Title:

Influence of albumin leakage on glycated albumin in patients with type 2 diabetes undergoing hemodialysis.

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The field of this research Artificial Kidney / Dialysis

A short running title: Albumin leakage and glycoalbumin in patients with diabetes undergoing hemodialysis.

Abstract Glycated albumin (GA) is recommended as a better glycemic indicator than HbA1c in patients undergoing hemodialysis, because red blood cell lifespan is generally faster than that in normal subjects and easily altered by blood loss and erythropoiesis-stimulating agent administration. However, GA can be also affected by protein loss in urine and hemodialysis fluid. Therefore, in this study, we investigated the effect of albumin leakage induced by hemodialysis on GA in a crossover manner. Nine patients undergoing hemodialysis with large or small amounts of albumin leakage were observed for nine months. Valuables indicating glycemic control, albumin and hemoglobin metabolism and nutritional status were evaluated. As a result, it was shown that albumin leakage could affect GA, but the effect was practically small considering the prescription of diabetic drugs. The correlation between HbA1c and blood glucose levels was similar with that between GA and blood glucose levels in our study. In conclusion, GA was a reliable indicator, even with the change of hemodialysis modality. The influence of clinically acceptable albumin leakage induced by hemodialysis on GA was negligible practically. We should recognize

that the preferable glycemic indicator in patients undergoing hemodialysis depends on the hemoglobin and albumin metabolism of each patient.

Key words Albumin・Hemodialysis・glycated albumin・HbA1c

Introduction

Glycated albumin (GA) is believed to be a more reliable marker for glycemic control in patients with end stage kidney disease than HbA1c. In addition to blood glucose levels, HbA1c is influenced by other factors including the lifespan of red blood cells, recombinant human erythropoietin administration, uremic environment and blood transfusion [1-4]. However, GA can also be affected by several conditions that influence albumin metabolism, such as chronic liver disease, thyroid dysfunction, and nephrotic syndrome [5,6]. Moreover, in patients undergoing peritoneal dialysis (PD), there is a relatively large amount of protein loss in dialysis fluid every day. Therefore, Watanabe et al showed that HbA1c was a preferable marker in patients undergoing PD, especially those who had a low serum albumin level or a large amount of protein loss in PD fluid [7]. Kobayashi et al reported that GA could underestimate glycemic status in patients undergoing PD [8].

Recently, hemodialysis (HD) using high flux dialyzers and on-line hemodiafiltration (HDF) have become familiar in removing not only low and middle molecular weight uremic toxins, but also albumin-binding ones. Especially, on-line HDF has been proven to be effective in decreasing uremic toxin-related symptoms. Moreover, HD using high flux dialyzers improved mortality compared to HD using low flux dialyzers, and high-efficiency postdilution on-line HDF could improve mortality compared to HD using high flux dialyzers. However, albumin leakage caused by HD using high flux dialyzers or on-line HDF is larger than that caused by conventional HD [9,10]. No one has estimated the effect of albumin leakage on GA.

Therefore, in this study, we investigated the effect of albumin leakage on GA in patients with type 2 diabetes undergoing HD or on-line HDF in a crossover manner. We also wanted to clarify how much difference of GA there was according to the amount of albumin leakage.

Materials and methods

Ethics Statement

All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. All subjects enrolled in this research have given their written informed consent which has been approved by the Research Ethics Committee of Kawashima Hospital. This protocol has been found acceptable by them. The registration number by the committee was 0291.

Design and subjects

This study started in May 2017, and included 16 patients undergoing HD or on-line HDF at Kawashima Hospital. Inclusion criteria were: (i) patients with type 2 diabetes; (ii) no hospitalization, no change of diabetic medication and dialysis modality for the previous 6 months; (iii) without obvious liver dysfunction, significant infection or malignancy. During the following nine month observation period, seven patients were excluded from this study due to a small amount of albumin leakage in the original dialysis setting (less than 1g/session) (n = 1),

diabetic medication change (n = 2), and hospitalization (n = 4). Finally, nine patients were analyzed. They were aged 50 to 74 years (mean ± SD, 64.7 ± 8.3 years). All of them were men. The duration of renal replacement therapy ranged from 0.6 to 11.1 years (7.0 ± 3.2 years). In the original setting, five underwent on-line HDF and four underwent HD. Every patient received four-hour dialysis started at the same time, three times per week. They had comorbidities such as cardiovascular disease (n = 2), cardiovascular disease and polycystic kidney disease (n = 1), peripheral artery disease (n = 1) and rheumatoid arthritis (n = 1). Two patients received diabetic injection therapy and four took oral antidiabetic drugs. Six took antihypertensive drugs including angiotensin II receptor blocker, calcium channel blocker and β-blocker. The clinical diagnoses of primary renal disease were diabetic kidney disease (n=8) and polycystic kidney disease (n=1).

As shown in Fig. 1a, patients were treated in the original dialysis settings for three months (pre-treatment with large albumin leakage: Pre-Large period). Albumin leakage was determined by collecting the whole dialysis waste liquid in the first dialysis session of the week. Albumin leakage ranged from 2.8

to 5.9 g/session (4.1 ± 1.2 g/session. n = 9.). Next, HD using a dialyzer of FB-210UPeco (Nipro, Osaka, Japan.) was performed for three months (treatment with small albumin leakage: Small period). Albumin leakage was small, ranging from 0.06 to 0.24 g/session (0.13 ± 0.06 g/session. n = 9.). Finally, the dialysis setting was returned to the original one for three months (post-treatment with large albumin leakage: Post-Large period). The dose of erythropoiesis-stimulating agent (ESA) in every patient was not changed through the observation period (none (n = 5), epoetin kappa 750 IU/ session (n = 2), darbepoetin alfa 30 μg/week (n = 1)) except one patient (darbepoetin alfa 520 in Pre-Large period→340 in Small period→155 μg/3 months in Post-Large period.).

Demographic and clinical characteristics were collected at enrollment. Blood samples for biochemical data were obtained from arteriovenous shunt just before starting the first dialysis session of the first week of each month. Normalized protein catabolic rate (n-PCR) and creatinine generation rate (CGR) were calculated using the method of Shinzato [11,12]. The biochemical data for

individuals were mean values of the results from three months in each period. The levels of casual blood glucose, GA, serum albumin, HbA1c (NGSP), hemoglobin, total cholesterol, cholinesterase, n-PCR and CGR were followed to evaluate the effects of albumin leakage.

Statistical analysis

All values are expressed as mean ± SD. Statistical analysis was performed using SPSS for Windows version 13.0 (SPSS, Inc., Chicago, IL, USA). The serological changes were analyzed using paired t-test. F-test was used for comparing the factors of the total deviation. Correlation was analyzed by Spearman's rank correlation. Spearman's coefficients were denoted by r_s. Significance was defined by *P* less than 0.05.

Results

Enrolled patients were observed for nine months (Fig. 1a). In Small

period, serum albumin levels became significantly higher accompanied with the lower levels of total cholesterol and choline esterase than those in Pre-Large and Post-Large periods, suggesting HD using a dialyzer of FB-210UPeco effectively suppressed albumin leakage, followed by the modulation of lipid metabolism and liver function, as well as patients with nephrotic syndrome (Fig 1b-d) [13].

Compared with Pre-Large and Small period, casual blood glucose levels were not different. GA did not increase, even though serum albumin levels became significantly higher. HbA1c and hemoglobin were not different. Regarding nutritional markers, CGR increased significantly in Small period.

Compared with Small and Post-Large period, casual blood glucose levels were not different. GA and serum albumin levels became significantly lower. However, the difference of mean GA between Small and Post-Large period was only 0.8%. HbA1c and hemoglobin were not different. Neither n-PCR nor CGR changed, indicating nutritional status was not different (Fig 1b,e-j).

The ratio of GA to HbA1c was higher in Small period, but not

significantly different between Pre-Large and Small periods (Fig 2a). The correlation between casual blood glucose levels and HbA1c in all periods was almost equal to that between casual blood glucose levels and GA in all periods (Fig 2b,c). The correlation between casual blood glucose levels and HbA1c or GA in each period was not significant (data not shown).

Discussion

In this study, we demonstrated that GA can be affected by dialysis-induced albumin leakage, but the effect of clinically acceptable albumin leakage on GA was not practically important to control diabetic medication.

To date, several middle weight proteins, such as β_2-microglobulin, interleukin-6, TNF-α and FGF-23, have been shown to be predictors for cardiovascular disease and/or mortality [14]. HD with high-flux dialyzers and on-line HDF are now the major prescriptions for patients undergoing dialysis, even though some amount of albumin leakage is inevitable. In this study, the

mean value of albumin leakage was 4.1 g/session, which was equivalent to 1.8 g/day. Therefore, albumin leakage in this study will be similar with the leakage in patients with nephrotic syndrome (protein loss 3.5g/day). Okada et al concluded that nephrotic-range proteinuria decreased GA values independent of glycemic status, while non-nephrotic range proteinuria did not influence GA values in diabetic CKD patients [6]. Our study results were consistent with this previous report. Theoretically speaking, the change of dialysis-induced albumin leakage affects albumin metabolism and GA. A significant difference of serum albumin levels and GA between Small and Post-Large period was observed, whereas we could not find a difference of GA between Pre-Large and Small period, even with a significant increase of serum albumin levels, probably due to minor conditional changes suggested by the significant difference of CGR. In addition, the GA change induced by albumin leakage was small and practically negligible considering a guide for diabetic treatment in patients undergoing HD (Fig 1) [15].

In patients undergoing PD, mean protein loss of 6 to 7.8 g/day can be more than that in patients with nephrotic syndrome [7,8]. Watanabe et al

investigated 71 patients undergoing PD and suggested HbA1c was correlated more closely to blood glucose levels than GA in their group. However, higher weekly doses of ESA could make HbA1c underestimate glycemic status. GA in the group with lower daily protein loss (<5.9 g/day) had better correlation with glucose levels than that in the group with higher protein loss (≥5.9 g/day). On the other hand, Abe et al investigated 20 patients undergoing PD and demonstrated that GA was associated with blood glucose levels better than HbA1c, although GA might underestimate glycemic status in patients undergoing PD because the ratio of GA to HbA1c was significantly lower than that in patients undergoing HD. We believe that the difference between the above two studies will be the diversity of red blood cell lifespan and albumin loss in the participants analyzed. In our study, the ratio of GA to HbA1c was slightly low in the period with large albumin leakage compared to that in Small period, suggesting GA could underestimate glycemic status in the period with large albumin leakage (Fig 2a). However, the correlation between blood glucose levels and HbA1c and that between blood glucose levels and GA in the period with small and large albumin leakage was

similar, maybe because ESA doses were relatively low and almost the same without apparent hemorrhage, hemolysis or impaired erythropoiesis, and the effect of albumin leakage change on GA was small during the observation period (Fig 2b,c). Therefore, even in patients undergoing HD or on-line HDF, if albumin leakage was clinically acceptable and red blood cell lifespan was stable with a relatively low amount of ESA use, both GA and HbA1c can be glycemic indicators, whereas both can underestimate glycemic status.

In summary, GA in patients undergoing HD or on-line HDF was practically reliable even if dialysis modality was changed. The preferable marker of glycemic status depends on the albumin and hemoglobin metabolism in each patient undergoing dialysis.

The strong point of this study is that patients were analyzed in a crossover manner so that we could neglect the effect of protein loss in urine. The limitation is that a small number of patients were evaluated without continuous blood glucose monitoring. In addition, we hypothesized that the condition of each patient including food intake and exercise habits was not different during the

observation period. To confirm the hypothesis, we monitored nutritional markers such as n-PCR and CGR in this study.

Conclusion

Clinically acceptable albumin leakage in HD or on-line HDF fluid could affect GA, but was not practically important in controlling diabetic medication. If albumin leakage was within a clinically acceptable level and red blood cell lifespan was stable with a low amount of ESA use, both GA and HbA1c would be correlated with glycemic status. We need to figure out which or what combination is the best among HbA1c, GA, and blood glucose levels to prescribe diabetic medication to each patient.

(2237 words)

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Vos FE, Schollum JB, Walker RJ. Glycated albumin is the preferred marker for assessing glycaemic control in advanced chronic kidney disease. NDT Plus. 2011;4:368-75.

2. Meyer L, Chantrel F, Imhoff O, Sissoko A, Serb L, Dorey F, Fleury D, Smagala A, Kepenekian L, Krummel T, Le Floch JP, Kessler L. Glycated albumin and continuous glucose monitoring to replace glycated haemoglobin in patients with diabetes treated with haemodialysis. Diabet Med. 2013;30:1388-9.

3. Fukami K, Shibata R, Nakayama H, Yamada K, Okuda S, Koga M. Serum albumin-adjusted glycated albumin is a better indicator of glycaemic control in diabetic patients with end-stage renal disease not on haemodialysis Ann Clin Biochem. 2015;52:488-96.

4. Sany D, Elshahawy Y, Anwar W. Glycated albumin versus glycated hemoglobin as glycemic indicator in hemodialysis patients with diabetes mellitus: variables that influence. Saudi J Kidney Dis Transpl 2013;24:260-73.

5. Yajima T, Yajima K, Hayashi M, Takahashi H, Yasuda K. Serum albumin-adjusted glycated albumin as a better indicator of glycemic control in type 2 diabetes mellitus patients with short duration of hemodialysis. Diabetes Res Clin Pract. 2017;130:148-53.

6. Okada T, Nakao T, Matsumoto H, Nagaoka Y, Tomaru R, Iwasawa H, Wada T. Influence of proteinuria on glycated albumin values in diabetic patients with chronic kidney disease. Intern Med. 2011;50:23-9.

7. Watanabe Y, Ohno Y, Inoue T, Takane H, Okada H, Suzuki H. Blood glucose levels in peritoneal dialysis are better reflected by HbA1c than by glycated albumin. Adv Perit Dial. 2014;30:75-82.

8. Kobayashi H, Abe M, Yoshida Y, Suzuki H, Maruyama N, Okada K. Glycated albumin versus glycated hemoglobin as a glycemic indicator in diabetic patients on peritoneal dialysis. Int J Mol Sci. 2016;17: E619.

9. Locatelli F, Manzoni C, Del Vecchio L, Cavalli A, Pontoriero G. Recent trials on hemodiafiltration. Contrib Nephrol. 2011;171:92-100.

10. Maduell F, Moreso F, Pons M et al. High-efficiency postdilution online hemodiafiltration reduces all-cause mortality in hemodialysis patients. J Am Soc Nephrol. 2013;24:487-97.

11. Shinzato T, Nakai S, Fujita Y et al. Determination of Kt/V and protein catabolic rate using pre- and postdialysis blood urea nitrogen concentrations. Nephron. 1994;67:280-90.

12. Shinzato T, Nakai S, Miwa M, Iwayama N, Takai I, Matsumoto Y, Morita H, Maeda K. New method to calculate creatinine generation rate using pre- and postdialysis creatinine concentrations. Artif Organs. 1997;21:864-72.

13. Nagai K, Tsuchida K, Hirose D, Michiwaki H, Hann M, Kanayama HO, Doi T, Minakuchi J. The effect of albumin leakage in hemodialysis patients on redox status of serum albumin. J Artif Organs. 2016;19:310-4.

14. Neirynck N, Vanholder R, Schepers E, Eloot S, Pletinck A, Glorieux G. An update on uremic toxins. Int Urol Nephrol. 2013;45:139-50.

15. Nakao T, Inaba M, Abe M, Kaizu K, Shima K, Babazono T, Tomo T, Hirakata H, Akizawa T; Japanese Society for Dialysis Therapy. Best practice for diabetic patients on hemodialysis 2012. Ther Apher Dial. 2015;19 Suppl 1:40-66.

Figure captions

Fig. 1. Effects of albumin leakage on glycated albumin and other valuables. (a) The scheme of experiments in observation period. Nine patients were treated in the original dialysis setting that caused 2.8 to 5.9 g albumin leakage per session for three months (Pre-Large period). Then, hemodialysis with <1.0 g albumin leakage was applied for three months (Small period). Upon conclusion of Small period, the modality was changed to the original setting for three months (Post-Large period). (b) Serum albumin levels increased significantly in Small period. (c,d) Total cholesterol (c) and cholinesterase (d) decreased in Small period. (e) Blood glucose levels did not change significantly during the observation period. (f) Glycated albumin increased in Small period. Especially Glycated albumin in Small period was significantly higher than that in Post-Large period. (g,h) HbA1c and hemoglobin were not different during the observation period. (i,j) Normalized protein catabolic rate (n-PCR) and creatinine generation rate (CGR) were not different during the observation period except CGR

between Pre-Large and Small period. All values are expressed as mean ± SD (n=9). *$P < 0.01$. **$P < 0.05$. M, month. Pre-L, Pre-Large period. S, Small period. Post-L, Post-Large period.

Fig 2. Relationship among HbA1c, glycated albumin and blood glucose levels.

(a) Ratio of glycated albumin to HbA1c. The ratio was higher in Small period, suggesting large albumin leakage could make glycated albumin underestimate glycemic status. (b,c) Relationship of HbA1c or glycated albumin with casual blood glucose levels. Spearman's coefficient between HbA1c and blood glucose levels was similar with that between glycated albumin and blood glucose levels. **$P < 0.05$. Pre-L, Pre-Large period. S, Small period. Post-L, Post-Large period.

Fig 1.

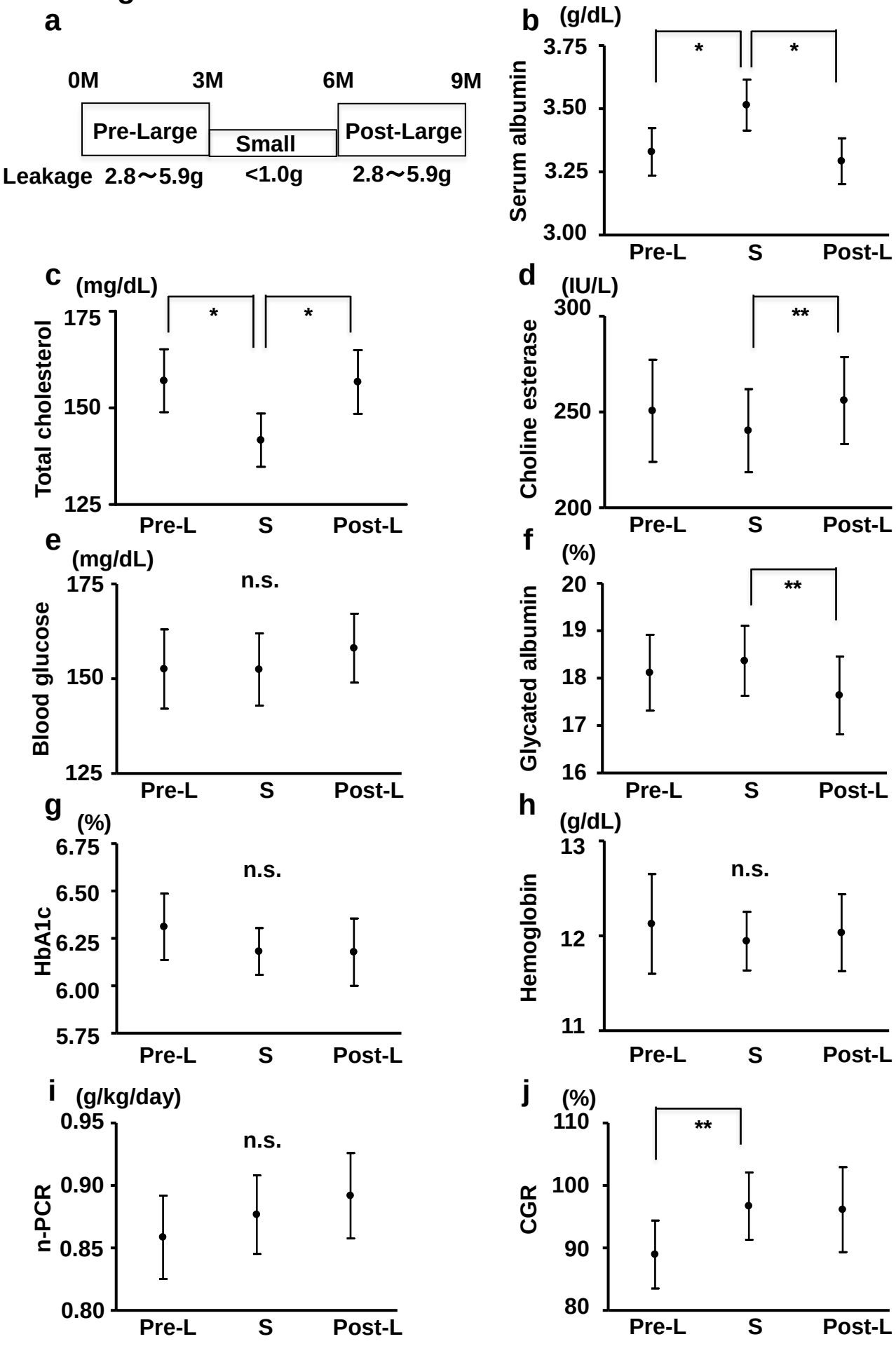
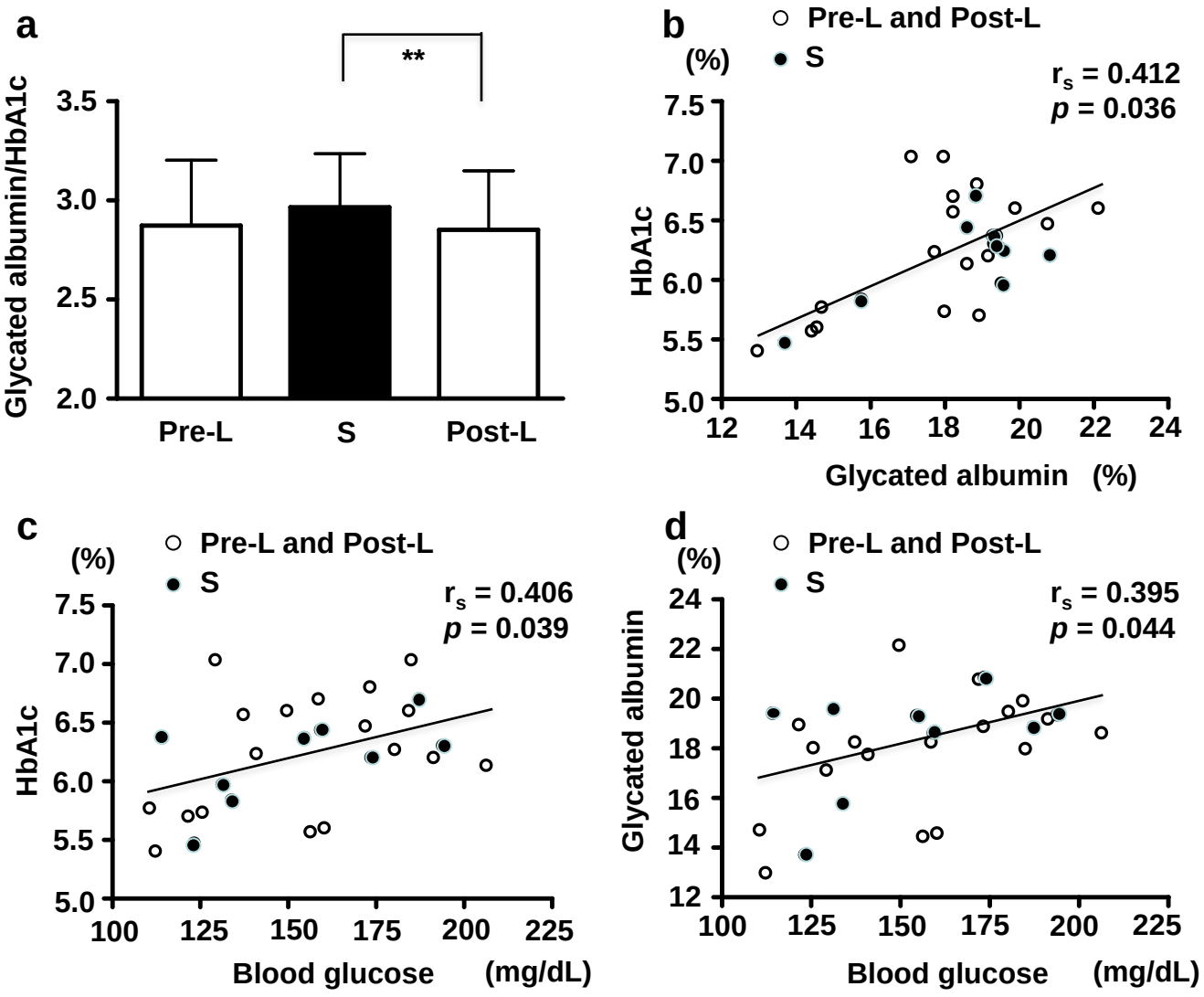

a
0M
3M
6M
9M
Pre-Large
Small
Post-Large
Leakage 2.8～5.9g
<1.0g
2.8～5.9g
b
(g/dL)
Serum albumin
3.75
3.50
3.25
3.00
*
*
Pre-L
S
Post-L
c
(mg/dL)
Total cholesterol
175
150
125
*
*
Pre-L
S
Post-L
d
(IU/L)
Choline esterase
300
250
200
**
Pre-L
S
Post-L
e
(mg/dL)
Blood glucose
175
150
125
n.s.
Pre-L
S
Post-L
f
(%)
Glycated albumin
20
19
18
17
16
**
Pre-L
S
Post-L
g
(%)
HbA1c
6.75
6.50
6.25
6.00
5.75
n.s.
Pre-L
S
Post-L
h
(g/dL)
Hemoglobin
13
12
11
n.s.
Pre-L
S
Post-L
i
(g/kg/day)
n-PCR
0.95
0.90
0.85
0.80
n.s.
Pre-L
S
Post-L
j
(%)
CGR
110
100
90
80
**
Pre-L
S
Post-L

Fig 2.


a
Glycated albumin/HbA1c
3.5
3.0
2.5
2.0
**
Pre-L
S
Post-L
b
Pre-L and Post-L
S
(%)
r_s = 0.412
p = 0.036
HbA1c
7.5
7.0
6.5
6.0
5.5
5.0
12
14
16
18
20
22
24
Glycated albumin (%)
c
Pre-L and Post-L
S
(%)
r_s = 0.406
p = 0.039
HbA1c
7.5
7.0
6.5
6.0
5.5
5.0
100
125
150
175
200
225
Blood glucose (mg/dL)
d
Pre-L and Post-L
S
(%)
r_s = 0.395
p = 0.044
Glycated albumin
24
22
20
18
16
14
12
100
125
150
175
200
225
Blood glucose (mg/dL)