EFFECT OF OXIDATIVE STRESS IN HEMODIALYSED PATIENTS

Attila Peti[1], Botond Csiky[2], Eszter Guth[2], Peter Kenyeres[3], Emese Mezosi[3], Gabor L. Kovacs[1], Laszlo Bajnok[3]

[1] Institute of Laboratory Medicine, School of Medicine, University of Pecs,

[2] FMC Dialysis Center, Pecs,

[3] 1st Department of Medicine, School of Medicine, University of Pecs, Hungary

Corresponding author's address

Laszlo Bajnok;
1st Department of Medicine, School of Medicine, University of Pecs, Hungary,
H-7624 Pecs, Ifjusag ut 13., Hungary;
Phone: 36 (72) 536-000/1771, Fax: 36 (72) 536-148;
Email: laszlo.bajnok@aok.pte.hu

Abstract

Aims, subjects and methods: Markers of oxidative stress and inflammatory activation of endothelium, as well as the adipose tissue secreted adipokines, e.g. adiponectin show altered pattern in renal failure. However, their internal relations have not been fully evaluated in this special patient population.

In our cross sectional study, beside the routine clinical and biochemical parameters, plasma malondialdehyde, glutathione (GSH), catalase, total peroxidase, as well as serum E-selectin and adiponectin were measured in 70 hemodialysed (HD) patients.

Results: GSH showed negative correlations with systolic and diastolic blood pressure (BP) values, while a positive one with HDL-cholesterol level, as expected. Interestingly, the level of sE-selectin was inversely correlated only with the age. In multiple regression analyses where anthropometric, BP and laboratory parameters were included and sE-selectin was the dependent variable, the inverse association between the age and level of sE-Selectin turned out being an independent factor.

Conclusions: In HD kidney failure patients of the biochemical cardiovascular risk markers those related to oxidative stress, endothelial dysfunction, or altered adipokine homeostasis are not necessarily strongly associated. Larger studies may be needed to confirm our novel observation, a negative and independent correlation of age to sE-Selectin level.

INTRODUCTION

Oxidative stress is high in patients treated in chronic hemodialysis (HD) program, as evidenced by increased lipid peroxidation, e.g. elevated malondialdehyde (MDA), while low antioxidant, e.g. decreased glutathione peroxidase levels [1-9]. Oxidative stress is further exacerbated by HD treatment itself [7]. Furthermore, level of MDA is significantly correlated with the severity of kidney dysfunction [6], as well as with the duration of HD program [9].

Moreover, the survival was independently predicted by MDA level [10]. Actually, Miller et al. (2006) found MDA level being the best marker for risk estimation of cardiovascular events in patients being on long-term HD [11]. MDA levels were also associated with the antioxidant paraoxonase-1 activity suggesting that patient with chronic kidney disease exhibits an oxidant-antioxidant imbalance related to high levels of atherosclerotic risk factors [8].

In opposite to MDA levels, studies measuring reduced glutathione (GSH), as another marker of oxidative stress gave conflicting result in HD patients. Chugh et al. (2000) found decreased [5], while Valentini et al. (2008), in opposite, elevated GSH levels in kidney failure [9]. In comparison with healthy subjects, among these patients Paul et al. (1993) showed a significantly lower activity of the enzyme glutathione peroxidase reverting back glutathione to reduced form (GSH) and an inverse correlation between MDA and GSH suggesting the existence of a mainly intracellular oxidizing stress [3].

Regarding the catalase, in HD patients, as compared to controls, some authors demonstrated decreased [4, 6], others increased activity [12], while Atamer et al. (2008) found no difference [8].

Although adiponectin as an adipose tissue derived cytokine having insulin sensitizing, anti-inflammatory, and anti-atherosclerotic properties, has a controversial role in chronic renal diseases [13]. Some studies carried out in kidney failure revealed a negative correlation between the serum adiponectin and levels of oxidative stress markers [14], similarly to patients without kidney failure [15].

Soluble E-selectin is a marker of inflammatory activation of the endothelium. An elevated serum level of this has been demonstrated in HD patients, probably due to both inadequate clearance, and enhanced synthesis/release of sE-selectin [16]. In cases without kidney failure, Miller et al. demonstrated that the level of sE-selectin correlated positively with body mass index (BMI) even if data were adjusted for age, sex, race, smoking habits, blood pressure, serum levels of lipids and insulin [11].

However, the relations of these factors in HD patients are far from optimally clarified yet. Our aim with this present study was to test further the possible associations of oxidative stress markers with other characteristics, e.g. sE-selectin, and adiponectin.

PATIENTS AND METHODS

Patients

The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and approved by the Local Ethics Committee. Subjects participating in the study gave written informed consent.

The main characteristics of 70 HD patients is shown in Table 1. Eighteen of HDPS had polycystic kidney disease, 13 diabetic nephropathy, 12 glomerulonephritis, 11 pyelonephritis, 8 ischemic renal disease, 4 idiopathic kidney atrophy, 2 nephrotic syndrome and 2 had earlier nephrectomy. Fifty one of them took antihypertensive medication, and 19 had diabetes.

None of the patients involved in the study had evidence of liver disease defined as alanine aminotransferase or aspartate aminotransferase >1.5 times the upper limit of normal range, thyroid disorders, infectious diseases or significant inflammation for 3 months prior to study, malignancy, coronary artery disease, blood pressure >160/100 mmHg, cerebral vascular disease, smoking, triglyceride levels higher than 4.5 mmol/L, alcoholism, drug dependence, pregnancy or lactation, anticoagulant, lipid lowering, glucocorticoid, oral contraceptive or sex hormone replacement medication.

The form of hemodialysed treatment was hemodiafiltration in every patient, except two of them in whom high flux hemodialysis treatment alone could be applied. Kt/V (dialysis efficiency) was calculated by the use of Kt/V = - ln (R 0.03-0.075 x UF/W) equation, where R = ratio of blood urea concentration measured after and before dialysis, UF =

volume of ultrafiltrate (l), W = body weight after dialysis (kg). Mean (lower/upper quartile) HD time of patients was 38 (16.7 / 62.7) months.

Biochemical analyses

Blood specimens for investigations were obtained before the dialysis sessions. For routine automated laboratory analyses Cobas Integra 700 Autoanalyzer was used (Roche, Schwitzerland). Concentrations of insulin were measured by electro-chemiluminescence immunoassay (Roche Elecsys). The insulin resistance index calculation was based on homeostasis model assessment (HOMA-IR) [17].

Commercially available sandwich enzyme immunoassays were used for the determination of serum concentration of adiponectin, and sE-selectin (Quantikine, R&D Systems, Minneapolis, MN, USA), and colorimetric assay for the measurement of total peroxidase activity (Oxystat, Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria).

Plasma levels of MDA were estimated by the thiobarbituric acid reactive substances (TBARS). For this basically the Matkovics method was used: whole blood was precipitated with a mixture of TCA and TBA and boiled, and the supernatant was measured by photometric method at 532 nm expressing the results in nmol/ml [18]. For the determination of blood GSH level, at first TCA was added, and then DTNB (5,5'-dithiobis 2 nitrobenzoic acid [Serva 20735]) to the supernatant of a low-temperature centrifugation. Photometric measurement was done at 412 nm expressing the results of GSH in nmol/ml [19]. During the determination of catalase enzyme activity from blood the decrease of peroxide concentration was detected at 240 nm (U.V. spectrophotometry on Perkin-Elmer Spectrophotometer) getting the results in BU/ml, where 1 BU means 1 g peroxide destruction per minute.

Statistical analysis

Statistical analyses were performed using the SPSS 11.0 software (SPSS, Inc., Chicago, IL, USA). Normality of distribution of data was tested by Kolmogorov-Smirnov test. Non-normally distributed parameters were transformed logarithmically to correct their skewed distributions. Data were expressed as means ± S.D. in case of normal distribution, and median (lower/upper quartile) in case of non-normal distribution. Values of $P < 0.05$ were considered statistically significant. Analyses of univariate correlations were done by Pearson's test and multivariate correlations were assessed by backward multiple regression analyses.

RESULTS

Table 1. contains the principal data of patients. The following parameters had to be transformed logarithmically: HD time, diastolic blood pressure (BP), plasma glucose, HOMA-IR, HDL-cholesterol (HDL-C), adiponectin, MDA, GSH, catalase and total peroxidase.

Table 1. Anthropometric and selected laboratory characteristics in the study patients

N	70
Age (y)[a]	56.2 ± 11.5
Female / male	37 / 33
BMI (kg/m2)[a]	26.4 ± 5.7
Waist (cm)[a]	99.8 ± 15.9
Dialysis efficiency (Kt/V)[a]	1.58 ± 0.26
HD time (months)[b]	38 (16.7 / 62.7)
Systolic BP (mmHg)[a]	126.9 ± 16.8
Diastolic BP (mmHg)[b]	79 (70 / 91)

Fibrinogen (g/l)[a]	4.2 ± 1.1
Plasma glucose (mmol/l)[b]	5.5 (5 / 6.7)
HOMA-IR (mU·mmol/l^2)[b]	3.5 (1.9 / 7.9)
Triglyceride (mmol/l)[a]	1.9 ± 0.9
Total cholesterol (mmol/l)[a]	4.6 ± 0.9
HDL-C (mmol/l)[b]	0.90 (0.8 / 1.2)
LDL-C (mmol/l)[a]	3.1 ± 0.9
Adiponectin (μg/ml)[b]	17.6 (13.4 / 27.6)
sE-Selectin (ng/ml)[a]	38.7 ± 15.9
MDA (nmol/ml)[b]	94.6 (84.9/107.1)
GSH (nmol/ml)[b]	120.8 (105.3/134.4)
Catalase (BE/ml)[b]	94 (73.1/128)
Total peroxidase (μmol/l)[b]	121.8 (68.2/215)

[a]: Normal distribution, data are mean ± SD. [b]:Non-normal distribution, data are median (lower/upper quartile).

Abbreviation: BP: blood pressure.``

Univariate correlations.

Pearson's correlations of oxidative stress markers and sE-Selectin with selected variables having special interest are demonstrated in Table 2 and 3, respectively.

Between the oxidative stress markers there was a weak internal association (actually, only catalase and MDA were associated to each other's). Of the relations of oxidative stress markers with clinical and/or laboratory parameters, it was the GSH that showed correlation: a negative one with BP values and a positive one with HDL-C level, as expected.

Interestingly, the level of sE-selectin showed correlation only with the age, namely an inverse one.

Table 2. Pearson's correlation coefficients of oxidative stress markers and selected variables

	MDA	GSH	Catalase	Total peroxidase
Age[a]	0.08	-0.09	-0.17	0.17
BMI[a]	0.05	-0.15	0.04	0.01
Waist[a]	0.03	-0.08	0.006	0.07
Systolic BP[a]	-0.001	-0.3*	0.004	0.06
Diastolic BP[b]	-0.03	-0.24*	0.131	0.06
Dialysis efficiency[a]	0.03	0.004	0.1	0.05
HOMA-IR[b]	-0.016	-0.09	-0.01	-0.001
Triglyceride[a]	0.1	-0.12	0.21	-0.09
HDL-C[b]	-0.1	0.25*	-0.16	0.08
LDL-C[a]	-0.1	0.2	-0.08	0.09
Adiponectin[b]	-0.15	0.06	-0.1	0.08
sE-Selectin[a]	-0.006	-0.05	0.14	0.09
MDA[b]	-	-0.14	0.29*	-0.08
GSH[b]	-0.14	-	-0.08	-0.18
Catalase[b]	0.29*	-0.08	-	0.05
Total peroxidase[b]	-0.08	-0.18	0.05	-

[a]: Normal distribution. [b]:Non-normal distribution.

*: P <0.05.

Abbreviation: BP: blood pressure.

Table 3. Pearson's correlation coefficients of sE-Selectin with selected variables

Age[a]	-0.40[**]
BMI[a]	-0.02
Waist[a]	-0.02
Systolic BP[a]	-0.04
Diastolic BP[b]	0.05
Dialysis efficiency[a]	0.01
sE-Selectin[a]	-
Plasma glucose[b]	-0.097
HOMA-IR[b]	0.003
Triglyceride[a]	0.06
HDL-C[b]	0.09
LDL-C[a]	-0.05
Adiponectin[b]	-0.06
MDA[b]	-0.006
GSH[b]	-0.05
Catalase[b]	0.14
Total peroxidase[b]	0.09

[a]: Normal distribution. [b]:Non-normal distribution.

*: P <0.05; **: P <0.01.

Abbreviation: BP: blood pressure.

Multivariate correlations.

Investigating the inverse connection between the age and level of sE-selectin, the latter was a dependent variable in a multiple regression model in which anthropometric, blood pressure and other laboratory variables were included. During these analyses, only age turned out being significant independent predictor of sE-Selectin (Table 4).

Table 4. Multiple regression analysis for sE-Selectin as a dependent variable

Variable	Model ($R^2 = 0.212$)		
	β	t	*P*
Age	**-0.403**	**-3.601**	**0.001**
Sex	0.096	0.820	**0.415**
BMI	0.086	0.74	**0.462**
Systolic BP	-0.088	-0.76	**0.45**
Kt/V	0.116	1.041	**0.302**
Adiponectin	-0.02	-0.15	**0.881**
PON1	0.116	1.034	**0.305**

Significant value indicated in bold; β is standardized regression coefficient.

Abbreviation: BP: blood pressure.

DISCUSSION

We applied multiple biochemical tests to approach the oxidative stress in order to eliminate false results. Moreover, the expected internal association between these markers helped us to exclude methodological troubles related

pitfalls. Despite these, we found no strong relationships between the oxidative stress and the other investigated cardiometabolic variables. Reduced glutathione as an antioxidant, as expected, showed correlations with other cardiovascular risk factors, namely, negative ones with both systolic and diastolic BP values, while a positive one with HDL-C.

Very few studies evaluated the coordinated behavior of oxidative stress markers, sE-Selectin and adiponectin in hemodialysed individuals. We could not confirm Lim et al.'s observation related to a negative correlation between the levels of oxidative stress markers and serum adiponectin in patients with kidney failure [14]. In our population no significant relation of adiponectin to any of the investigated oxidative variables could be demonstrated.

A novel finding of our work is a negative and independent correlation between age and sE-Selectin level. This is in contrast with a study carried out in patients with preserved kidney function that demostrated a positive correlation between sE-selectin and age [20]. Currently our observation cannot be convincingly explained by the available information, but it may be related to a profoundly different patient population.

Our negative results might derive from the limited number of investigated subjects and low statistical power. However, the size of our study group (n=70) was typical for such a study and in accordance to the capacity of a single hemodialysis centre.

CONCLUSION

In kidney failure and hemodialysis of the biochemical cardiovascular risk markers those related to oxidative stress, endothelial dysfunction, or altered adipokine homeostasis are not necessarily strongly associated. Larger studies may be needed to confirm our novel observation, a negative and independent correlation of age to sE-Selectin level.

ACKNOWLEDGMENT

References

1. Dirican M, Sarandol E, Serdar Z, Ocak N, Dilek K. Oxidative status and prevalent cardiovascular disease in patients with chronic renal failure treated by hemodialysis. Clin Nephrol. 2007; 68(3):144-50.
2. Durak I, Kaçmaz M, Elgün S, Oztürk HS. Oxidative stress in patients with chronic renal failure: effects of hemodialysis. Med Princ Pract. 2004; 13(2):84-7.
3. Paul JL, Sall ND, Soni T, Poignet JL, Lindenbaum A, Man NK, Moatti N, Raichvarg D. Lipid peroxidation abnormalities in hemodialyzed patients. Nephron 1993; 64(1):106-9.
4. Lukoseviciene R, Ziaukiene G, Urbeliene J, Kalpokaite Z, Glemziene I. A study of oxidative stress markers and antioxidant system activity in the serum of hemodialysis patients. Medicina (Kaunas) 2003; 39 Suppl 1:115-8.
5. Chugh SN, Jain S, Agrawal N, Sharma A. Evaluation of oxidative stress before and after haemodialysis in chronic renal failure. J Assoc Physicians India. 2000; 48(10):981-4.
6. Mimić-Oka J, Simić T, Djukanović L, Reljić Z, Davicević Z. Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. Clin Nephrol. 1999; 51(4):233-41.
7. Loughrey CM, Young IS, Lightbody JH, McMaster D, McNamee PT, Trimble ER. Oxidative stress in haemodialysis. QJM 1994; 87(11):679-83.
8. Atamer A, Kocyigit Y, Ecder SA, Selek S, Ilhan N, Ecder T, Atamer Y. Effect of oxidative stress on antioxidant enzyme activities, homocysteine and lipoproteins in chronic kidney disease. J Nephrol. 2008; 21(6):924-30.
9. Valentini J, Grotto D, Paniz C, Roehrs M, Burg G, Garcia SC. The influence of the hemodialysis treatment time under oxidative stress biomarkers in chronic renal failure patients. Biomed Pharmacother. 2008; 62(6):378-82.
10. Scott B, Deman A, Peeters P, Van den Branden C, Stolear JC, Van Camp G, Verbeelen. Cardiac troponin T and malondialdehyde modified plasma lipids in haemodialysis patients. Nephrol Dial Transplant. 2003; 18(4):737-

42.

11. Miller MA, Cappuccio FP: Cellular adhesion molecules and their relationship with measures of obesity and metabolic syndrome in a multiethnic population. Int J Obes (Lond). 2006; 30: 1176-1182.

12. Fatouros IG, Pasadakis P, Sovatzidis A, Chatzinikolaou A, Panagoutsos S, Sivridis D, Michailidis I, Douroudos I, Taxildaris K, Vargemezis V. Acute exercise may exacerbate oxidative stress response in hemodialysis patients. Nephron Clin Pract 2008; 109(2):c55-64.

13. Guebre-Egziabher F, Drai J, Fouque D: Adiponectin and chronic kidney disease. J Ren Nutr 2007;17:9-12.

14. Lim PS, Chen SL, Wu MY, et al. Association of plasma adiponectin levels with oxidative stress in hemodialysis patients. Blood Purif. 2007; 25: 362-369.

15. Katsuki A, Suematsu M, Gabazza EC, et al. Increased oxidative stress is associated with decreased circulating levels of adiponectin in Japanese metabolically obese, normal-weight men with normal glucose tolerance. Diabetes Res Clin Pract. 2006;73:310-4.

16. Bonomini M, Reale M, Santarelli P, et al. Serum levels of soluble adhesion molecules in chronic renal failure and dialysis patients. Nephron 1998; 79: 399-407.

17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and ß-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-9.

18. Placer ZA, Cushman LL, Johnson BC: Estimation of product of lipid peroxidation (malondialdehyde) in biochemical systems. Anal Biochem 1966; 15:359-64.

19. Sedlak J, Lindsay RH: Estimation of total protein-bound and non-protein sulphydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25:192-205.

20. De Caterina R, Ghiadoni L, Taddei S, et al. Soluble E-selectin in essential hypertension: a correlate of vascular structural changes. Am J Hypertens. 2001; 14: 259-266.