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ORIGINAL ARTICLE

Effect of high flux versus low flux hemodialysis on serum beta-2 microglobulin, advanced oxidation protein products and protein carbonyl

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Abstract *Background:* The uremic syndrome is characterized by the retention of toxins that contribute to the associated morbidity and mortality. The heterogeneity of uremic toxins with respect to their molecular weight and protein binding may affect their removal by hemodialysis.
Objectives: To investigate the effect of high flux versus low flux hemodialysis (HD) on serum beta-2-microglobulin (B2MG) and advanced oxidation protein products (AOPP) as uremic toxins and protein carbonyl (PCO) as an oxidative stress marker in chronic HD patients.
Methods: Twenty patients on chronic low flux HD were recruited from El-Shefaa Hospital in Alexandria. At the start of the study, all patients were switched to high flux HD for 8 weeks (period A), followed by 2 weeks low flux HD as a wash out period. Then, the patients had another 8 weeks of low flux HD (period B). Pre-dialysis serum samples were taken at beginning and end of period A (pre-high flux and post-high flux) and at beginning and end of period B (pre-low flux and post-low flux). B2MG was measured by ELISA, AOPP and PCO were assayed spectrophotometrically.

Abbreviations: AOPP, advanced oxidation protein products; HD, hemodialysis; B2MG, beta-2 microglobulin; PCO, protein carbonyl.

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Results: Post-high flux B2MG and PCO levels were significantly lower than pre-high flux levels ($p = 0.031$ and 0.036, respectively), but no significant change was observed in AOPP ($p = 0.8$). Post-low flux B2MG, AOPP and PCO were all significantly higher than pre-low flux levels ($p = 0.0002$, 0.019, and 0.0002, respectively). Post-low flux B2MG and PCO were significantly higher than post-high flux levels ($p < 0.0001$and $p = 0.0005$, respectively) but no significant difference was observed in AOPP ($p = 0.112$). Pre-high flux PCO positively correlated with AOPP ($p = 0.013$) and B2MG ($p = 0.01$). Post-high flux PCO positively correlated with B2MG ($p = 0.036$).

Conclusion: High flux hemodialysis results in the reduction of serum beta-2 microglobulin and protein carbonyl whereas advanced oxidation protein products are not affected. On the other hand, low flux hemodialysis is associated with increased levels of the three markers. High flux hemodialysis thus allows better removal of uremic toxins than low flux hemodialysis.

1. Introduction

The uremic syndrome is characterized by the retention of toxins that adversely affect various metabolic processes and contribute to the associated morbidity and mortality. According to their sizes and hydrophobicity, uremic toxins may be: (a) water-soluble, small molecules (molecular weight < 500 Da) such as urea. These are easily removed by any dialysis approach. (b) Middle molecules with a molecular weight > 500 Da. (c) Protein-bound hydrophobic solutes such as phenolic compounds.[1,2]

Middle molecules include beta-2 microglobulin (B2MG), advanced glycation endproducts (AGE), advanced oxidation protein products (AOPP), and other peptides. High flux dialysis using membranes that have large pores may allow more efficient removal of these large molecules than low flux dialysis that uses membranes with smaller pore sizes.[2]

B2MG forms the beta chain of the human leukocyte antigen class I molecule. It is present on the surface of most nucleated cells and in most biological fluids.[3] It is mainly excreted by the kidney with negligible extra-renal elimination. B2MG has a molecular weight of 11.8 kDa and has been widely used for assessment of dialysis efficiency as regards middle molecule removal.[5] Longstanding increases in serum B2MG levels are associated with the development of dialysis-related amyloidosis in patients on chronic hemodialysis.[4,6]

During dialysis, blood contact with dialyzer membrane results in the activation of mononuclear cells and neutrophils. This results in the generation of large amounts of reactive oxygen species. The resulting oxidative stress is implicated in the accelerated atherogenesis and increased cardiovascular disease seen in patients on chronic hemodialysis.[7–9]

Advanced oxidation protein products (AOPP) are uremic toxins created during oxidative stress through the reaction of chlorinated oxidants with plasma proteins.[10] Oxidation of amino acid residues such as tyrosine leading to formation of dityrosine, protein cross-linking and fragmentation as well as loss of enzymatic or other functional properties.[7,11] Advanced oxidation protein products have variable molecular weights depending on the ligand to which they are linked.[1] Besides being markers of oxidative stress, AOPP appear to have a pro-inflammatory action.[12]

Oxidation of carbohydrates and lipids may lead to the production of reactive carbonyl groups (RCOs). The association of reactive oxygen species (ROS) and RCOs with proteins results in the formation of carbonylated dysfunctional proteins in plasma and tissues.[11] Protein carbonyl (PCO) content is considered a good marker for oxidative stress-dependent cellular damage.[13] A general increase in plasma protein carbonyls in uremic patients has been reported.[8,14] Besides, protein carbonyls are precursors of advanced glycation end-products (AGE) which constitute a middle molecule toxin that plays a vital role in the progression of renal failure.[15,16]

Since the heterogeneity of middle molecule uremic toxins with respect to their molecular weight and protein binding may affect their removal by hemodialysis, this work was carried out to investigate the effect of high flux versus low flux hemodialysis on serum levels of uremic toxins beta-2 microglobulin and advanced oxidation protein products as well as the oxidative stress marker protein carbonyl in chronic hemodialysis patients.

2. Methods

Twenty patients (16 males and four females) with end stage renal disease (ESRD) undergoing chronic regular hemodialysis were recruited from El-Shefaa Hospital in Alexandria, Egypt. All patients were in a stable clinical condition. A group of 20 healthy age-matched subjects were included as a control group. Smokers and subjects receiving anti-inflammatory or antioxidant therapy were excluded from the study.

Informed consent was taken from all participants in the study. The study was approved by the ethics committee of the Faculty of Medicine, Alexandria University.

2.1. Study design

Before starting the study, all patients had been on chronic regular low flux HD. At the beginning of the study, all patients were switched to high flux HD for a period of 8 weeks (period A). This was followed by a wash-out period (period W) of 2 weeks on low flux HD to exclude carry-over effects. Patients were then maintained on low flux HD for a further period of 8 weeks (period B). Since different membrane types may have different effects on the oxidative status in HD patients,[17] both high flux and low flux membranes were made of the same material (synthetic polyamide blend membranes: polyflux 170H and polyflux 17L respectively, Gambro Co, USA).

All patients were dialyzed 3 times/week using bicarbonate dialysate. Dialysate flow rate was 500 mL/min. The duration

of each session varied from 4–6 h. Blood flow rate ranged between 250–400 mL/min with a mean of 323 ± 42.2 mL/min. Duration of HD sessions and blood flow rate were varied to reach a urea reduction ratio (URR) of > 65%.[18] All patients were on a maintenance dose of human recombinant erythropoietin after reaching target hemoglobin level (11–12 g/dL). No blood transfusion was given to any patient during the study period.

Blood samples were taken before the first dialysis session of the high flux period A (pre-high flux), another pre-dialysis sample was taken at the end of this period (post-high flux). A third pre-dialysis sample was taken at the beginning of period B (pre-low flux) and a fourth pre-dialysis sample was taken at the end of this period (post-low flux).

Full history taking and clinical examination were carried out. In addition, complete blood picture was performed/4 weeks. Erythropoietin dose was adjusted to maintain hemoglobin level at 11–12 g/dL. Pre- and post-dialysis serum urea was measured monthly once to calculate urea reduction ratio: $URR = (urea_{pre\text{-}dialysis} - urea_{post\text{-}dialysis})/urea_{pre\text{-}dialysis} \times 100$.

The following laboratory tests were done on each of the four venous blood samples obtained from each patient and once for the control subjects:

2.1.1. Beta-2-microglobulin assay

This was done by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (DRG® Microglobulin, beta-2 EIA Kit, DRG International, Inc., USA).

2.1.2. Advanced oxidation protein products (AOPP)[10]

AOPP were measured spectrophotometrically. In 96-well microtiter plates, 200 μL serum (diluted 1:5 in phosphate-buffered saline) was acidified using 20 μL acetic acid. Absorbance of the reaction mixture was read immediately at 340 nm against a blank containing 200 μL PBS, 10 μL 1.16 mol/L potassium iodide, and 20 μL acetic acid. AOPP content was determined using a standard curve constructed with chloramine-T standard solutions (Sigma–Aldrich, USA). AOPP concentrations are expressed in μmol/L chloramine-T equivalents.

2.1.3. Protein carbonyl[19]

Protein carbonyl was measured spectrophotometrically using OxiSelect protein carbonyl spectrophotometric kit (Cell Biolabs, Inc., USA). The test is based on derivitization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH). The amount of protein-hydrozone produced is measured spectrophotometrically at 375 nm. Carbonyl levels are expressed as nmol/mg of protein.

2.1.3.1. Procedure. Serum protein was assayed on the Dimension RXL chemistry autoanalyzer (Siemens Healthcare Diagnostics, Inc., USA) and was adjusted to 1 mg/mL using distilled water. One milliliter of DNPH was added to 250 μL of diluted serum. After incubation, protein was precipitated using 1.25 mL trichloracetic acid 20%. The protein pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v) to remove any free DNPH and was solubilized in 250 μL 1 M NaOH. Absorbance was read at 370 nm. Carbonyl content was calculated using molar extinction coefficient of DNPH (22×10^3 mol^{-1} cm^{-1}). Since some protein may be lost during the washing steps, the protein content of the final solubilized pellet was measured by the Lowry method.[20] Carbonyl content was standardized to protein content and was expressed as nmol/mg protein.

2.2. Statistical analysis

Data were analyzed using STATVIEW software version 5.1 for Windows (SAS Institute Inc., NC, USA). Comparisons between patients and controls were done using Mann Whitney test. Changes in studied parameters within the patients group were analyzed using Wilcoxon signed rank test. Spearman correlation test was used to investigate the correlation between the studied variables. Statistical significance was considered at p value < 0.05.

3. Results

The clinical characteristics of 20 patients on chronic hemodialysis are depicted in Table 1. Hypertension was the most common cause of chronic kidney disease (30%), while the cause was not identified in 15% of patients. Three patients (15%) had cardiomegaly and two patients (10%) had diabetic retinopathy. Table 2 presents baseline biochemical data of HD patients versus controls. Serum B2MG, AOPP and PCO were significantly higher in HD patients than in controls ($p < 0.0001$).

Significantly lower levels of serum B2MG and PCO were observed at the end of the high flux dialysis period (post-high flux) compared to pre-high flux levels. ($p = 0.031$ and 0.036, respectively). On the other hand, no statistically significant change was observed in AOPP levels during this period ($p = 0.8$). (Table 3). The mean percent change (± standard error of the mean, SEM) in B2MG, AOPP and protein carbonyl during this period was −7.97 ± 6.84%, 1.82 ± 3.63% and −7.69 ± 5.04%, respectively (Fig. 1).

Levels of the studied parameters were comparable at the start of high flux (period A) and low flux (period B) HD, indicating the adequacy of the intervening washout period (B2MG: $p = 0.313$, AOPP: $p = 0.472$, protein carbonyl: $p = 0.66$).

Significantly higher levels of B2MG, AOPP and protein carbonyl were observed at the end of 8 weeks of low flux HD (post-low flux) compared to their levels at the beginning of this period (pre-low flux), ($p = 0.0002$, 0.019 and 0.0002, respectively) (Table 3). Mean% change (± standard error of the mean, SEM) during period B for B2MG, AOPP and PCO was: 30.31 ± 6.43%, 9.13 ± 3.27% and 31.03 ± 5.05%, respectively (Fig. 1).

Post-low flux levels of B2MG and protein carbonyls were significantly higher than post-high flux levels ($p < 0.0001$ and $p = 0.0005$, respectively). On the other hand, no significant difference was observed in AOPP levels ($p = 0.112$). No significant change was observed in serum urea level after high flux ($p = 0.41$) or low flux hemodialysis ($p = 0.57$).

At the baseline, a positive correlation was observed between pre-high flux protein carbonyl and AOPP ($r = 0.569$, $p = 0.013$). Protein carbonyl was also correlated with B2MG ($r = 0.591$, $p = 0.01$). At the end of period A, post-high flux protein carbonyl positively correlated with B2MG ($r = 0.421$, $p = 0.036$). No other correlations were detected between the studied parameters.

Table 1 Characteristics of 20 patients on chronic hemodialysis.

Age (years)	61.8 ± 7.5 (45–75)
Gender	
Male	16 (80%)
Female	4 (20%)
Duration of CKD (years)	6.7 ± 4.3(2–17)
Duration of HD (years)	4.3 ± 2.7 (1–10)
Cause of CKD	
Hypertension	6 (30%)
Gout	4 (20%)
Diabetes mellitus	3 (15%)
Ostructive uropathy	3 (15%)
Unknown	3 (15%)
Polycystic kidney	1 (5%)
Systolic blood pressure (mmHg)	130.0 ± 20.3 (80–160)
Diastolic blood pressure (mmHg)	79.0 ± 8.5 (60–90)

Results are expressed as mean ± SD (minimum–maximum) and as number (%).
CKD: chronic kidney disease, HD: hemodialysis.

4. Discussion

Some of the morbidity associated with chronic hemodialysis such as hypertension and cardiovascular disease is thought to result from retention of middle molecule uremic toxins that are poorly removed by hemodialysis using low-flux membranes with small pores. Recent clinical studies suggest that enhancing the removal of these compounds has a beneficial effect on survival and the quality of life of patients with end stage renal disease. This can be accomplished by using high-flux dialysis membranes with large pore sizes, increasing dialysis frequency or prolonging the dialysis session.[21]

In the present study, we explored the effect of high flux versus low flux hemodialysis on serum levels of beta-2 microglobulin and advanced oxidation protein products as representative middle molecules. We also investigated the levels of the oxidative stress marker protein carbonyl.

In agreement with previous reports[22,23] our study showed that at the start of the study, baseline pre-dialysis serum levels of B2MG, are considerably higher in chronic HD patients than in controls. It has been suggested that elevated B2MG concentrations lead to dialysis-related amyloidosis and comorbidities that contribute to early mortality.[6,24,25] Okuno et al.[25] concluded that serum beta-2-microglobulin level is a significant independent predictor of mortality in hemodialysis patients, suggesting the clinical importance of lowering serum beta-2-microglobulin in these patients.

In the present study, serum B2MG levels showed a significant decrease after 8 weeks on hemodialysis using high-flux membranes whereas the use of low-flux dialysis resulted in increased levels. Similarly, several studies demonstrated that

Table 2 Baseline biochemical data and uremic toxins in HD patients versus controls.

	Patients (n = 20)	Controls (n = 20)	p Value
Hemoglobin (g/dL)	12 ± 0.6	12.3 ± 0.5	0.15
S. urea (mg/dL)	147.4 ± 33.4	28.7 ± 6.4	< 0.0001*
S. creatinine (mg/dL)	8.9 ± 2.4	0.8 ± 0.17	< 0.0001*
S. B2MG (mg/L)	75.4 ± 18.3	1.29 ± 0.28	< 0.0001*
S. AOPP (μmol/L)	85.9 ± 11.3	33.4 ± 7.9	< 0.0001*
S. PCO (nmol/mg protein)	1.45 ± 0.3	0.97 ± 0.28	< 0.0001*

Results are expressed as mean ± standard deviation. Data were analyzed by Mann–Whitney test. AOPP: advanced oxidation protein products, B2MG: beta-2 microglobulin, PCO: protein carbonyl, HD: hemodialysis.
* Statistically significance at $p < 0.05$.

Table 3 B2MG, AOPP and protein carbonyls in 20 patients before and after high flux and low flux HD.

	High flux HD (period A, 8 weeks)		Low flux HD (period B, 8 weeks)	
	Pre-high flux	Post-high flux	Pre-low flux	Post-low flux
B2MG (mg/L)				
Mean ± SD	75.4 ± 18.3	64.9 ± 9.5	70.5 ± 5.6	91.6 ± 20.4
p Value	0.031*		0.0002*	
AOPP (μmol/L)				
Mean ± SD	85.9 ± 11.3	86.5 ± 12.2	84.7 ± 14.2	91.3 ± 13.1
p Value	0.8		0.019*	
PCO (nmol/mg protein)				
Mean ± SD	1.45 ± 0.29	1.3 ± 0.27	1.39 ± 0.25	1.81 ± 0.35
p Value	0.036*		0.0002*	

Data were analyzed using Wilcoxon signed rank test. AOPP: advanced oxidation protein products, B2MG: beta-2 microglobulin, PCO: protein carbonyl, HD: hemodialysis.
* Statistically significance at $p < 0.05$.

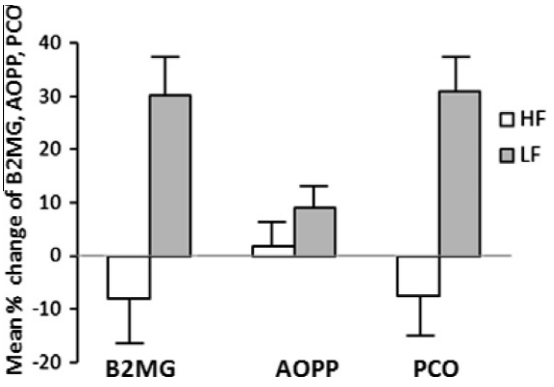

Figure 1 Mean% change in B2MG, AOPP and PCO in response to high flux and low flux HD. Results are shown as mean ± standard error of the mean (SEM). HF: high flux HD, LF: low flux HD.

pre-dialysis B2MG level was significantly lower with the use of high flux dialyzers than with low-flux dialyzers.[26–28]

Due to its negligible extra-renal clearance from the circulation, B2MG has been used as a marker of uremic toxins in the "middle molecule" range, in particular for the assessment of dialysis efficiency.[4] However, beta-2-microglobulin levels are not only the result of clearance but also of inflammation, which is a hallmark of uremic toxicity. In addition, several other middle molecules are bound to proteins and, therefore, their kinetic behavior and inter-compartmental transport is quite different from that of beta-2-microglobulin.[3,29] It is, therefore, questionable if B2MG alone is sufficient for the evaluation of adequate middle molecule removal in HD patients. It is probably useful to measure other middle-molecule substances in addition to B2MG.

In the present study, AOPP were higher in HD patients than in controls. The elevated levels of AOPP in uremic patients, especially those on HD therapy, have been previously reported.[7,30] In the present study, plasma AOPP levels were not changed in response to 8 weeks high flux hemodialysis. A similar observation was reported by Ward et al.[31] Bordoni et al.[32] observed that plasma carbonyl residues but not AOPP were significantly lowered in patients on high flux HD. They also noted that neither of the two markers decreased in patients on low flux therapy. The absence of an effect of high flux HD on AOPP level may be related to the large molecular weight of AOPP molecules which are the result of damage to albumin as well as proteins of higher molecular weights.

In uremic patients, particularly those undergoing maintenance HD, proteins may undergo a variety of modifications including carbonylation. This results in defective protein function, formation of advanced glycation end products and cellular dysfunction.[33] In the present study, patients on chronic HD had significantly higher levels of PCO content than control subjects. Similar results have been reported by other investigators.[11,14,33,34] Carbonyl stress has been shown to contribute to long-term complications associated with chronic renal failure and hemodialysis such as dialysis-related amyloidosis and accelerated atherosclerosis.[35]

Carbonyl stress in HD patients' results not only from uremia, hemodialysis therapy may also have a relevant impact on carbonyl balance. During HD, biochemical reactivity following contact of blood with the dialysis membrane and the loss of antioxidant substances, may promote carbonyl formation via an increase of oxidative stress.[36–38] On the other hand, HD therapy may reduce carbonyl overload by removing low molecular weight reactive carbonyl compounds. Pavone et al.[33] demonstrated that HD membranes possess the ability to adsorb and carbonylate plasma proteins and concluded that hemodialytic membranes may affect the carbonyl balance in chronic uremic patients. The level of protein carbonyl is, therefore, a net result of the balance between increased production and removal.

In the present study, serum protein carbonyl was significantly reduced at the end of the high-flux hemodialysis period while levels significantly increased by the end of low-flux hemodialysis period. It is possible that in the case of low flux dialysis, the production of reactive carbonyl groups exceeded the ability of the dialysis membranes to remove these groups. This probably resulted in carbonylation of proteins whose molecular weights are much larger than the range that could be effectively removed by low flux membranes.[33]

A positive correlation was observed in the present study between PCO content and B2MG levels both at baseline and 8 weeks after high flux HD. An explanation of this relation may be provided by the work of Miyata et al.[39] who reported that AGE-modified B2MG binds to AGE receptors on monocytes, leading to production of cytokines and reactive oxygen species, which in turn enhance tissue toxicity.

In conclusion, serum beta-2 microglobulin and protein carbonyls were significantly reduced in response to high flux hemodialysis, whereas advanced oxidation protein products were not affected. On the other hand, hemodialysis using low flux membranes resulted in significantly increased levels of the three markers. Using high flux hemodialysis thus allows improved removal of a wider spectrum of uremic toxins which may improve the quality of life and reduce the morbidity and mortality in patients on chronic hemodialysis.

References

1. Vanholder R, Van Laecke S, Glorieux G. The middle-molecule hypothesis 30 years after: lost and rediscovered in the universe of uremic toxicity. *J Nephrol* 2008;**21**:146–60.
2. Vanholder R, De Smet R, Glorieux G, et al. Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney Int* 2003;**63**:1934–43.
3. Drueke TB, Massy ZA. Beta2-microglobulin. *Semin Dial* 2009;**22**:378–80.
4. Vanholder R, Eloot S, Van Biesen W. Do we need new indicators of dialysis adequacy based on middle-molecule removal? *Nat Clin Pract Nephrol* 2008;**4**:174–5.
5. European best practice guidelines expert group on hemodialysis. European renal association, European best practice guidelines for haemodialysis (part 1), section II: haemodialysis adequacy: II.2 Haemodialysis dose quantification: middle molecules (MM). Nephrol Dial Transplant 2002;17:21–3.
6. Yamamoto S, Kazama JJ, Narita I, Naiki H, Gejyo F. Recent progress in understanding dialysis-related amyloidosis. *Bone* 2009;**45**(Suppl. 1):S39–42.
7. Coskun C, Kural A, Doventas Y, et al. Hemodialysis and protein oxidation products. *Ann NY Acad Sci* 2007;**1100**:404–8.
8. Dursun B, Dursun E, Suleymanlar G, et al. Carotid artery intima-media thickness correlates with oxidative stress in chronic haemodialysis patients with accelerated atherosclerosis. *Nephrol Dial Transplant* 2008;**23**:1697–703.

9. Himmelfarb J. Uremic toxicity, oxidative stress, and hemodialysis as renal replacement therapy. *Semin Dial* 2009;**22**:636–43.
10. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, et al. Advanced protein oxidation products as a marker of oxidative stress in uremia. *Kidney Int* 1996;**49**:1304–13.
11. Himmelfarb J, McMongale E, McMenamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney Int* 2000;**28**:2571–8.
12. Witko-Sarsat V, Friedlander M, Nguyen KT, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998;**161**:2524–32.
13. Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Milzani A. Protein carbonylation in human diseases. *Trends Mol Med* 2003;**9**: 169–76.
14. Koken T, Serteser M, Kahraman A, Gokce C, Demir S. Changes in serum markers of oxidative stress with varying periods of haemodialysis. *Nephrology* 2004;**9**:77–82.
15. Schwedler S, Schinzel R, Vaith P, Wanner C. Inflammation and advanced glycation end products in uremia: simple coexistence, potentiation or causal relationship? *Kidney Int* 2001;**59**(78):S32–6.
16. D'Agati V, Schmidt AM. RAGE and the pathogenesis of chronic kidney disease. *Nat Rev Nephrol* 2010;**6**:352–60.
17. Wu CC, Chen JS, Wu WM, et al. Myeloperoxidase serves as a marker of oxidative stress during single haemodialysis session using two different biocompatible dialysis membranes. *Nephrol Dial Transplant* 2005;**20**:1134–9.
18. Gotch FA. Evolution of the single-pool urea kinetic model. *Semin Dial* 2001;**14**:252–6.
19. Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol* 1994;**233**:346–57.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;**193**:265–75.
21. Vanholder R, Glorieux G, Van Biesen W. Advantages of new hemodialysis membranes and equipment. *Nephron Clin Pract* 2010;**114**:c165–72.
22. Traut M, Haufe CC, Eismann U, Deppisch RM, Stein G, Wolf G. Increased binding of beta-2-microglobulin to blood cells in dialysis patients treated with high-flux dialyzers compared with low-flux membranes contributed to reduced beta-2-microglobulin concentrations. Results of a cross-over study. *Blood Purif* 2007;**25**: 432–40.
23. Mumtaz A, Anees M, Bilal M, Ibrahim M. Beta-2 microglobulin levels in hemodialysis patients. *Saudi J Kidney Dis Transpl* 2010;**21**:701–6.
24. Cheung AK, Rocco MV, Yan G, et al. Serum beta-2 microglobulin levels predict mortality in dialysis patients: results of the HEMO study. *J Am Soc Nephrol* 2006;**17**:546–55.
25. Okuno S, Ishimura E, Kohno K, et al. Serum beta2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. *Nephrol Dial Transplant* 2009;**24**:571–7.
26. Ayli M, Ayli D, Azak A, et al. The effect of high-flux hemodialysis on dialysis-associated amyloidosis. *Ren Fail* 2005;**27**:31–4.
27. Grooteman MPC, Gritters M, Wauters IM, et al. Nub. Patient characteristics rather than the type of dialyser predict the variability of endothelial derived surface molecules in chronic haemodialysis patients. *Nephrol Dial Transplant* 2005;**20**:2751–8.
28. Li PK, Cheng YL, Leung CB, et al. Effect of membrane permeability on inflammation and arterial stiffness: a randomized trial. *Clin J Am Soc Nephrol* 2010;**5**:652–8.
29. Ward RA, Greene T, Hartmann B, Samtleben W. Resistance to intercompartmental mass transfer limits beta2-microglobulin removal by post-dilution hemodiafiltration. *Kidney Int* 2006;**69**: 1431–7.
30. Kalousova M, Sulkova S, Fialova L, et al. Glycoxidation and inflammation in chronic haemodialysis patients. *Nephrol Dial Transplant* 2003;**18**:2577–81.
31. Ward RA, Ouseph R, Mcleish KR. Effects of high-flux hemodialysis on oxidant stress. *Kidney Int* 2003;**63**:353–9.
32. Bordoni V, Piroddi M, Galli F, et al. Oxidant and carbonyl stress-related apoptosis in end-stage kidney disease: impact of membrane flux. *Blood Purif* 2006;**24**:149–56.
33. Pavone B, Sirolli V, Bucci S, et al. Adsorption and carbonylation of plasma proteins by dialyser membrane material: in vitro and in vivo proteomics investigations. *Blood Transfus* 2010;**8**(Suppl. 3): s113–9.
34. Raj DS, Dominic EA, Pai A, et al. Skeletal muscle, cytokines, and oxidative stress in end-stage renal disease. *Kidney Int* 2005;**68**: 2338–44.
35. Miyata T, Saito A, Kurokawa K, de Strihou C. Advanced glycation and lipoxidation end products: reactive carbonyl compounds-related uraemic toxicity. *Nephrol Dial Transplant* 2001;**16**(Suppl. 4):8–11.
36. Dursun E, Dursun B, Suleymanlar G, Ozben T. Carbonyl stress in chronic renal failure: the effect of haemodialysis. *Ann Clin Biochem* 2005;**42**:64–6.
37. Mitrogianni Z, Barbouti A, Galaris D, Siamopoulos KC. Oxidative modification of albumin in predialysis, hemodialysis, and peritoneal dialysis patients. *Nephron Clin Pract* 2009;**113**:234–40.
38. Koca T, Berber A, Koca HB, Demir TA, Koken T. Effects of hemodialysis period on levels of blood trace elements and oxidative stress. *Clin Exp Nephrol* 2010;**14**:463–8.
39. Miyata T, Hori O, Zhang J, et al. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 1996;**98**: 1088–94.