〈Original Article〉

EC-SOD levels in pre-dialysis sera and the relationship with HOMA-R

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Summary The study patients were characterized as having diabetic nephropathy, which was renal insufficiency with glomerular deterioration in the majority of cases.

Focusing on a combination of extracellular SOD (EC-SOD) and insulin resistance index (HOMA-R) as a new marker in dialysis samples (N = 48), we examined its usefulness as an index of disease status.

EC-SOD in ng/mL (*y*-axis, real measured value) was determined by the method of Adachi et al., and HOMA-R (*x*-axis, dimensionless relative value) by the method of Matthews et al. The results yielded a correlation coefficient of $r = 0.740$ ($p < 0.001$) and regression equation $y = 22.3x + 108.1$, which indicated usefulness. Because the number of studied cases was less than 50 and statistical power was insufficient, this report only presents an account of our findings.

Key words: EC-SOD, Pre-dialysis sera, HOMA-R, Adachi method, Matthews method

1. Introduction

In the seventy years since the end of World War Ⅱ, Japan has experienced unprecedented economic growth and consequently entered an era of low fertility and population aging. This has changed the lives of people physically, functionally, and even culturally. Increasing longevity demanded radical revision of the healthcare system, as lifestyle-related diseases in elderly citizens emerged as a major theme. Various

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types of diseases affecting elderly citizens each have different characteristics. Diabetic nephropathy, in particular, is a complex disease characterized by specific features. The prevalence of diabetic nephropathy has increased rapidly in recent years, almost overtaking that of glomerular nephropathy. Many patients are falling into conditions requiring hemodialysis due to the advancement of disease, and they are usually assessed by the following three types of disease characterization tests[1].

One is periodic tests to monitor the effect of hemodialysis, the second is disease-specific tests to check for abnormal changes in the disease, and the third is screening tests for speedy detection of sudden changes in disease status. Of these broad classes of tests, periodic tests play the central role.

The main purposes of hemodialysis tests in general are the evaluation of dialysis adequacy and the early detection of complications. In the practice of hemodialysis treatment in Japan, pre-dialysis tests are performed customarily before a dialysis session following two days of no dialysis. We used the measurement parameters to assess the effect of hemodialysis as described below. Electrolytes and metals (Na, K, Cl, Ca, i-P), protein and N compounds (TP, Alb, Crea, BUN), and hematology values (Hb, Ht) have long been used as common indices. In order to understand disease status in greater detail, we

Table 1 Age and clinical laboratory test values of pre-hemodialysis patients
The data of patients who had EC-SOD level more than 400 ng/ml were eliminated from the analysis.

N=48

Components		pre-tests	Correlation (pre vs post)	
		$\bar{x}$±SD	Pre/post	t-test
1 Age	years	55.4±11.2		
2 BMI	kg/m^2	20.5±1.9		
3 Systolic blood pressurs	mmHg	144.7±21.1		
4 GOT	U/L	12.6±5.3	0.903	p<0.001
5 BUN	mg/dL	66.1±14.9	0.813	p<0.001
6 Crea	mg/dL	11.4±2.7	0.894	p<0.001
7 Ca	mg/dL	8.8±1.1	0.689	NS
8 i-P	mg/dL	5.8±1.7	0.714	p<0.001
9 Fasting plasma glucose	mg/dL	113.6±25.9	0.541	p<0.001
10 HbA1c	%(NGSP)	5.4±1.2	0.933	NS
11 TG	mg/dL	92.1±56.2	0.746	p<0.001
12 TC	mg/dL	141.5±36.4	0.906	p<0.001
13 HDL-C	mg/dL	43.1±12.8	0.889	p<0.001

Table 2 The difference and ratios of HOMA-R related values between pre- and post- hemodialysis patients.
The data of patients who had EC-SOD level more than 400 ng/mL were eliminated from the analysis.

N=48

studies		$\bar{x}$±SD		post/pre
		Pre-Hemodialysis	Post-Hemodialysis	
Basic HOMA-R		4.49±2.01		
1 EC-SOD	(ng/mL)	208.3±60.6	253.0±75.7	1.21
2 LPL mass	(ng/mL)	60.6±12.9	74.0±19.3	1.22
3 FFA	(μEq/L)	541.8±458.7	1,893.2±982.9	3.50
4 GA	(%)	16.9±3.7	16.3±3.6	0.96

devised the following strategy.

As we considered it important to select indices for the detection of insulin resistance and the avoidance of its aggravation, we measured extracellular SOD (EC-SOD)[2] as the main focus of our attention and also the insulin resistance index (HOMA-R)[3] and lipoprotein mass status (LPLmass)[4]. We examined how these parameters reflected disease status and the interrelationships among them.

2. Subjects and Methods

1. Tests on Enrolled Dialysis Patients (Tables 1 and 2)

Forty-eight outpatients on hemodialysis who had undergone routine clinical tests were randomly selected and the population trends of relevant laboratory test values were analyzed.

We calculated the dispersion (x±SD) of the values for 13 mandatory test items and reported the pre/post-dialysis ratios. Remarkable differences were observed in BUN, Crea, Ca, i-P, TG, and HDL-C (Table 1). In this study, we excluded the mutant-type cases with EC-SOD of 400 ng/mL or more, so that we could grasp the correlation with disease status.

The pre/post-dialysis ratio in this analysis was 1.21 for EC-SOD, 1.22 for LPLmass, 3.50 for FFA, and 0.96 for GA. We paid attention to the stability of EC-SOD and LPLmass and the absolute value of GA. Because solute components in the blood of dialysis patients are eliminated from the body through diffusion and filtration, selection of the time of sampling was an important key to the correct evaluation of blood levels. Considering the fact that pre-dialysis tests are usually performed two days after a dialysis session, we conducted tests following the same schedule.

2. Methods

Quality control (QC) is a major theme for the tests on hemodialysis patients. Although the laboratory room maintains techniques that consistently control measurement errors to a certain level, it is essential that we have a testing system satisfying clinical needs. There must be a specialized testing system based on prearranged coordination between dialysis tests and the clinical practice of dialysis apart from the system for routine testing.

In particular, dialysis tests are performed as strict prearranged tests (e.g., time of blood sampling) according to rules agreed on with clinical practice. The testing staff performs laboratory tests with accurate understanding of within-day and day-to-day varia-

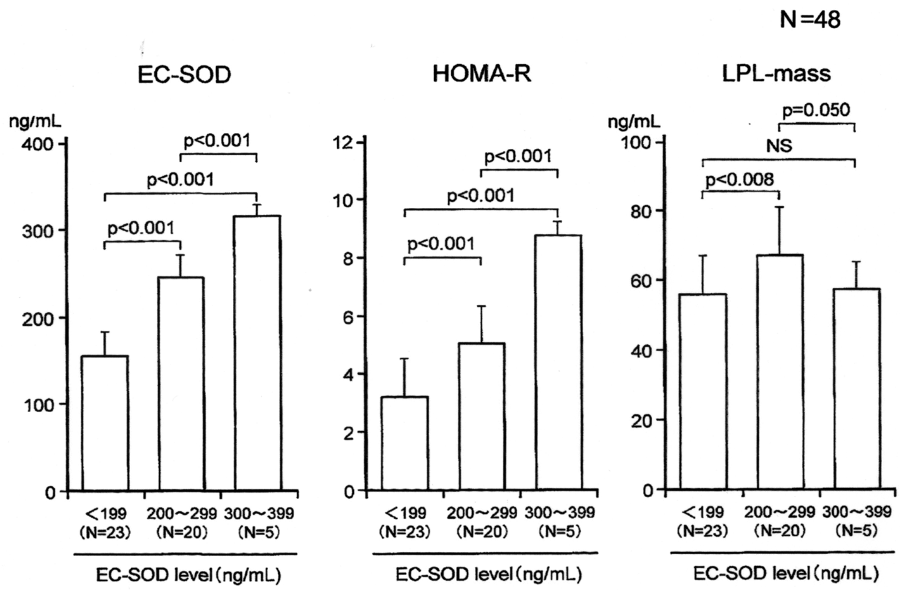

Fig. 1 The comparison of EC-SOD, HOMA-R and LPL-mass levels between three patinet groups defined by EC-SOD levels.

tions. While thorough quality control must of course be performed, the difference in quality that occurs between the specimens from the dialysis testing room and those from the routine testing room makes it necessary to grasp population trends, mode values, and other characteristics.

In this study, the $\overline{x} \pm SD$ increased remarkably for BUN and Crea, and slightly for blood pressure and i-P. In addition, an increase in albumin was observed after dialysis.

3. Results

1) EC-SOD Levels (Table 2)

The population trends of EC-SOD levels were examined in 48 patients excluding the cases with 400 ng/mL or higher EC-SOD, which were considered to comprise EC-SOD mutant patients.

HOMA-R (method of Matthews et al.)[5] showed a moderate insulin resistance index at 4.49±2.01. The ratio between the pre- and post-dialysis values for EC-SOD was 1.21. The similar ratio for LPLmass was 1.22, that for FFA was 3.50, and that for GA was 0.96. While LPLmass showed the same behavior as EC-SOD, the data for FFA suggested the presence of a background process undergoing substantial biochemical changes. GA showed no changes partly because of the effect of glycation.

2) Behavior of Relevant Parameters Relative to Three Levels of EC-SOD (Fig. 1)

The standard value for EC-SOD as used in the method of Adachi et al.[6] is 73.1±16.7 ng/mL (N = 53). When the data grouped according to the three levels of EC-SOD (<199, 200-299, and 300-399 ng/mL) were compared, the mean concentrations of EC-SOD were 155, 244, and 315 ng/mL, respectively, increasing significantly in a kinetic manner.

On the other hand, HOMA-R in comparison among the three levels of EC-SOD also increased significantly from 3.2 to 5.0 and 8.7, while LPLmass only showed a non-significant increasing tendency.

3) Behavior of Relevant Parameters Relative to Three Levels of HOMA-R (Fig. 2)

HOMA-R can be characterized as a relative laboratory marker.

Maehata et al.[3] reported that the standard value (x±1SD) for HOMA-R was 1.57 and the cut-off value was 2.00.

The concentrations of EC-SOD as compared among the three levels of HOMA-R (<3, 3-5, and >5) showed significant increases from 161 to 192 and 267 ng/mL.

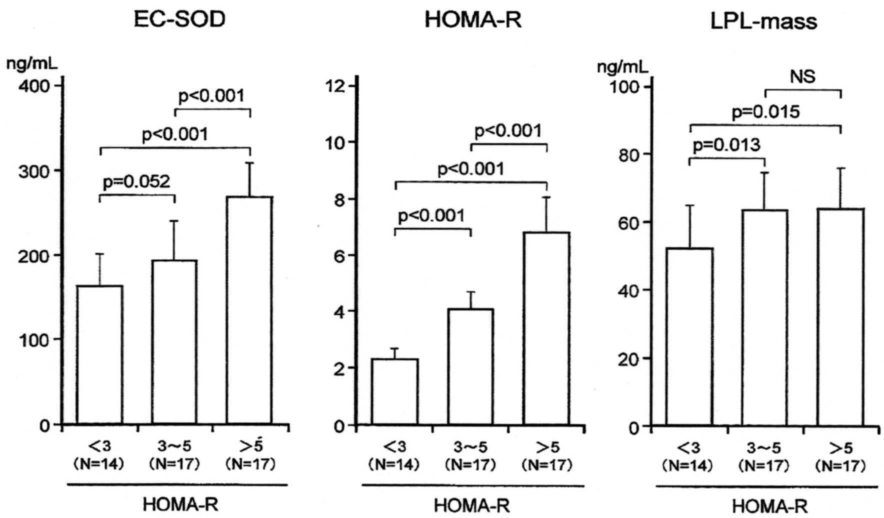

Fig. 2 The comparison of EC-SOD, HOMA-R and LPL-mass levels between three opatient groups defined by HOMA-R levels.

On the other hand, HOMA-R in comparison among the three levels showed significant stepwise increases from 2.3 to 4.1 and 6.8, following a similar pattern.

LPLmass only showed a non-significant increasing tendency.

4) Behavior of Relevant Parameters Relative to Three Levels of Years on Dialysis (Fig. 3)

We examined the behavior of three parameters (EC-SOD, HOMA-R, and LPLmass) in comparison among the three levels of years on dialysis (<10 years, 10-20 years, and >20 years). The number of cases falling under the three dialysis period categories was 25, 15, and 8, respectively.

No significant changes were seen in the three parameters examined.

5) Correlation between Pre-dialysis EC-SOD and HOMA-R (Fig. 4)

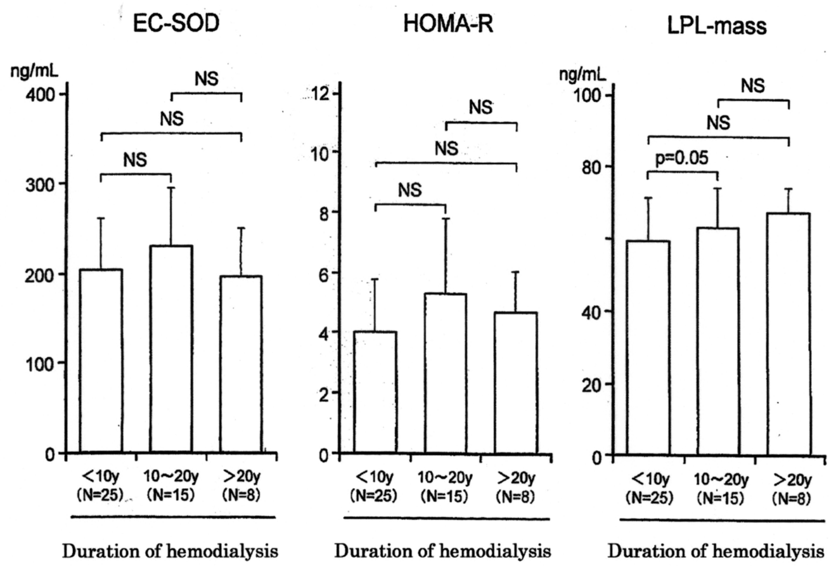

Fig. 3 The comparison of EC-SOD, HOMA-R and LPL-mass levels between three patient groups defined by duration of hemodialysis.

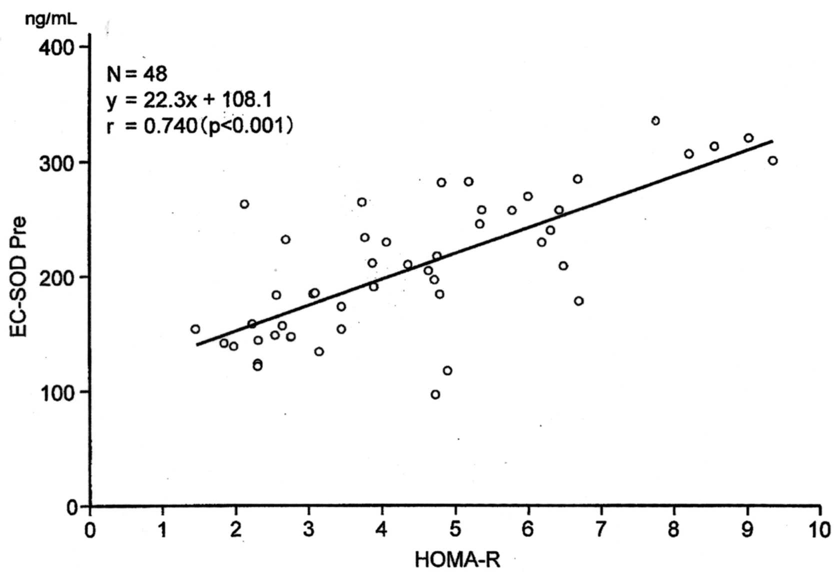

Fig. 4 The correlation between HOMA and EC-SOD levels in the pre-hemodialysis serum.

When pre-dialysis EC-SOD of 48 cases was used as the basis for comparison, the correlation between this parameter and HOMA-R was significant with a correlation coefficient $r = 0.740$ ($p < 0.001$) (Fig. 4).

On the other hand, weak, albeit significant correlations were found between HOMA-R and LPLmass (pre) with $r = 0.253$ ($p < 0.001$) and between LPLmass (pre) and FFA (pre) with $r = 0.236$ ($p < 0.05$). Neither of these two weak correlations was useful as a laboratory test, being only indicative of a tendency. The correlation between pre-dialysis EC-SOD and HOMA-R with $r = 0.740$ seemed good, and was considered useful despite the result that its contribution ratio was less than 60%.

4. Discussion

The rapid socioeconomic progress of Japan after World War Ⅱ was so remarkable that it changed the dietary life of the Japanese people and forcibly modified their body constitution. In particular, the accelerated introduction of a Western style diet, combined with the increase in fat intake from restaurant foods, upset the nutrient balance, leading to the unwanted establishment of the physical characteristics of the present-day Japanese.

In the past, the long history of eating poorly digestive rice as the staple food created long intestines in Japanese people, low lactase activity, and low insulin secretion capacity, which predisposes them to the development of diabetes mellitus.[7] In other words, the pancreas of the Japanese with low insulin secretion capacity has become exhausted from the dependence on modernized diet, resulting in a situation where overeating and obesity promote the development of diabetes mellitus.

This phenomenon is observed markedly in the middle- and high-age populations. Unless the Japanese return to traditional Japanese foods that are compatible with their original body constitution, they are likely to suffer food-related problems and inevitable causation of diseases.

When we look at the national statistics of the per capita daily intake of nutrients[8] in 1950, 1970, and 2010, energy intake decreased from 2,098 to 2,210 and 1,849, and protein and carbohydrates followed a similar pattern. In contrast, fat intake increased from 18.3 to 46.5 and 53.7, emerging as a cause of problems. The rapid economic growth of Japan unwittingly produced an aging society and contributed to the prevalence of obesity.

The mean life of the Japanese this year increased to 86.61 years in females (ranking the first in the world) and 80.21 years in males (the fifth in the world), confirming the advent of "an era of longevity" supported by the progress of healthcare technologies.

However, diseases in elderly persons tend to involve multiple organs, and many of them are manifested in the form of the geriatric syndrome presenting nonspecific symptoms. This situation demands radical revision of the diseases in elderly people. In this respect, one of the most important health conditions in Japan is age-related disorders in dialysis patients. The mean age of patients has exceeded 60 years, and the most frequent problem in these patients is renal insufficiency resulting from diabetic nephropathy. This situation is emerging as a major healthcare issue in the country.

In this study, the randomly selected 48 dialysis patients had a mean age of 55.4±11.2 years, and were monitored using Gluc, HbA1c, and GA as the indices for blood glucose control. Many patients on dialysis have pathologies causing oliguria or anuria, in which solutes that should be excreted via the kidneys tend to accumulate in the body. Because hemodialysis artificially removes the solutes requiring excretion using a regimen with two or three sessions in a week, the assessment of the adequacy of this procedure crucially requires appropriate definition of sampling conditions.

The test items examined before and after dialysis include electrolytes and metals (Na, K, Cl, Ca, i-P), N components (BUN, Crea), proteins (total protein, albumin), liver enzymes (GOT, GPT), glycated substances (GA), and anemia markers (Hb, Ht). *Management of Diabetic Patients on Hemodialysis 2012*, the guidelines published by the Japanese Society for Dialysis Therapy[9] stipulates laboratory tests for this purpose basically using the above-mentioned markers.

We focused our attention on the possibility that biological substances related to active oxygen species may provide useful information as diabetes markers. First, we examined the dependence of SOD activity on EC-SOD[10] concentration in the patients with high SOD activity. Our report[2] demonstrated the usefulness of EC-SOD as a nephropathy marker, yielding promising results concerning disease characterization and the efficacy of dialysis therapy.

As a next step, the present study investigated the measurement of EC-SOD levels in dialysis patients in terms of its meaning and efficacy. In our methodology, EC-SOD levels were stratified into three groups (<199, 200-299, and 300-399 ng/mL), the distribution of EC-SOD levels among these groups was confirmed, and then the analysis was focused on the relationships with HOMA-R and LPLmass. The levels in the 48 dialysis patients studied were associated with increases in BUN (mean 66.1 mg/dL), Crea (mean 11.4 mg/dL), and i-P (mean 5.8 mg/dL) and a decrease in Ca (mean 8.8 mg/dL).

First, we examined the pathological level focusing on EC-SOD (Figs. 1, 2, and 3).

We stratified EC-SOD levels into three groups and, using this as the basis for comparison, we graded HOMA-R and LPLmass in terms of disease identification and assessed significance. EC-SOD and HOMA-R were significantly ($p < 0.001$) positive for identification, showing clearly stepwise increases. Namely, both significantly showed kinetic behavior, and were considered promising as a marker.

Finally, we looked for the correlations that might detect pathologic reactions. The correlation between EC-SOD (y) and pre-dialysis HOMA-R (x) ($N = 48$) was $r = 0.74$ with the regression equation $y = 22.3x + 108.1$. EC-SOD was a real value, while HOMA-R was a relative value, and the contribution ratio in this case was as low as 54%. Because of this reason and because the sample size was 48 cases, we consider that this result barely satisfies the needs in dialysis therapy[11]. As the intercepts on the correlation diagram in this analysis were different from each other ($x \neq y$), we set the aim as a significant correlation.

On the other hand, the correlation between HOMA-R and LPLmass (pre) was $r = 0.253$ ($p < 0.001$), and the correlation between LPLmass (pre) and FFA (pre) was $r = 0.236$ ($p < 0.05$). These correlations were considered to be weak, and therefore we adopted only the correlation between EC-SOD (pre) and HOMA-R.

5. Conclusion

Monitoring of disease status using pre- and post-dialysis tests is an essential part of treatment of patients on hemodialysis, and the tests must be performed in a system tailored to actual needs and endorsed by adequate quality control. Such a system should comply with the testing guidelines stipulated by the Japanese Society for Dialysis Therapy.

In this study, we designed a simple dilution method as an attempt to introduce a new dialysis test. This method used EC-SOD (y-axis, Adachi's method), an extracellular heparin-binding substance that is an enzyme reacting with active oxygen species, as a standard. Pre-dialysis serum was diluted simply and the activity level was examined in relation to HOMA-R (x-axis, Matthews' method).

A significant ($P < 0.001$) linear relationship with a correlation coefficient $r = 0.740$ and a constant positive bias ($y = 22.3x + 108.1$) was revealed. Our method was found to trace EC-SOD positive conversion and its progression significantly up to the HOMA-R value of 6.5. In this study, we demonstrated its efficacy as a direct method in the analysis of dialysis specimens, proposed a new test method for use in dialysis therapy, and made it possible to develop a system with improved accuracy.

References

1. Kato A: How to Read and Interpret Laboratory Test Data in Dialysis. pp. 1-7, Chugai-Igakusha, 2014.
2. Maehata E, Adachi T, Inoue M, Yano M, Shimomura H, Shiba T, et al.: High blood superoxide dismutase (SOD) states in patients with diabetes mellitus-dependence on extracellular (EC)-SOD.: Dependence on Extracellular (EC)-SOD. J Japan Diab Soc, 44(12): 935-941, 2001.
3. Maehata E, Yano M, Shiba T, Yamakado M, Inoue M, Suzuki S: Methods for the evaluation of insulin resistance, insulin resistance index (HOMA-R method). Japanese Journal of Clinical Medicine, 60(suppl. 8):

341-350, 2002.

4. Kobayashi J, Hashimoto H, IFukamachi I, Tashiro J, Shirai K, et.al.: Lipoprotein lipase mass and activity in severe hypertriglyceridemia. Clin Chim Acta, 216: 113-123, 1003.
5. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Trecher OF and Turner RC: Homeostasis model assessment: insulin resistance and beta β -cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28: 412-419, 1985.
6. Adachi T, Ohta H, Yamada H, Futenma A, Kato K and Hirano K: Quantitative analysis of extracellular-superoxide dismutase in serum and urine by ELISA with monoclonal antibody. Clin Chim Acta, 21: 89-102, 1992.
7. Sato T: Supplement Bible for Ages 50 and Over. pp. 20-22, Kodansha, 2010.
8. Present State of National Health and Nutrition − From the Report of the National Health and Nutrition Survey in Japan, 2010, by the Ministry of Health, Labour and Welfare, Appendix 2, Dai-ichi Shuppan, 2013.
9. Management of Diabetic Patients on Hemodialysis 2012. Journal of the Japanese Society for Dialysis Therapy, 46(3): 311-357, 2013.
10. Adachi T: New Information on Extracellular SOD in "New Information on SOD (Structure, Function, and Relevance to Diagnosis and Treatment)", Katsube Y and Taniguchi N (eds.), pp. 129-141, Medical Tribune, 1990.
11. Kitaoka T: Knowledge of Renal Failure and Dialysis Therapy. pp. 145-156, Nanzando, 2014.