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Original article

Influence of dialysis modalities on removal efficiency of serum AGE-Peptides determined by FIA

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ABSTRACT

To compare the removal efficiency for advanced glycosylation end products-peptides (AGE-P) from serum of patients with end stage renal disease (ESRD) by different type of dialysis membrane and modalities. Methods: 78 patients with ESRD were randomly divided into three groups: polysulfon membrane (F6), bichloroacetate cellulose membrane (CA150), hemophane membrane (HE). They were on hemodialysis（HD） with these three different types of low-flux (LF) membranes. Among these people, 66 patients were randomly divided into three groups: HD with LF membranes, HD with High-flux (HF) membranes, HDF. The serum AGE-P concentrations were measured by using flow injection assay (FIA) at the time before and after HD or HDF. Results: In LF-HD, the reduction rate of AGE-P is not significantly different among F6, CA150 and HE. The significant decrease of serum AGE-P was observed after HDF comparing with LF-HD. The significant reduction of serum AGE-P was observed after HDF treatment comparing with LF-HD, but not statistically significant with HF-HD. Conclusions: The serum AGE-P level in patients with ESRD is elevated and can be removed by HD and HDF. HF-HD or HDF was superior to remove the AGE-P in dialysis patients.

1. Introduction

The accumulation of advanced glycosylation end products (AGEs) in the patients with ERSD is related to the development of various long-term complications (i.e. cardiovascular disease) [1, 2] and complications of the uremic syndrome [3]. The reduction of serum AGEs result in a decrease in morbidity and mortality of cardiovascular complications in ESRD. Human serum AGE-P is shown to retain strong cross-linking activity with collagen in vitro. AGE-P in human circulation may represent a thus far unrecognized class of reactive and potentially toxic substances which can exacerbate extra renal vascular pathology, through their covalent attachment onto matrix proteins. Therefore AGE-P are highly reactive species and can interact with tissue and circulating proteins, leading to tissue modification and impaired protein functionality [4]. Our previous study proved by FIA that HD decreased serum AGE-P levels in patients with ESRD [5], but which kind of dialysis modalities are better for clearance of AGE-P has not been investigated [6]. In present study, we compare the removal efficiency of AGE-P from serum of patients with ESRD by different type of HD membranes and modalities.

2. Materials and Methods

2.1.Study Patients

A total of 78 patients on three times per week maintenance HD (45 males and 33 females) either out-patient clinic or in Department of nephrology of Zhongda Hospital in Southeast University in Nanjing were studied. All were on HD with the same membrane type for three months or more. Their mean age was 60.1 ± 10.0 (SD) years. All these patients with ESRD were randomly divided into three groups: polysulfon membrane (F6, surface area 1.3m^2, ultrafiltrate coefficient 5.5ml·mmHg^{-1}·h^{-1}, Fresenius Medical Care, Germany), bichloroacetate cellulose

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(CA150, surface area 1.5m^2, ultrafiltrate coefficient 6.8ml·mmHg-1·h^{-1}, Baxter, USA), hemophane membrane (HE, surface area 1.5m^2, ultrafiltrate coefficient 8.7ml·$mmHg^{-1}$·h^{-1}, Cobe, USA). They were on HD with these three different types of LF membranes. Among these people, 66 patients (35 males and 31females) were randomly divided into three groups: HD with LF membranes (F6, surface area 1.3m^2, ultrafiltrate coefficient 5.5ml·$mmHg^{-1}$·h^{-1}, Fresenius Medical Care, Germany), HD with HF membranes (F60, surface area 1.3m^2, ultrafiltrate coefficient 40ml·$mmHg^{-1}$·h^{-1}, Fresenius Medical Care, Germany), HDF（F60）. Their mean age was 59.0 ± 9.0 (SD) years. The baseline characteristics of the study participants are shown in Table 1 and Table 2. All the baseline characteristics (age, sex, duration of HD prior to the study , ALB, BMI, Ccr , Kt/Vurea, β2MG , blood glucose、 serum AGE-P level before HD)were similar in the 3 study groups in Table 1 and Table 2.

Table1. Baseline characteristics of the study groups in the first stage (x±s)

Characteristic	F6 (n=26)	CA150 (n=26)	HE (n=26)	P
Primary renal disease				
Diabetes	6	3	2	
Hypertension	3	6	1	
Glomerulonephritis	11	12	9	
Cystic kidney	2	1	1	
Tubulointerstitial nephritis	2	0	6	
Other	2	4	7	
Age (year)	59.8 ± 9.4	61.3 ± 10.2	59.1 ± 9.8	NS
Sex (Male/Female)	16 / 10	15 / 11	14 / 12	NS
Duration of haemo dialysis (month)	25.4 ± 11.2	24.9 ±12.8	25.9±10.7	NS
Albumin （g/L^{-1}）	36.9 ± 0.8	35.8 ± 0.9	36.2 ± 0.4	NS
BMI（kg/m^{-2}）	21.8 ± 3.2	23.3 ± 2.1	22.9 ± 2.5	NS
Creatinine clearance (ml/min^{-1})	5.4 ± 2.1	4.9 ± 2.3	5.1 ± 2.0	NS
Kt/Vurea	1.4 ± 0.1	1.6 ± 0.3	1.5 ± 0.3	NS
β2－microglobulin（mg/L^{-1}）	23.1 ± 8.3	24.5 ± 7.4	23.9 ± 8.5	NS
Glucose (pre-dialysis, mmol/ L^{-1})	6.3 ± 0.3	5.8 ± 0.9	6.1 ± 0.7	NS
AGE-P（pre-dialysis, u/ml^{-1}）	6.39 ± 1.36	5.81 ± 1.49	5.80±1.60	NS

Values are expressed as mean ± SD or percentage. The data were analyzed by one-way analysis of variance. NS indicates not significant.

Table2. Baseline characteristics of the study groups in the second stage (x±s)

Characteristic	LF-HD F6 (n=22)	HF-HD F60(n=22)	HF-HD F60 (n=22)	P
Primary renal disease				
Diabetes	3	2	1	
Hypertension	3	3	1	
Glomerulonephritis	10	12	9	
Cystic kidney	2	1	1	
Tubulointerstitial nephritis	2	0	4	
Other	2	4	6	
Age (year)	58.8 ± 9.4	58.3 ± 11.3	60.1 ± 8.5	NS
Sex (Male/Female)	12 / 10	11 / 11	12 / 10	NS
Duration of haem odialysis (Month)	26.4 ± 11.2	24.2 ±13.5	26.5 ± 11.2	NS
Albumin （g/L^{-1}）	34.9 ± 0.6	37.5 ± 0.6	37.8 ± 0.7	NS
BMI（kg/m^{-2}）	21.8 ± 2.2	22.4 ± 2.9	22.1 ± 3.1	NS
Creatinine clearance (ml/min^{-1})	5.5 ± 2.3	4.7 ± 2.4	5.0 ± 2.2	NS
Kt/Vurea	1.4 ± 0.1	1.5 ± 0.2	1.5 ± 0.3	NS
β2－microglobulin（mg/L^{-1}）	22.1 ± 8.6	22.8 ± 7.5	22.9 ± 7.2	NS
Glucose (pre-dialysis, mmol/ L^{-1})	6.2 ± 0.3	5.9 ± 0.7	6.1 ± 0.5	NS
AGE-P（pre-dialysis, u/ml^{-1}）	6.56 ± 0.92	6.16 ± 0.57	6.12 ± 0.55	NS

Values are expressed as mean ± sd or percentage. The data were analyzed by one-way analysis of variance. NS indicates not significant.

The serum AGE-P concentrations were measured by flow injection assay (FIA) at the time before and after HD or HDF.
The serum AGE-P concentrations of 33 healthy volunteers (18 males and 15 females) were also been measured by flow injection assay (FIA).

The reduction rate of AGE-P was calculated as follows:
AGE-P(%)= [AGE-P(pre-therapy)− AGE-P(post-therapy)]/ AGE-P(pre-therapy)×100%

2.2.Dialysis treatment modalities

LF-HD was performed as follows: blood flow 200250 ml.min^{-1}; dialysate flow 400-500 ml.min^{-1} using bicarbonate dialysate; treatment time 4h; HF-HD/ HDF was performed as follows: blood flow 250300 ml.min^{-1}; dialysate flow 500 ml/min using bicarbonate dialysate; treatment time 4h. No food and no coffee or tea was ingested during dialysis sessions.

2.3.Preparation of AGEP-BSA

BSA or HSA 50 gL^{-1} were incubated with D-glucose 0.5 molL^{-1} in the 0.2 molL^{-1} phosphate buffer saline (PBS, pH 7.4) at 37^{0} for 90 d. After incubation, dialysis against PBS was carried out to remove unbound glucose. Fluorescence spectra recorded using a 650-60 fluorospectrometer (Hitachi, Japan). Sephadex G-200 purified AGEP-BSA. The method of Bradford was used for quantification of proteins. The molecular mass of AGEP-BSA detected by mass spectrogram chromatogram was less than 10KD, showing the presence of a wide set of peptides, mostly less than 3KD (detected by pro. Zilin Sun, unpublished).

2.4. Quantitative fluorescence spectroscopy

Serum sample 50 μl was diluted into PBS 5.0 ml. After the samples were filtrated through 0.22 μm filters, fluorescence intensity of it was measured with a 650-60 fluorospectrometer at an excitation wavelength of 370 nm and an emission wavelength of 440 nm. Various dilutions of purified AGEP-BSA were used as calibrator and the sample AGE-P levels were calculated according the standard curve. The AGE-P value was defined as Uml^{-1}, 1.0 Uml^{-1} equal to 1.0 mgL^{-1} AGEP-BSA.

2.5. Flow injection assay

The 20 μl of serum samples were mixed with trichloroacetic acid (0.15 molL^{-1}) 480 μl and chloroform 100 μl in microcentrifuge tubes. The tubes were shaken vigorously to complete the precipitation of proteins and to extract lipids to organic phase and then were centrifuged (10 min, 13000×g). The 20 μl of the aqueous layer was injected to sample injector (loop 20 μl) of high performance liquid chromatography. Water flow rate was at 0.5 mlmin^{-1} and spectrofluorometric detector was set with emission wavelength at 440 nm and excitation wavelength at 370 nm for detection of AGE-P. The samples were analyzed in triplicate and peak height mode was used for signal measurement. Standard AGE-P (obtained by hydrolysis of AGEP-BSA with proteinase K) were diluted as 0.1, 0.5, 1, 5, 10, 50, 100 mgL^{-1} and performed for preparing calibration curve and calculating sample AGE-P as above described. The value was also defined as Uml^{-1}, 1.0 Uml^{-1} equal to standard AGE-P obtained from hydrolysis of 1.0 mgL^{-1} AGEP-BSA.

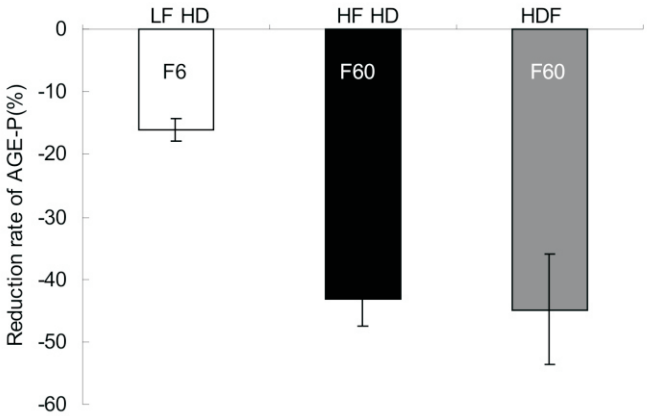
2.6. Statistic analysis

Data were expressed as means ± sd ($\bar{x}$ ±s). The serum AGE-P concentrations were compared between healthy volunteers and patients with end stage renal disease by paired t-test. The individual characteristics and the reduction rate of AGE-P were compared among groups of patients on HD with three different membranes and groups of patients on different modalities by one-way analysis of variance (with F tests).

3. Results

The reduction rate of AGE-P between groups of patients on different modalities. The significant decrease of serum AGE-P was observed after HF-HD comparing with LF-HD (43.09±4.35% vs 16.05±2.52%, P<0.05). The significant reduction of serum AGE-P was observed after HDF treatment comparing with LF-HD (44.82±8.90% vs 16.05±2.52%， P<0.05), but not significantly different from HF-HD. (Figure 1)

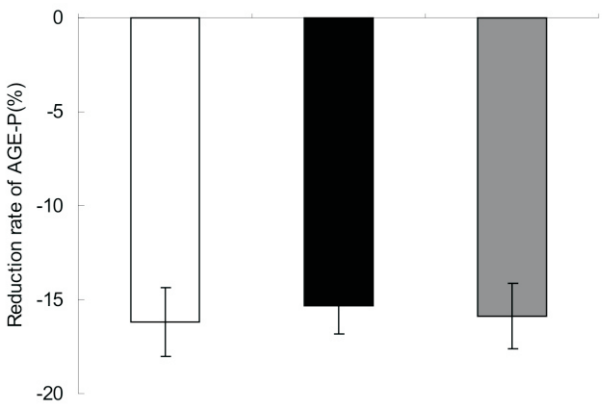
Figure1. The reduction rate of AGE-P with three different types of HD modalities.



*P<0.05 vs LF HD; #P>0.05 vs Group HDF

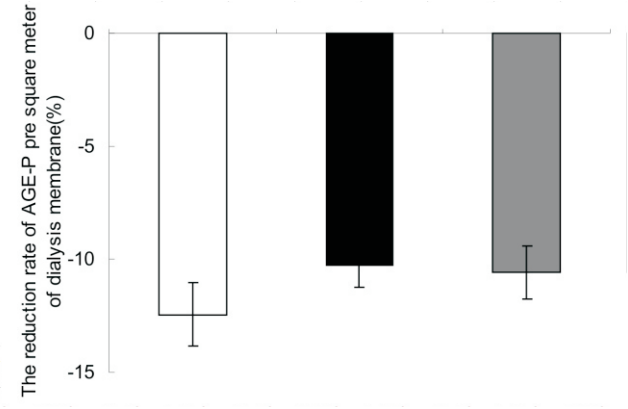
The reduction rate of AGE-P between groups of patients on HD with different low-flux membranes In LF-HD, the reduction rate of AGE-P is no statistical difference among F6, CA150 and HE(16.19±1.79%； 15.34±1.48%； 15.90±1.74%, respectively). (Figure 2) For per unit area of dialysis membrane the significant reduction rate of serum AGE-P was observed in group F6 comparing with group CA150 and HE（ 12.45±1.38% vs 10.23±0.98%, P<0.05； 12.45±1.38% vs 10.60±1.16%， P<0.05）, but not significantly different between the latter two. (Figure 3)

Figure2. The reduction rate of AGE-P with three different types of LF membranes.



No significant different among the three groups (P>0.05)

Figure3. The reduction rate of AGE-P pre square meter of dialysis membrane with three different types of LF membranes.



*P<0.05 vs F6

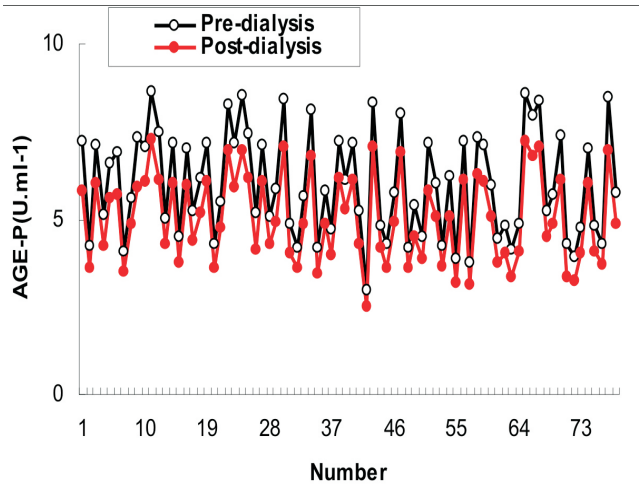
The serum AGE-P levels of controls and patients with ESRD The serum AGE-P levels in patients with ESRD were higher than those in controls (Table 3). The AGE-P level of each individual with ESRD in this study decreased after HD and HDF (Figure 4, 5), but it still significantly increased than controls.

Table3. The serum AGE-P levels of Group Control and Group ESRD (x±s)

Characteristic	Control (n=33)	ESRD (n=78)		ESRD (n=66)	
		Pre-dialysis	Post-dialysis	Pre-dialysis	Post-dialysis
AGE-P (u.ml-1)	1.24±0.43	6.00±1.49*	5.05±1.25*	6.28±0.71*	4.13±1.16*

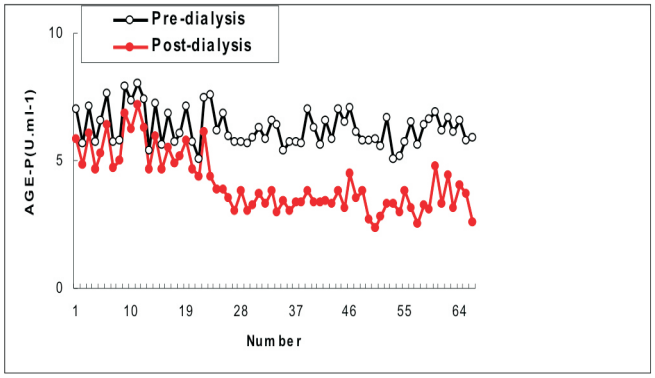
*P<0.05 vs control group.

Figure4. Pre- and post-therapy levels of AGE-P in individual patients with ESRD on HD with three different LF membranes.



Group F6: Number 1-26; Group CA150: Number 27-52; Group HE: Number 53-78

Figure 5. Pre- and post-therapy levels of AGE-P in individual patients with ESRD on different modalities.



Group LF HD: Number 1-22; Group HF HD: Number 23-44; Group HDF: Number 45-66

4. Discussion

Our study was designed to evaluate the removal efficiency of AGE-P determined by FIA from serum of patients with ESRD by different type of HD membranes and modalities. The most striking observation of this study is that the serum AGE-P level determined by FIA in patients with ESRD is elevated and can be removed by HD and HDF. The major factor effect on removal efficiency is dialysis modality.

In this study the serum AGE-P decreased after HD with all three different LF members, and there was no difference in reduction rate of AGE-P among the three groups. In various studies it has been demonstrated that removal of AGEs by dialysis is inadequate, mostly due to the fact that the majority of AGEs are protein-bound. It is know that LF-HD entails mainly diffusive to eliminate uremic material. The molecular weights less than 500 Da, characterized by a high degree of water solubility and the absence of protein binding, which can be removed by LF effectively. Thus, only small percentages of total pentosidine can be removed by dialysis [7]. Furthermore, it could be shown that the chemical nature of the dialysis membranes may have a higher impact on AGEs levels than the pore size [8]. We choose three deferent LF-HD membranes, which almost are in the same pore size to investigate if the chemical nature of it can effect on the reduction rate of AGE-P. There was no difference in reduction rate of AGE-P among the three LF membranes. But for per unit area of dialysis membrane the significant reduction of serum AGE-P was observed in-group F6 comparing with group CA150 and HE, but no statistical difference between group CA150 and HE. It may be accounting for the different absorption of the dialysis member, which is connected, with the chemical nature of dialysis membrane. For AGE-P can cross-linking widely with tissue, circulating proteins and combine with each other. Maybe we should find some dialysis membrane, which can combine with AGEs easily for example dialysis membrane surface covered specific anti-AGE antiboby in order to eliminate it efficiently.

Since dialysis modalities such as haemofiltration and HDF can influence serum AGE levels, which has been reported to remove peptides more efficiently than HF-HD [9]. Our study wasdesigned to focus on comparing the removal efficiency for advanced glycosylation end products-peptides (AGE-P) from

serum of patients with end stage renal disease (ESRD) by different type modalities with dialysis membrane of the same chemical nature and area. We confirm that the significant decrease of serum AGE-P was observed after HF-HD and HDF comparing with LF-HD. There is slightly higher reduction rate of AGE-P in-group HDF than group HF-HD, but no statistical significance.

HF-HD entails diffusive and convective mass transfer, and thus provides a convenient procedure to assess whether reactive groups are in filterable low molecular weight or non-filterable high molecular weight fractions. Isolated ultrafiltration allows the assessment of pure convective mass transfer depending on the sieving profile of the membrane. For the molecular mass range of AGE-P is wide, which covers both low molecular uremia (free pentosidine is 379 Da) and middle molecular uremia [10, 11], while the primary is middle molecular uremia, which can partly explain why the serum AGE-P level in patients with ESRD is removed by HF-HD and HDF effectively.

A number of studies have demonstrated that the serum AGEs levels in patients with end stage renal disease were higher than those in controls, either complicated with DM or not Gugliucci et al [12]. investigate that ESRD patients had a 3-fold increase in serum AGEs and a striking 10-fold increase in low molecular weight (<10 kDa) AGEs [13]. We used circulating AGE-P as the marker to assess the removal effect of hemodialysis because i. low-molecular but not high-molecular AGEs are removed by dialysis, ii low-molecular-weight AGE-P are highly reactive species and can interact with tissue and circulating proteins, leading to tissue modification and impaired protein functionality, which is essential and interested us. But determination of AGE-P by FIA just represented the low molecular weight of AGE-P which can produce fluorescence， not including immidazolone, CML, pyrroline [14]. To determine AGEs by ELISA， the anti-AGE antibody can not react with early Amadori products, pentosidine or caproyl pyrraline as well as with glycated proteins incubated under antioxidative conditions，so which can not include all the heterogeneity of AGE compounds， but the two methods may be complementary for each other [15].

5. Conclusion

In summary, our investigations revealed the following novel insights: (i) the serum AGE-P level determined by FIA in patients with ESRD is elevated and removed by HD and HDF. (ii) For removal efficiency, the major factor is dialysis modality. More work should be done on how to eliminate AGE-P efficiently and persistently.

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6. References

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