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Suitability of α_1-microglobulin reduction rate as a biomarker of removal efficiency of online hemodiafiltration: a retrospective cohort study

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Abstract

Background: Online hemodiafiltration (OL-HDF), whether in pre-dilution OL-HDF (pre-HDF) or post-dilution OL-HDF (post-HDF), is conducted to efficiently remove low molecular weight proteins from the blood of patients requiring dialysis. β_2-microglobulin (β_2-MG) and α_1-microglobulin (α_1-MG) are used as biomarkers to evaluate removal efficiency of OL-HDF.
We aimed to evaluate the relationship between β_2-MG and α_1-MG reduction rates and the amount of albumin leakage. Furthermore, we statistically analyzed the relationship between the α_1-MG reduction rate and α_1-MG removal amount, and its suitability as a biomarker for evaluating the removal efficiency of OL-HDF.

Methods: We collected the results of regularly conducted routine evaluations to assess the efficiency of OL-HDF from cases of patients undergoing maintenance dialysis at our clinic from 2018 to 2019. Data on was collected on both pre-HDF and post-HDF sessions. β_2-MG and α_1-MG reduction rates were analyzed. Regression analysis on reduction rates showed a significant correlation between the α_1-MG reduction rate and the α_1-MG removal amount.

Results: We conducted 435 tests on OL-HDF efficiency in 87 cases undergoing maintenance dialysis at our clinic in 2018 and 2019. There were 80.7 ± 4.5% for the β_2-MG reduction rate, 33.8 ± 9.4% for the α_1-MG reduction rate, and 3.9 ± 1.8 g/s for the amount of albumin leakage. There was no correlation between the β_2-MG reduction rate and the α_1-MG reduction rate, or between the amount of albumin leakage and β_2-MG reduction rate.

Conclusion: α_1-MG reduction rate was found to correlate with its removal amount, demonstrating its suitability as a biomarker for evaluating the removal efficiency of OL-HDF.

Trial registration: Retrospectively registered.

Keywords: α_1-microglobulin, Reduction rate of α_1-microglobulin, Online hemodiafiltration, Biomarker for evaluation of removal efficiency

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Background

Online hemodiafiltration (OL-HDF), whether in predilution OL-HDF (pre-HDF) or post-dilution OL-HDF (post-HDF), is conducted to efficiently remove low molecular weight proteins (LMWP) (molecular weight [MW]: 10,000–55,000), particularly larger LMWPs (MW > 30,000), from blood of dialysis patients. This is accomplished by increasing the convection volume from high-volume replacement fluid. Biomarkers are important for evaluating the efficiency of OL-HDF. The small molecular size of β_2-microglobulin (β_2-MG) (MW: 11,800, Stokes radius: 16A) allows for removal dependent on both diffusion and convection. Therefore, β_2-MG cannot be used as a biomarker to accurately assess OL-HDF removal efficiency.

α_1-Microglobulin (α_1-MG) (MW: 33,000, Stokes radius: 28.4A) is mainly removed by convection in dialysis therapy with a more suitable molecular size; hence, it has often been used as a biomarker to assess the efficiency of removal of middle- and large-MW solutes in Japan for over 30 years. Similarly, we reported a strong correlation between changes in the clinical symptoms of dialysis patients and changes in the α_1-MG reduction rate [1, 2]. We also reported that OL-HDF should aim for a β_2-MG reduction rate of 80% and an α_1-MG reduction rate of over 35% when performed to treat complications in long-term dialysis patients [3, 4].

There is a twofold difference in molecular weight between albumin (alb) (MW: 66,000, Stokes radius: 35.5A) and α_1-MG, with a difference in Stokes radius of 20% [5]. Thus, it is impossible to separate α_1-MG and alb based on differences in molecular size alone and remove them using current dialysis membranes. Some degree of alb leakage is inevitable when removing α_1-MG at high efficiency. There is no report examining the appropriateness of the reduction rate of the α_1-MG reduction rate as a biomarker using statistical analysis. In this study, we evaluated the relationship between β_2-MG and α_1-MG reduction rates and the amount of alb leakage, as well as the relationship between the reduction rates of β_2-MG and α_1-MG based on the results of OL-HDF removal efficiency tests regularly conducted as routine examinations in 2018 and 2019. Furthermore, we statistically analyzed the relationship between the α_1-MG reduction rate and the α_1-MG removal amount, and we investigated whether this was a suitable biomarker for evaluating the removal efficiency of OL-HDF.

Methods

Four hundred thirty-five tests for evaluating OL-HDF efficiency in 87 patients undergoing maintenance dialysis at our clinic between January 2018 and December 2019 (male/female: 60/27, age: 60.5 ± 12.0 years, dry weight: 60.5 ± 13.0 kg, dialysis vintage: 146.0 ± 130.9 months). Pre- and post-HDF comprised 304 and 131 sessions, respectively. Blood flow rate (Qb) was 261.5 ± 28.5 mL/min, total dialysate flow rate (Qd total) was 400 mL/min for 22 cases and 500 mL/min for 413 cases, and replacement fluid volume was 48.7 ± 8.2 L/session(s) for pre-HDF and 14.2 ± 3.1 L/s for post-HDF. Treatment time was 4.1 ± 0.2 h/s (Table 1). Different types of hemodiafilters commercially available in Japan were used. The obtained test values were used to investigate the relationship between β_2-MG and α_1-MG reduction rates and the amount of alb leakage, as well as the relationship between the β_2-MG reduction rate and α_1-MG reduction rate.

Thereafter, the relationship between the α_1-MG reduction rate and its removal amount was analyzed, and whether the reduction rate was suitable for evaluating the removal efficiency was investigated. This was done by setting a 95% prediction interval of the regression curve created from the α_1-MG removal amount (horizontal axis, mg) and reduction rate (vertical axis, %), and extracting test values that were outliers from this interval from the regression analysis. Values with reduction rates that were lower than the lower limit or higher than the upper limit of the calculated 95% prediction interval were defined as outlier test values. A histogram and Q-Q plot was used to assess if a set of data have a normal distribution. Continuous variables were expressed as the mean ± standard deviation (SD) and categorical variables as frequencies. A P value of < 0.05 was considered statistically significant, and all P values were two-sided. All statistical analyses were performed using SPSS statistics ver. 23.0 (IBM Japan, Ltd., Tokyo, Japan) software.

Post-dialysis values used to calculate the β_2-MG and α_1-MG reduction rates were values that were corrected by the hematocrit level to exclude concentration effects. The waste dialysate was partially stored throughout the

Table 1 Patients background and treatment mode

Total patient count	87
Age (years)	60.5 ± 12.0
Dialysis vintage (months)	146 ± 130.9
Sex, M/F	60/27
Body weight (kg)	58.9 ± 13.0
Total number of evaluations	435
Qb (mL/min)	261.5 ± 28.5
Qd total (mL/min)	400: 22 cases 500: 413 cases
Treatment time (h)	4.1 ± 0.2
Replacement fluid (L/session)	Pre: 48.7 ± 8.2 Post: 14.2 ± 3.1
Hemodiafilters	Various kinds, 10 types
Membrane area (m^2)	2.1 ± 0.1

Table 2 Hemodiafilter Specs from NIKKISO CO., LTD., Tokyo, Japan; Nipro CO., Osaka Japan; Toray Medical Co., Ltd., Tokyo Japan; ASAHI KASEI Medical Co., Ltd., Tokyo Japan; Gambro Dialysatoren GmbH, Hechingen, Germany; Fresenius Medical Care Japan K.K., Fukuoka, Japan

	Membrane material	Manufacturer	SC	
			β_2-MG	Albumin
GDF	PEPA	Nikkiso	0.87	0.03
GDF-M	PEPA		0.88	0.01
MFX-U eco	PES	Nipro	0.91	0.01
FIX-S eco	ATA		0.93	0.01
FIX-U eco	ATA		1.05	0.02
NVF-P	PS	Toray Medical	0.9	0.013
NVF-H	PS		0.9	0.009
ABH-PA	PS	AsahiKASEI Medical	0.78	≤ 0.01
Polyflux-H	PEAS	GAMBRO	0.82	0.0022
FX-HDF	PS	Fresenius Medical Care	0.8	0.004

From each company's catalog

dialysis session at a rate of 2 L/h, after which the total amount stored was well-mixed, and part of this was used to measure the β_2-MG and α_1-MG amounts removed and the amount of alb leakage.

This study has been approved by our institutional committee on human and/or animal research. All patients provided informed written consent.

Results

We conducted 435 tests on OL-HDF efficiency in 87 cases undergoing maintenance dialysis at our clinic in 2018 and 2019 (male/female: 60/27, age: 60.5 ± 12.0 years, dry weight: 60.5 ± 13.0 kg, dialysis vintage: 146.0 ± 130.9 months). Pre-HDF comprised 304 sessions, and post-HDF comprised 131 sessions. Qb was 261.5 ± 28.5 mL/min, Qd total was 400 mL/min for 22 cases and 500 mL/min for 413 cases, and replacement fluid volume was 48.7 ± 8.2 L/session(s) for pre-HDF and 14.2 ± 3.1 L/s for post-HDF. Treatment time was 4.1 ± 0.2 h/s (Table 1). Ten types of hemodiafilters commercially available in Japan were used (Table 2). The membrane surface area was 2.1 ± 0.1 m^2.

The results of all 435 tests (mean ± SD) were 80.7 ± 4.5% for the β_2-MG reduction rate, 33.8 ± 9.4% for the α_1-MG reduction rate, and 3.9 ± 1.8 g/s for the amount of alb leakage. There were six cases in ten sessions

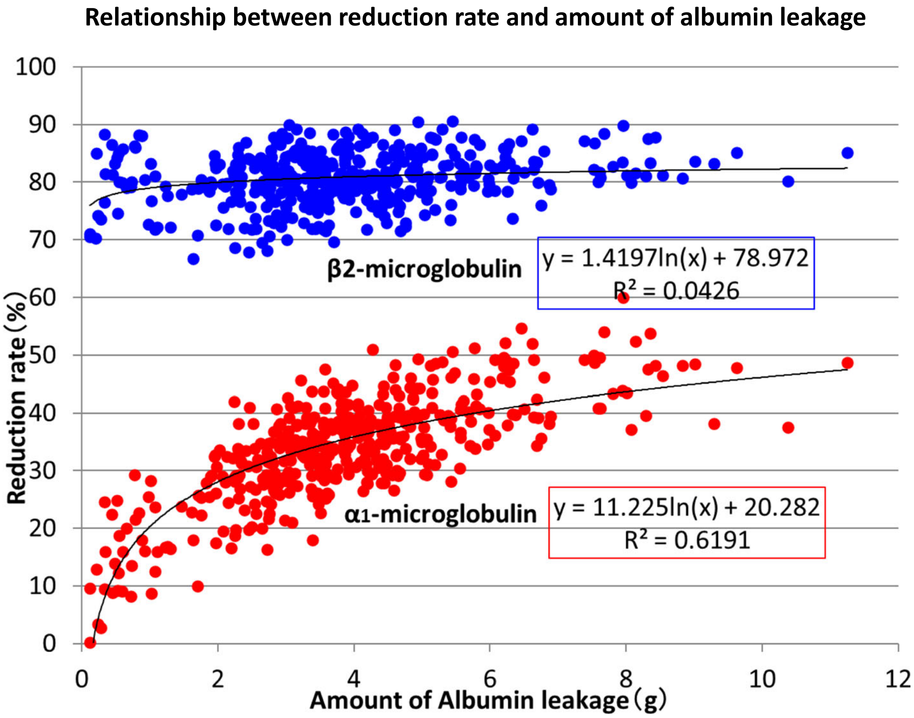

Fig. 1 Relationship between reduction rate and amount of albumin leakage. No significant correlation is found between the amount of alb leakage and β_2-MG reduction rate. Since β_2-MG is primarily removed by diffusion as well as convection, this indicates that high-efficiency removal is possible with little to no alb leakage. α_1-MG cannot be removed with high efficiency without some degree of alb leakage. Furthermore, as 30–50% of α_1-MG is bound to dimeric IgA in renal failure, the maximum reduction rate is around 60%

where the α_1-MG reduction rate was > 50%, and the maximum α_1-MG reduction rate value was 60.0%. No significant correlation was observed between the amount of alb leakage and β_2-MG reduction rate (Fig. 1). There was a slightly strong significant correlation between the amount of alb leakage and α_1-MG reduction rate. The removal dynamics of alb leakage and α_1-MG removal were almost identical (Fig. 1). There was no correlation between the β_2-MG reduction rate and the α_1-MG reduction rate (Fig. 2).

Results of the regression analysis on reduction rates showed a significant correlation between the α_1-MG reduction rate and the α_1-MG removal amount (Table 3). Additionally, 17 out of the 435 total sessions (3.9%) were outlier values from the 95% prediction interval of the regression curve (Fig. 3). It was shown from these analyses that it was possible to appropriately evaluate the removal efficiency of OL-HDF by investigating the reduction rate without determining the removal amount.

Discussion

In this study, we evaluated the relationship between β_2-MG, α_1-MG reduction rates, and amount of alb leakage, as well as the relationship between the reduction rates of β_2-MG and α_1-MG. There was no correlation between the β_2-MG reduction rate and the α_1-MG reduction rate, nor between the amount of alb leakage and β_2-MG reduction rate. We found that alb leakage was inevitable when removing α_1-MG at high efficiency. Further, we revealed that there was a significant correlation between the α_1-MG reduction rate and the α_1-MG removal amount. Our study showed that α_1-MG reduction rate was a suitable biomarker for evaluating the efficiency of removal of OL-HDF.

In Japan, α_1-MG is widely used as a biomarker for evaluating the performance of removal efficiency of OL-HDF for several reasons: (1) optimal molecular size, (2) low likelihood of measurement errors due to its non-negligible blood concentration, (3) low likelihood of concentration changes under physiological conditions, (4) stable synthesis rate, (5) accumulation in renal failure, and (6) convection-based removal. α_1-MG is primarily synthesized in the liver. Under physiological conditions, 50% of it is present in the blood in free form, while 50% is bound with dimeric immunoglobulin A (IgA) [6]. It is estimated that free α_1-MG increases to around 70% because its excretion from the kidneys is reduced during renal dysfunction, but there are no detailed reports on these dynamics. α_1-MG has strong antioxidant activity and has been reported as a protective molecule by scavenging free radicals, binding to heme, and undergoing reduction reactions when exposed to oxidative stresses

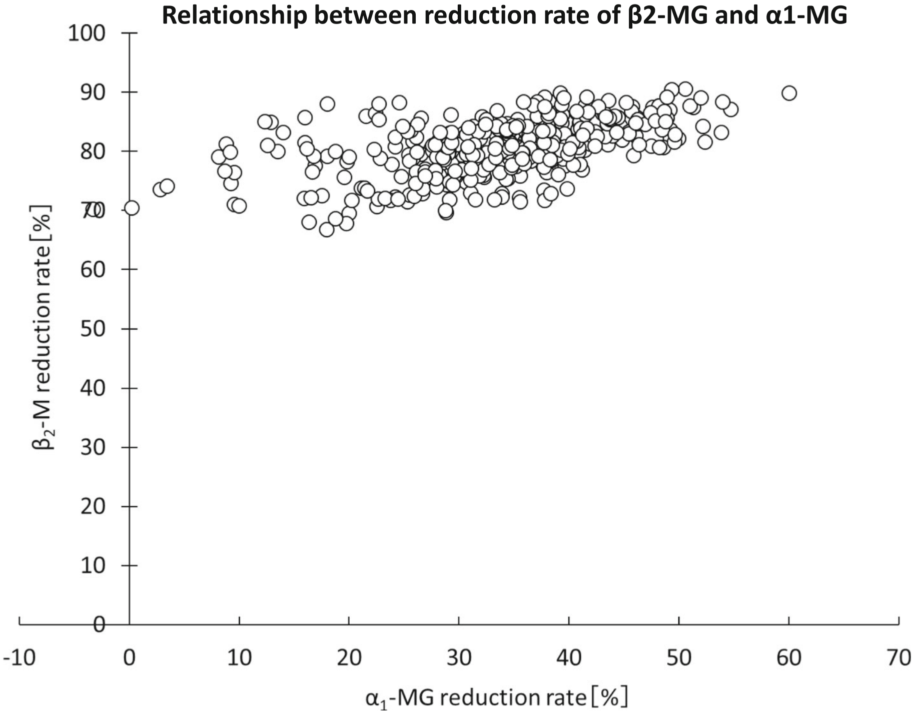

Fig. 2 Relationship between reduction rate of β_2-MG and α_1-MG. Since there is no correlation between β_2-MG and α_1-MG reduction rates, the advantages of OL-HDF cannot be evaluated with β_2-MG reduction rate alone. As efficient removal of larger LMWPs is an objective of OL-HDF, efficiency tests that use α_1-MG as a biomarker are necessary

Table 3 Results of regression analysis on reduction rates. To evaluate the association between removal amount and reduction rate, and to identify cases that fell outside of the association, we performed a regression analysis with reduction rate as the dependent variable and removal amount as the independent variable. The removal amount was log-transformed

Parameter	Regression coefficient (β)	S.E.	*t*	*p*
(Constant)	− 42.467	3.490	− 12.17	< 0.0001
Ln (reduction rate)	15.638	0.713	21.93	< 0.0001

Ln natural logarithm transform, $R^2 = 0.526$

[7–11]. The antioxidant activity of α_1-MG in dialysis patients may be a topic of future studies.

The MW of IgA is 160,000; hence, the combined MW of the resulting α_1-MG-IgA complex is 350,000, which is impossible to remove using dialysis. Since free α_1-MG is the target in dialysis therapy and total α_1-MG is usually measured, the reduction rate is affected by the binding affinity of α_1-MG and IgA. Consequently, the α_1-MG reduction rate calculated with the pre- and post-dialysis values do not appropriately indicate the removal efficiency of OL-HDF.

Therefore, we investigated whether the α_1-MG reduction rate and the α_1-MG removal amount obtained from the 435 OL-HDF sessions were significantly correlated. Results showed that there was a significant correlation between the two measures. However, there were 17 test values outside the 95% prediction interval of the regression curve. Specifically, outliers were observed below the lower limit in seven patients across seven sessions, whereas outliers were observed over the upper limit in five patients across ten sessions. Test conditions when outlier values were obtained may be a topic of future studies.

Measuring the amount of a given solute removed during dialysis is complicated due to the need for the installation of a device that is capable of continuously collecting waste fluid from a drainage line of the patient monitoring system. However, the reduction rate can be calculated simply by collecting blood before and after dialysis and calculating the reduction rate using the following equation:

$$\mathrm{RR} = \left(1 - \frac{(1-\mathrm{Ht}_{\mathrm{post}})\mathrm{Ht}_{\mathrm{pre}}C_{\mathrm{post}}}{(1-\mathrm{Ht}_{\mathrm{pre}})\mathrm{Ht}_{\mathrm{post}}C_{\mathrm{pre}}}\right) \times 100\ (\%)$$

Where Ht is hematocrit and *C* is concentration. This enables the evaluation of removal efficiency of middle- and large-MW solutes.

It is essential in the evaluation of high-efficiency OL-HDF to investigate the removal efficiency of solutes with

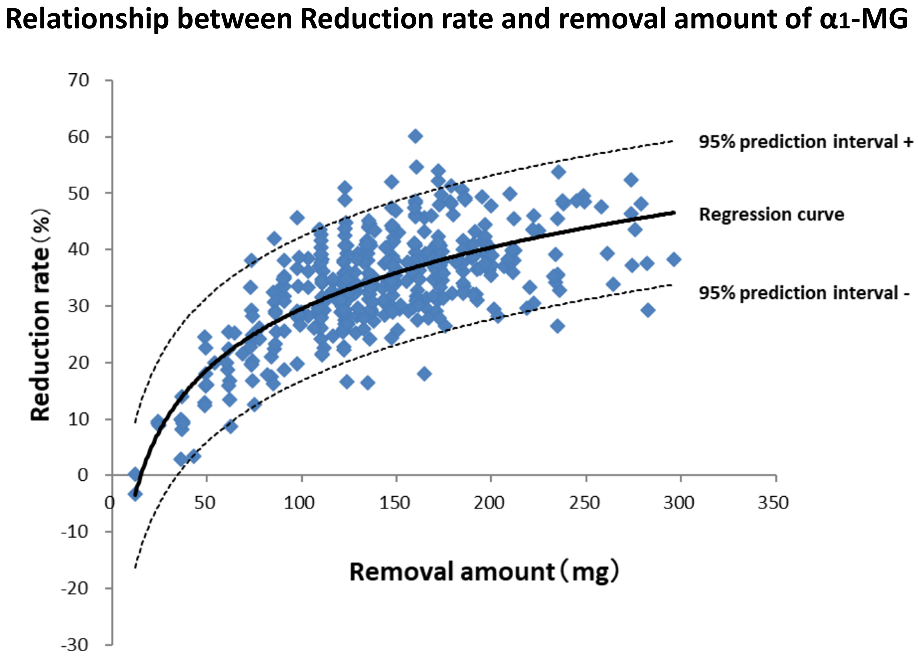

Fig. 3 Relationship between Reduction rate and removal amount of α_1-MG. From the results of the regression analysis, a logarithmic approximation curve including the 95% prediction interval is created with the removal amount as the horizontal axis and the reduction rate as the vertical axis. Values with reduction rates that fell outside the 95% prediction interval are defined as outlier cases. There are 17 outliers (3.9%) in this study

a MW of 30,000–50,000 using α_1-MG as a biomarker. Our study supported the use of α_1-MG reduction rate to be an appropriate evaluation method indicating that past accumulated data can be applied to future studies.

Several reports investigated the effects of OL-HDF on a patient's survival [12–14]. Kikuchi et al. [15] reported that pre-HDF with high-replacement fluid volume had a more favorable effect on patient's survival than hemodialysis and pre-HDF with low-replacement fluid volume. However, both reports only investigated the impacts of replacement fluid volume on a patient's survival, and neither mentioned specific numerical values for removal efficiency. In Europe, α_1-MG has been used for over five years as a biomarker for removal performance when evaluating OL-HDF or high-performance dialyzers, and the evaluation of dialysis efficiency using α_1-MG reduction rate has become increasingly common [16, 17]. The groundbreaking JAMREDS study, which was started in Japan this spring, examined the effect of not only replacement fluid volume but also α_1-MG reduction rate on patient survival of OL-HDF. Hence, the usefulness of removing middle- and large-MW toxins is anticipated to become even more apparent in the future.

The limitation of this study was that it was performed in a single facility, which can lead to selection bias.

Conclusions

We found that α_1 reduction rate may be used as a valid biomarker to evaluate the removal efficiency of OL-HDF. Furthermore, some alb leakage is inevitable when removing α_1-MG at high efficiency.

Abbreviations

alb: Albumin; HD: Hemodialysis; IgA: Immunoglobulin A; LMWP: Low molecular weight protein; MW: Molecular weight; OL-HDF: Online hemodiafiltration; pre-HDF: Pre-dilution online hemodiafiltration; post-HDF: Post-dilution online hemodiafiltration; SD: Standard deviation; Qb: Blood flow rate; Qd total: Total dialysate flow rate; α_1-MG: α_1-microglobulin; β_2-MG: β_2-microglobulin

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Authors' contributions

KS designed the study and drafted the manuscript. HH and YK acquired and analyzed the data. TS designed the study. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study has been approved by our institutional committee on human and/or animal research. All patients provided informed written consent.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest

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