The Effectiveness of On-Line Hemodiafiltration on beta-2 Microglobulin Clearance in End Stage Renal Disease

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Objective: *To compare β_2-microglobulin (β_2M) clearance between on-line hemodiafiltration (HDF) and high flux demodialysis (HFHD).*
Material and Method: *The total, convection/diffusion, and membrane adsorption components of β_2M clearance in 10 hemodialysis patients treated with on-line HDF, at the replacement fluid rates of 75 (HDF75) and 125 (HDF125) mL/min, were determined and compared with HFHD.*
Results: *The total β_2M clearance in the HDF 125 group was significantly higher than the HDF75 group (124.5 ± 4.4 vs 101.3 ± 4.1 mL/min; $p < 0.05$); both values were much greater than the HFHD group ($p < 0.01$). The convection/diffusion was the major portion of total β_2M clearance in all three groups. The values of convection/diffusion and membrane adsorption in both HDF groups were about 2 and 3 times, respectively, of the HFHD group ($p < 0.01$). Both components of β_2M clearance in the HDF125 group did not statistically differ from the HDF75 group, however; the value of convection/diffusion clearance in HDF125 was more than in the HDF75 group. Regarding Kt/Vurea and phosphate clearance, there were no significant differences among the study groups.*
Conclusion: *On-line HDF could provide more β_2M clearance than HFHD by increasing both the convection/diffusion, and membrane adsorption clearances. HDF125 provided more total β_2M clearance than HDF75 from the convection/diffusion mechanism while the adsorptive mechanisms were equal.*

Keywords: *Hemodiafiltration, High flux hemodialysis, β_2-microglobulin, Convection/diffusion, Membrane adsorption*

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End stage renal disease (ESRD) patients have retention of numerous uremic toxins(1-3). Most of the current hemodialysis (HD) modalities, which mainly provide diffusion, can effectively eliminate small molecular weight uremic toxins. Large molecular meight uremic toxins (LMWUT) including β_2-Microglobulin (β_2M), culprits for morbidity and mortality in HD patients(4-8), cannot be adequately dialyzed by HD treatments(9). Hemodiafiltration (HDF) is becoming a popular therapeutic modality because the procedure could provide convection clearance, which is a physiological process in the normal kidney(10) and diffusion process. The greater replacement fluid rate could induce more LMWUT clearance(11).

Indeed, the total β_2M clearance occurring in any HD modalities consists of 2 portions: convection/diffusion and membrane adsorption(12). Most previous studies regarding on-line HDF determined β_2Mclearance by direct dialysate measurement. This represents only the convection/diffusion but not the membrane adsorption component. Thus, the actual total β_2M clearance in HDF, which comprises convection/diffusion and adsorption components, remains incompletely un explored. There is no available data regarding the membrane adsorption component in HDF compared

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between different replacement fluid rates of HDF itself and HFHD. Moreover, whether the replacement fluid rates affect the magnitude of membrane adsorption of HDF remains unknown.

The present study was carried out in HD patients treated with on-line HDF to assess and compares the total, proportion of diffusion/convection and membrane adsorption of β_2M clearance between the two different replacement fluid rates, 75 and 125 mL/min.

Material and Method

Patients

This prospective study was conducted, at King Chulalongkorn Memorial Hospital, Bangkok Thailand, in 10 stable ESRD patients who were treated with twice-a-week high-flux hemodialysis for more than 6 months and had vascular access flow rate of more than 350 mL/min. The study was approved by the Ethics Research Committee, Faculty of Medicine, Chulalongkorn University. Each patient participating in the study gave written consent. The patients were excluded from the study if they had symptomatic cardiovascular diseases, history of previous severe intradialytic hypotension, hematocrit levels above 45%, vascular access recirculation of more than 5%, and infection/inflammation conditions within 2 weeks.

Study design

Each enrolled patient was assigned to receive three different dialysis modalities: HFHD, hemodiafiltration with 75 ml/min post-dilution replacement rate (HDF75), and hemodiafiltration with 125 ml/min post-dilution replacement rate (HDF125). Each dialysis modality was performed three times in each patient and the average values of various parameters were determined and used in statistical comparison. All dialysis sessions were carried out by the Fresenius4008H machine using a new high-flux polysulfone (F80S) hemodialyzer. The dialysis fluid quality was tested monthly and complied with the ultrapure criteria according to the European Pharmacopoeia[13].

In each dialysis session, the mean values of total clearance and reduction ratio of β_2M were determined. Then, convection/diffusion and membrane adsorption clearances of β_2M clearances were assessed. Other small solute clearances including urea and phosphate were also measured.

Sample collection and measurement

In each study session, blood flow rate was measured at hours 1, 2, 3, and 4 of the treatment and blood samples were taken simultaneously from the blood inlet and outlet dialyzer ports. The dialysate samples were collected at hours 1, 2, 3, and 4 for calculation of convection/diffusion clearance.

All the blood samples were centrifuged and the sera were sent to the laboratory within 24 hours, otherwise they were refrigerated at -70 C. The levels of β_2M were quantified by COBAS CORE β_2M EIA (Roche Diagnostics GmbH, Mannheim, Germany).

The values of total β_2M clearance were determined by direct measurement of the blood side clearance[12] while the convection/diffusion β_2M clearance was determined by direct dialysate measurement. Thus, the membrane adsorption clearance was calculated from the difference between the above two values as the following equations[12]:

1) Total (blood side) β_2M clearance

$$= \frac{(\text{Plasma inlet flow x } CB_{in}) - (\text{Plasma outlet flow x } CB_{out})}{CB_{in}}$$

2) Convection/Diffusion (dialysate side) β_2M clearance

$$= \frac{\text{Total } \beta_2\text{M in dialysate sample in 10 minutes}}{CB_{in} \text{ x Time of dialysate sample collection}}$$

3) Membrane adsorption β_2M clearance

= Total β_2M clearance - Convection/Diffusion b_2M clearance

Where,	Plasma inlet flow	=	QB_{in} x (1-Hct_{in}/100) x Fp_{in}
	Plasma outlet flow	=	QB_{out} x (1-Hct_{out}/100) x Fp_{out}
	CB	=	β_2M levels in plasma (mg/L)
	QB	=	Effective blood flow rate (mL/min)
		=	QBn x [1 - (QBn - 200)/2000]; QBn = nominal blood flow
	Hct	=	Hematocrit
	Fp	=	Protein fraction
		=	(1-0.0107 x total protein concentration; g/dL)
	in	=	inlet dialyser port
	out	=	outlet dialyser port

These parameters were determined hourly at hours 1, 2, 3, and 4 during dialysis session and then, the mean values were obtained and used in the calculation.

The β_2M reduction ratio was derived and corrected by Bergstrom's method[14].

Urea clearance was determined by single-pool Kt/V calculated from the second generation Daugirdas equation[15]. Phosphate clearance was measured on an assumption that there was no membrane adsorption clearance as demonstrated in the previous study[16].

Statistical analysis

All data were expressed as mean $\pm$ SE values of 10 participating patients. The statistical difference among the values in each hour of treatment was analyzed by repeated ANOVA. All statistical testing were performed by using the SPSS statistical package (version 11.0 for Windows, SPSS Inc, Chicago, IL). The results were statistically significant when $p < 0.05$.

Results

Basic patient characteristics

Of the ten patients who completed the present study, there were 5 male and 5 female subjects. The mean patients' age was 58.2 $\pm$ 14.7 years. The average hematocrit was 36.2 $\pm$ 2.7%. The mean residual renal function was 2.2 $\pm$ 5.3 mL/min. The causes of ESRD in these patients were diabetes mellitus (20%), hypertension (20%), lupus nephritis (10%), obstructive uropathy (10%), autosomal dominant polycystic kidney disease (10%), chronic glomerulonephritis (10%), and unknown (20%).

Dialysis data

As illustrated in Table 1, there were no significant differences in the baseline dialysis data among the three study groups. All patients tolerated well without any intradialytic complications during the present study.

As depicted in. Fig. 1, the total β_2M clearance of the HDF125 group was significantly higher than the HDF75 group (124.5 $\pm$ 4.4 mL/min vs. 101.3 $\pm$ 4.1 mL/min, respectively, $p < 0.05$). Of interest, both values were much greater than the HFHD group (44.4 $\pm$ 2.7 mL/min, $p < 0.01$).

The convection/diffusion clearance of β_2M in the HDF125 group was slightly more than in the HDF75 group but the statistical significance was not attained (76.9 $\pm$ 5.6 mL/min vs 56.9 $\pm$ 6.9 mL/min, $p = 0.058$). However, both values were much higher than the HFHD group (29.9 $\pm$ 2.3 mL/min, $p < 0.01$). The membrane adsorption clearance of β_2M in the HDF125 group did not differ from the HDF75 group (47.6 $\pm$ 6.9 mL/min vs. 44.4 $\pm$ 5.0 mL/min; NS). Again, both values were much greater than the HFHD group (14.5 $\pm$ 2.3 mL/min, $p < 0.05$).

The values of hourly total β_2M clearance of each dialysis modality, assessed within each study session, are illustrated in Fig. 2. There were no significant differences among the values of hours 1, 2, 3, and 4 in each dialysis modality.

The β_2M reduction ratio in the HDF125 group was not statistically significant from the HDF75 group (NS) but both were significantly higher than the HFHD group ($p < 0.01$) (Fig. 3).

The values of single-pool Kt/V urea in the HFHD, HDF75, and HDF125 groups were 2.1 $\pm$ 0.1, 2.3 $\pm$ 0.1, and 2.3 $\pm$ 0.2, respectively, all of which were not significantly different. Also, the clearances of phosphate were not different among the three study groups (235.9 $\pm$ 23.2, 258.7 $\pm$ 28.9, and 258.8 $\pm$ 23.4 mL/min, respectively; NS).

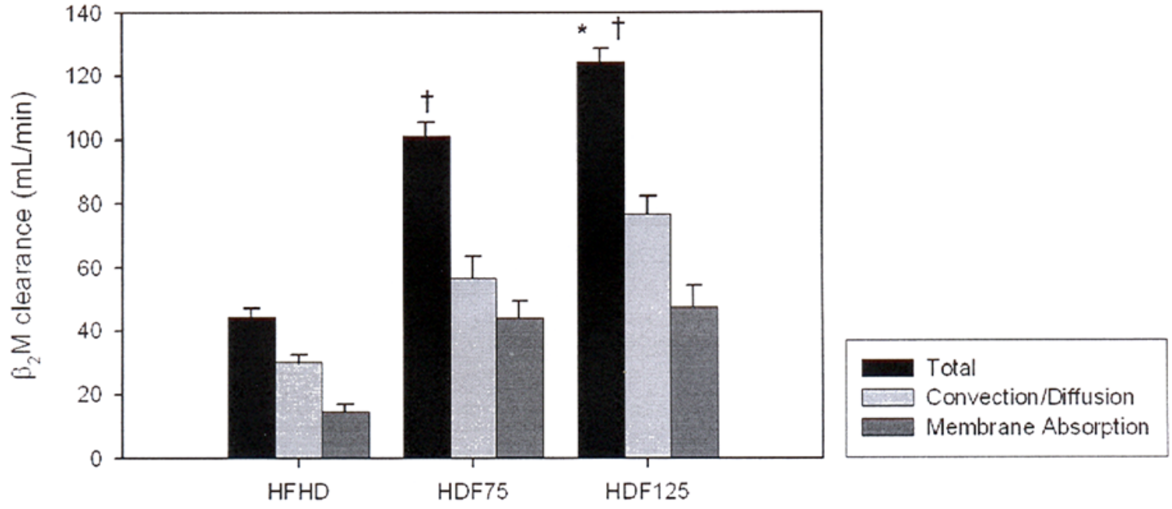
Table 1. Dialysis data

Dialysis data	HFHD	HDF75	HDF125
Pre-dialysis body weight (kg)	57.1$\pm$4.0	57.1$\pm$4.2	57.4$\pm$4.1
Post-dialysis body weight (kg)	54.1$\pm$4.0	54.3$\pm$4.1	54.1$\pm$4.0
Body weight loss (kg)	2.9$\pm$0.2	2.8$\pm$0.3	3.3$\pm$0.2
Ultrafiltration fluid (Litre)	3.5$\pm$0.3	3.1$\pm$0.3	3.4$\pm$0.1
Blood flow rate (mL/min)	420.0$\pm$13.2	426.0$\pm$13.1	430.0$\pm$13.3
Pre-dialysis BUN (mg/dL)	71.1$\pm$6.5	66.7$\pm$5.8	78.1$\pm$6.1*
Pre-dialysis β_2M (mg/L)	31.1$\pm$3.1	29.6$\pm$3.2	30.0$\pm$3.3
Pre-dialysis hematocrit (%)	36.2$\pm$0.8	36.4$\pm$0.9	36.0$\pm$1.2
Replacement fluid rate (mL/min)	-	65.0$\pm$0.8	119.8$\pm$0.6

Data were presented as mean $\pm$ SE

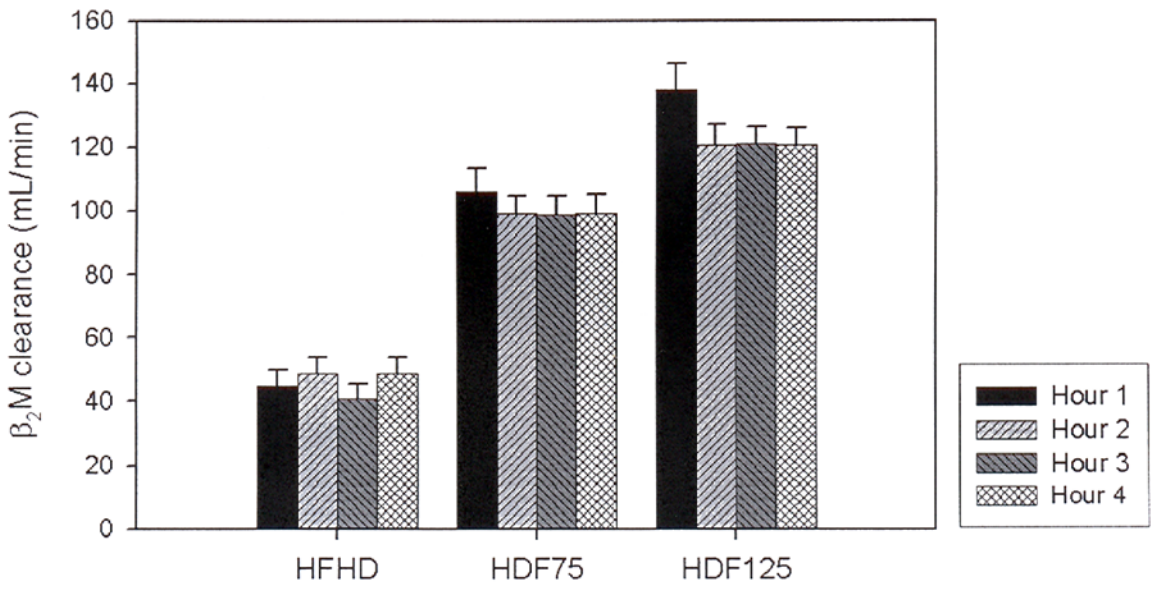
*$p = 0.044$ vs HDF75

Abbreviations: HFHD = high flux hemodialysis,
HDF75 = hemodiafiltration with replacement fluid rate of 75 mL/min,
HDF125 = hemodiafiltration with replacement fluid rate of 125 mL/min



* $p < 0.05$ vs HDF75, $p < 0.01$ vs HFHD

Fig. 1 The total, convection/diffusion, and membrane adsorption β_2-microglobulin clearances

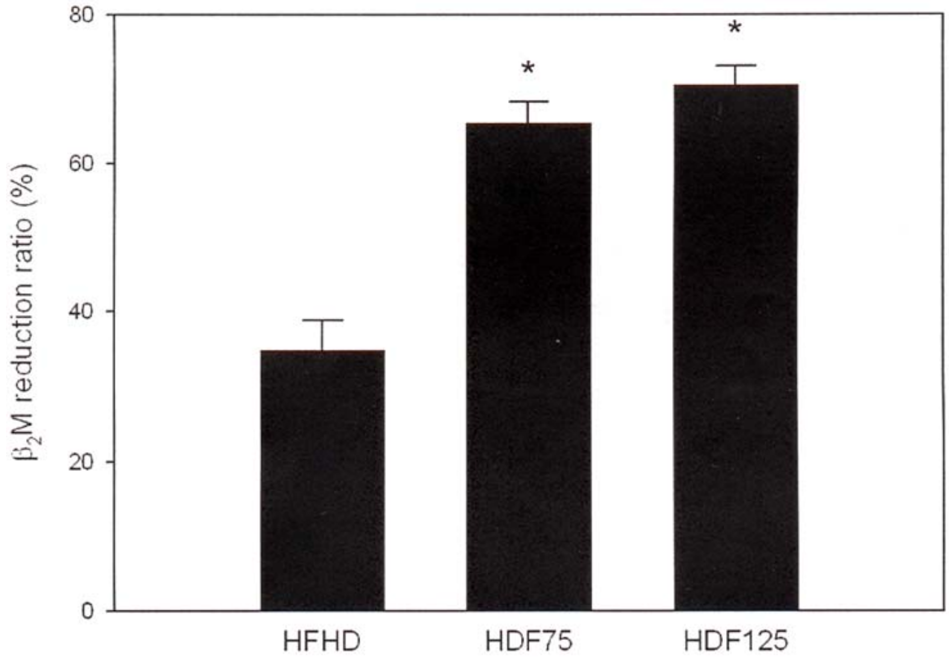


Abbreviations: HFHD = high flux hemodiaysis,
HDF75 = hemodiafiltration with replacement fluid rate of 75 mL/min,
HDF125 = hemodiafiltration with replacement fluid rate of 125 mL/min

Fig. 2 Hourly total β_2M clearance in HFHD, HDF75 and HDF125

Discussion

The present study has demonstrated that on-line HDF with post-dilution replacement rates of 75 as well as 125 mL/min produced higher β_2M clearance and β_2M reduction ratio than HFHD and could yield comparable small solute clearance. Regarding the on-line HDF, the higher replacement rate, 125 mL/min, provided more total β_2M clearance than the lower rate



Abbreviations: HFHD = high flux hemodiaysis,
HDF75 = hemodiafiltration with replacement fluid rate of 75 mL/min,
HDF125 = hemodiafiltration with replacement fluid rate of 125 mL/min
* $p < 0.01$ vs HFHD

Fig. 3 β_2M reduction ratio in each treatment group

(75 mL/min), although, the reduction ratios were not different. This superiority of total β_2M clearance was the consequence from the greater convection/diffusion clearances whereas the adsorptive clearances were equal. Practically, the results of the present study are in agreement with the previous studies that the on-line HDF is safe for HD patients(11,16-19).

The superior total β_2M clearance of on-line HDF over HFHD was demonstrated by previous studies(11,18). One study had shown that the β_2M clearance of on-line HDF with post-dilution replacement rate of 80 mL/min was higher than of HFHD (61 vs 38 mL/min, $p < 0.001$)(11). Another study had demonstrated that when the replacement fluid rate was elevated from 60 to 120 mL/min, the β_2M clearances were increased from 63.8 to 158.3 mL/min, both values were much higher than HFHD(18). However, these clearance data were derived from direct dialysate measurement that did not include membrane adsorption clearance component. Thus, only the convection/diffusion component but not the total clearance was determined.

The β_2M adsorptive clearance in HDF and direct comparison of the values among different modalities has not been studied yet. A previous study explored transmembranous transport and adsorption of β_2M during hemodialysis using polysulfone, polyacrylonitrile, polymethylmethacrylate, and cuprammonium rayon membranes. It found that polysulfone (using F60, Fresenius at blood flow 200 mL/min) had a 17% adsorption of β_2M(20). In a recent study, the mass of β_2M adsorption rate was explored by postdilution on-line HDF using 1.89 m^2 polysulphone membrane with blood flow 465 $\pm$ 5.0 mL/min, dialysate flow 800 mL/min and infusion rate 103.6 $\pm$ 12.3 mL/min(21). The result was 13.5% when compared with the total mass of β_2M removal rate at 30 minutes after starting the on-line HDF. These results were different from the present study where β_2M adsorption clearance was 32.7% for HFHD, 38.2% for HDF125 and 43.8% for HDF75. The present study used plasma water volume for calculation of total blood side β_2M clearance. This complied with the kinetics of β_2M in the blood circulation(12,22). In contrast, in the two earlier studies(20,21), blood volume instead of plasma volume was utilized and this would

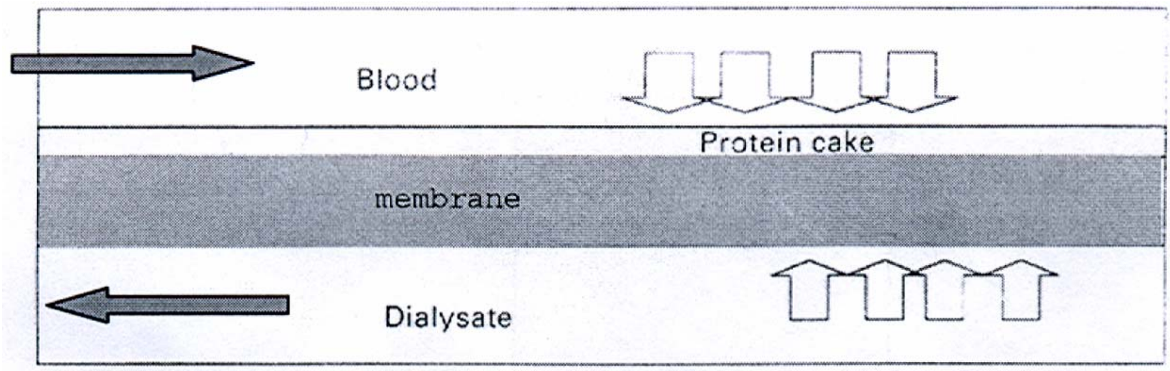


Fig. 4 Protein cake effect

result in overestimation of total blood side β_2M clearance and would lower percent of β_2M adsorption.

Interestingly, the present study is the first to demonstrate an equal membrane adsorption clearance in the two different replacement fluid rates of on-line HDF (Fig. 1). Furthermore, the membrane adsorption clearance value of on-line HDF was much higher than HFHD (Fig. 1). This may indicate more complex mechanisms other than a simple adsorption to material surface that should result in an equal membrane adsorption clearance among the three treatments. The more β_2M passing through the membrane by convection of on-line HDF compared with HFHD may be an explanation of more adsorption. The "Protein cake effect" may be another explanation for this phenomenon (Fig. 4). It is well known that HFHD has a back filtration within a dialyzer, especially the blood outlet side. However, if one would imagine the convection process occurring in a dialyzer, the on-line HDF has an enormous convection while the back filtration could never occur resulting in a thicker protein cake, including β_2M the entire dialyzer length. However, the authors still do not know what the lowest replacement rate is that maximizes the membrane adsorption clearance.

The β_2M reduction ratio is another parameter used in representing middle molecule removal, as clearly demonstrated earlier by Lornoy et al[11]. The β_2M reduction ratio was higher in on-line HDF than HFHD in both studies. HDF125 had a higher total β_2M clearance than HDF75 and the reduction ratio had a similar trend but did not reach statistical significance. The same study had demonstrated the equal β_2M reduction ratio between HDF100 and HDF120 despite the significantly higher clearance in HDF120. Long-term study may be warranted to determine a recommendation for an optimal replacement fluid rate.

The values of dialysis adequacy were equal between HDF125 and HDF75. They both were approximately 10% higher than HFHD. This is comparable with the previous study by Kerr et al[17].

In the present study, phosphate clearance did not significantly differ from the three treatment modalities. This observation is different from an earlier report, by Zehnder et al[16] showing that HDF could provide a higher phosphate clearance than HFHD. Such results might be explained by the higher prescribed dialysate flow rate in HDF group than HFHD group (500 vs 800 mL/min).

In summary, on-line HDF provides salutary β_2M clearance. The β_2M clearance magnitude directly correlated to the rates of fluid replacement. Membrane adsorption clearance, an important mechanism of total β_2M clearance, is equal in the two different fluid replacement rates.

References

1. Vanholder R, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, et al. Uremic toxicity: present state of the art. Int J Artif Organs 2001; 24: 695-725.
2. Vanholder R, De Smet R, Lameire NH. Redesigning the map of uremic toxins. Contrib Nephrol 2001; 42-70.
3. Vanholder R, De Smet R, Hsu C, Vogeleere P, Ringoir S. Uremic toxicity: the middle molecule hypothesis revisited. Semin Nephrol 1994; 14: 205-18.
4. Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, et al. Effect of dialysis dose and membrane flux in maintenance hemodialysis. N Engl J Med 2002; 347: 2010-9.
5. Hakim RM, Wingard RL, Husni L, Parker RA, Parker TF III. The effect of membrane biocompatibility on

plasma beta 2-microglobulin levels in chronic hemodialysis patients. J Am Soc Nephrol 1996; 7: 472-8.
6. Kuchle C, Fricke H, Held E, Schiffl H. High-flux hemodialysis postpones clinical manifestation of dialysis-related amyloidosis. Am J Nephrol 1996; 16: 484-8.
7. Locatelli F, Marcelli D, Conte F, Limido A, Malberti F, Spotti D. Comparison of mortality in ESRD patients on convective and diffusive extracorporeal treatments. The Registro Lombardo Dialisi E Trapianto. Kidney Int 1999; 55: 286-93.
8. Locatelli F, Mastrangelo F, Redaelli B, Ronco C, Marcelli D, La Greca G, et al. Effects of different membranes and dialysis technologies on patient treatment tolerance and nutritional parameters. The Italian Cooperative Dialysis Study Group. Kidney Int 1996; 50: 1293-302.
9. Floege J, Ketteler M. beta2-microglobulin-derived amyloidosis: an update. Kidney Int Suppl 2001; 78: S164-71.
10. Canaud B, Bosc JY, Leray H, Stec F, Argiles A, Leblanc M, et al. On-line haemodiafiltration: state of the art. Nephrol Dial Transplant 1998; 13 (Suppl 5): 3-11.
11. Lornoy W, Becaus I, Billiouw JM, Sierens L, Van Malderen P, D'Haenens P. On-line haemodiafiltration. Remarkable removal of beta2-microglobulin. Long-term clinical observations. Nephrol Dial Transplant 2000; 15(Suppl 1): 49-54.
12. Locatelli F, Di Filippo S, Manzoni C. Removal of small and middle molecules by convective techniques. Nephrol Dial Transplant 2000; 15(Suppl 2): 37-44.
13. The EBPG Expert Group on Haemodialysis. European best practice guidelines for haemodialysis (Part I): Section IV. Dialysis fluid purity. Nephrol Dial Transplant 2002; 17(Suppl 7): 45-62.
14. Bergstrom J, Wehle B. No change in corrected beta 2-microglobulin concentration after cuprophane haemodialysis. Lancet 1987; 1: 628-9.
15. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. J Am Soc Nephrol 1993; 4: 1205-13.
16. Zehnder C, Gutzwiller JP, Renggli K. Hemodiafiltration - a new treatment option for hyperphosphatemia in hemodialysis patients. Clin Nephrol 1999; 52: 152-9.
17. Kerr PB, Argiles A, Flavier JL, Canaud B, Mion CM. Comparison of hemodialysis and hemodiafiltration: a long-term longitudinal study. Kidney Int 1992; 41: 1035-40.
18. Ward RA, Schmidt B, Hullin J, Hillebrand GF, Samtleben W. A comparison of on-line hemodiafiltration and high-flux hemodialysis: a prospective clinical study. J Am Soc Nephrol 2000; 11: 2344-50.
19. Wizemann V, Lotz C, Techert F, Uthoff S. On-line haemodiafiltration versus low-flux haemodialysis. A prospective randomized study. Nephrol Dial Transplant 2000; 15(Suppl 1): 43-8.
20. Klinke B, Rockel A, Abdelhamid S, Fiegel P, Walb D. Transmembranous transport and adsorption of beta-2-microglobulin during hemodialysis using polysulfone, polyacrylonitrile, polymethylmethacrylate and cuprammonium rayon membranes. Int J Artif Organs 1989; 12: 697-702.
21. Garcia H, Hernandez-Jaras J, Maduell F, Yago M, Calvo C, Ferrero JA. Interaction of the mechanisms of beta 2-microglobulin convection, diffusion, and adsorption in line hemodiafiltration. Nefrologia 2000; 20: 59-65.
22. Odell RA, Slowiaczek P, Moran JE, Schindhelm K. Beta 2-microglobulin kinetics in end-stage renal failure. Kidney Int 1991; 39: 909-19.

ประสิทธิภาพของการรักษาด้วยวิธีออนไลน์ฮีโมไดอาฟิลเตรชั่นในการขจัดสารเบต้าทูไมโครโกลบูลินในโรคไตวายเรื้อรังระยะสุดท้าย

อัญชนะ พานิช, ขจร ตีรณธนากุล, เกื้อเกียรติ ประดิษฐ์พรศิลป์, สมชาย เอี่ยมอ่อง

วัตถุประสงค์: *เพื่อเปรียบเทียบการขจัดสารเบต้าทูไมโครโกลบูลินระหว่างการทำการรักษาด้วยวิธีออนไลน์ฮีโมไดอาฟิลเตรชั่น และการฟอกเลือดชนิดไฮฟลักฮีโมไดอาไลซิส*
วัสดุและวิธีการ: *ทำการศึกษาในผู้ป่วยไตวายเรื้อรังระยะสุดท้าย 10 ราย ซึ่งได้รับการรักษาโดยวิธีการรักษาทั้งสองทำการตรวจหาค่าการขจัดสารเบต้าทูไมโครโกลบูลิน สารยูเรีย และฟอสเฟต*
ผลการศึกษา: *การขจัดสารเบต้าทูไมโครโกลบูลินโดยวิธีออนไลน์ฮีโมไดอาฟิลเตรชั่นสูงกว่าการฟอกเลือดชนิดไฮฟลักฮีโมไดอาไลซิส โดยเมื่อเพิ่มอัตราการทดแทนสารน้ำจาก 75 เป็น 125 มล./นาที จะเพิ่มประสิทธิภาพการขจัดสารมากขึ้น พบว่าส่วนการพา / การแพร่เป็นปัจจัยสำคัญมากกว่าการดูดซึมสารของเมมเบรนในการขจัดสารเบต้าทูไมโครโกลบูลิน ไม่พบความแตกต่างของค่าการขจัดสารยูเรียและฟอสเฟตระหว่างวิธีการทั้งสอง*
สรุป: *การรักษาด้วยวิธีออนไลน์ฮีโมไดอาฟิลเตรชั่นสามารถขจัดสารเบต้าทูไมโครโกลบูลินได้มากกว่าการฟอกเลือดชนิดไฮฟลักฮีโมไดอาไลซิส โดยเพิ่มทั้งส่วนการพา / การแพร่ และการดูดซึมสารของเมมเบรน*