substance that reacts with thiobarbituric acid. The immunoassay by Biomedica gruppe, Vienna, Austria was used to examine the LDD-ox and anti–LDL-ox antibodies (anti LDL-ox Ab). Student t-test was used (p<0.05) for statistical analysis.

RESULTS

ESRD patients demonstrated a higher value of TAS (1.63±0.1 mmol/L (before HD)) compared to the control group (1.23±0.03 mmol/L) (p<0.001). After HD, the TAO value decreased to 1.53±0.1 mmol/L (p<0.05) (Fig. 3).

The antioxidative enzymes SOD (1264 ± 124 U/g) and GPx (54.8± 12 U/g Hb) did not demonstrate statistical significance compared to the control group (Fig. 4, Fig. 5).

Lipid peroxidation resulted in a higher value of TAS (4.52±0.22 μmol/L) compared to the control group (3.81±0.18 μmol/L) (p<0.01) (Fig. 6). The TAS value increased (220±125 mU/ml) compared to the control (201±83 mU/ml) for Anti LDL-ox Ab (p<0.05) (Fig. 7).

DISCUSSION

ESRD patients exhibit an imbalance of pro-oxidant and anti-oxidant systems, which leads to OS and its consequences. Nguyen et al, 1985 first identified increased FR in the circulation of patients during HD. The activation of phagocyte oxidative metabolism is induced by the haemodialysis membrane (8). Thus, inflammatory response is an important source of increased OS and endothelial dysfunction in ESRD patients. This means that inflammation, OS and atherosclerosis are closely related and have much in common. The TAC is based on small plasma molecules, such as vitamins (A, C, E), uric acid, bilirubin, transferrin, ceruloplasmin, etc. The TAC level increased despite OS in ESRD patients. This finding is consistent with the results published by Montazerifar F et al, 2010 (9), in which the TAC level and vitamin A were significantly higher than the controls. In spite of increased TAC in these patients, the depletion of key chain breaking antioxidants is apparent, which may induce accelerated atherogenesis in these patients (10, 11). In this study, the TAC level was higher for the ESRD patients than...
the controls. The level decreased after HD, but it remained higher than the controls. The higher TAC value may be related to concomitant fluctuations of plasma urates. This finding requires further study and interpretation. There is a strong relationship between the TAC and the MDA, haemoglobin, haematocrit and the serum albumin level (12, 13, 14). No changes were observed for the antioxidative enzymes SOD and Gpx in HD patients. Knap B, et al. (15) reported a decrease in SOD activity and no difference in Gpx activity compared to controls; however, MDA and LDL-ox were increased in HD patients. This result indicated the presence of OS. Despite no change in antioxidative enzymes and increased TAC, HD patients demonstrated an impaired balance of FR production and scavenger, which means that the antioxidative defence was not appropriate. Antioxidant loss, through membranes during the HD process, may be related to higher LP level. This increased level in HD patients affects atherosclerosis development (16). An increased level of LDL-ox in HD patients confirmed that FR impaired the LDL molecule through oxidation. This impairment might have occurred because of the released myeloperoxidase from activated neutrophils (from the HD process) (17). Both LP and LDL-ox demonstrated elevated values in HD patients compared to the controls. OS is progressive and may induce complications such as the dysfunction of lipids and other molecules, accelerated tissue or cell apoptosis and endothelial impairment with possible cardiovascular morbidity. These last parameters are important biomarkers for the severity of OS, and the morbidity and mortality of these patients. Future studies are needed to clarify the appropriate biomarkers that define a HD patient’s condition, and to demonstrate the benefits of antioxidative therapy.

REFERENCES