ORIGINAL ARTICLE

Study of the Role of Erythrocyte Glutathione Transferase as A potential Marker of Haemodialysis Adequacy

Nagwa M. Mowafy [a], Muhammad A. B. Khedr [a], Farag K. F. Ebrahim [b], Mohamed A. S. Aleysawy [a,*]

[a] Department of Clinical Pathology, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt
[b] Department of Internal Medicine, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt

Abstract

Background: ***The high prevalence of complications and fatalities caused by end-stage renal disease (ESRD) makes it a major issue in global public health. Over the past three decades, the prevalence of chronic kidney disease (CKD) has increased dramatically on a global scale.***

Aim and objectives: ***Our goal is to find out whether erythrocyte glutathione transferase (E-GST) is just another urea measurement that goes along with Kt/V hemodialysis (HD) or if it's a new way to tell if dialysis is working well.***

Patients and methods: ***In this cross-sectional study, which lasted from August 2023 to August 2024 and was structured according to hemodialysis mode, forty patients with end-stage renal disease (ESRD) who were regularly treated at the hemodialysis units of Al-Hussein and Said Galal Al-Azhar university hospitals were included. Patients were divided into two equal groups according to gender and age.***

Results: ***When it comes to measuring hemodialysis adequacy, E-GST is just as effective as URR and Kt/V Urea. It serves as an additional indicator that reflects URR and Kt/V values, and there is no statistically significant distinction between traditional HD and post-dilutional online hemofiltration (OL-HDF) (P-value>0.05).***

Conclusion: ***Hemodialysis adequacy and the elimination of toxins in multiple sessions by different hemodialysis modalities can be expressed by the amount of E-GST, a hemodialysis patient marker that is both sensitive and specific.***

Keywords: E-GST; Haemodialysis adequacy

1. Introduction

One of the most important issues in public health around the world, end-stage renal disease (ESRD), is responsible for a significant number of deaths and illnesses.[1]

Over the past three decades, the prevalence of chronic kidney disease (CKD) has increased dramatically on a global scale.[2]

Its current estimated prevalence in the global population is 11% to 13%3

The elimination of urea has traditionally been used to evaluate the solute removal during dialysis. Nevertheless, it is considerably more challenging to eliminate protein-bound solutes and larger intermediate molecules (MW≥1000 Da), and the persistence of these substances has been linked to numerous clinical manifestations of uremia. In addition, they are believed to have a significant impact on the negative consequences of hemodialysis.[3]

The current standard for discussing hemodialysis dose and appropriateness is Kt/V urea, which represents the fractional dialyzer whole blood clearance of urea.[4]

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* Corresponding author at: Clinical Pathology, Faculty of Medicine for Boys, Al-Azhar University, Cairo, Egypt.
E-mail address: fmohammedaleysawy@gmail.com (M. A. S. Aleysawy).

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Kt/V urea in dialysis is only a surrogate for small molecule clearance; it does not reveal anything about intermediate or big molecule clearance, according to recent research; therefore, it does not accurately reflect the removal of other fluids and solutes.[5]

The purpose of this research was to confirm whether E-GST is an additional urea parameter to the Kt/V ratio or a new indicator of hemodialysis sufficiency in various dialysis methods.

2. Patients and methods

This cross-sectional study analyzed 40 ESRD patients who were regularly treated with hemodialysis at the hemodialysis units of Al-Hussein and Said Galal Al-Azhar University hospitals from August 2023 to August 2024. The patients were categorized according to the mode of hemodialysis they were undergoing. Two age- and sex-matched groups of patients were formed: Twenty patients undergoing standard hemodialysis (Group A), twenty patients undergoing high-dose filtration (HDF) (Group B), and twenty healthy individuals (Control) were included. Next, patients were divided into two groups based on Kt/V: one group was given appropriate dialysis (Kt/V>1.3), and the other was given inadequate dialysis (Kt/V≤1.3)

Inclusion Criteria

Adults with ESRD who have been taking regular HD for at least six months and who are receiving therapy three times a week for a total of four hours were included in the study.

Exclusion Criteria:

Liver disease and hyperbilirubinemia.

Methods:

After obtaining a complete well-informed consent, from all subjects they were subjected to comprehensive clinical workup including full clinical history, clinical examinations and laboratory investigations which include the following: The laboratory tests include an iron panel (serum iron, total bilirubin, direct bilirubin serum creatinine, and TIBC), a full blood count (CBC), and a battery of chemical tests. Prior to and after dialysis, each patient's urea, single pool Kt/V urea, and urea reduction ratio (URR) were all measured. Before the hemodialysis treatment began, the enzyme-linked immunosorbent assay (ELISA) was used to measure the blood erythrocyte glutathione transferase levels of all patients and controls.

Sampling:

The complete blood count (CBC) requires three milliliters of venous blood on EDTA and five milliliters of venous blood on a plain tube.

Calculation of Result:

Establish a reference curve by best-fitting data points from a graph showing concentration (X-axis) and average optical density (OD) of all standards (Y-axis). The best way to do computations like this is with computer-based curve-fitting tools, and regression analysis can help you identify the best-fit line.

Statistical analysis:

For data editing, coding, and tabulation, we relied on IBM's Statistical Package for the Social Sciences (2017, Released). Download the latest version of IBM SPSS Statistics for Windows from IBM Corp. in Armonk, New York, USA. The version is 25.0. Standard deviation (±SD) and mean (S) were used for parametric numerical data. When dealing with nonparametric numerical data, the median and interquartile range were used. Data that was not numerical was represented using percentages and frequencies. In order to verify that the data followed a normal distribution, the Shapiro-Wilk test was used. A chi-square test was used to observe whether there was a link between the two qualitative variables. To check for a statistically significant difference in the parametric variable, we used the Student T-Test to compare the two groups' means. The paired T-Test was used to compare two time periods and find out if a parametric variable changed significantly.

We used the one-way ANOVA test to see if the research groups' parametric variables differed significantly from one another. We used the Kruskal-Wallis test to see if any of the research groups' nonparametric variables differed significantly from one another. Study participants used Pearson's correlation analysis to rate the strength of the association between two numerical variables.

Quantitative diagnostic tests that divide cases into two groups can be usefully assessed for specificity and sensitivity using the ROC curve. Our goal was to identify the cutoff number that would maximize the area under the curve (AUC). We used logistic regression analysis to predict risk factors when the dependent variable was categorical. Probability ratios (ORs) are a useful tool for estimating the strength of a relationship between two variables. In order to determine the likelihood of an occurrence given an exposure, one can use the odds ratio (OR), which compares the odds with and without the exposure. OR=1 Variable exposure has no effect on outcome probability.

3. Results

Table 1. Comparing the demographic data of hemodialysis patients with Kt/V ≤ 1.3 and Kt/V > 1.3

	CONTROL N=20		KT/V>1.3 N=20		KT/V≤1.3 N=20		TEST	P1	PAIRWISE		
	No.	%	No.	%	No.	%			p2	p3	p4
SEX											
MALE	10	50.0	9	45.0	12	60.0	χ2=0.934	0.627	NS	NS	NS
FEMALE	10	50.0	11	55.0	8	40.0					
AGE(YEARS)							F=5.086*	0.009*	0.030*	0.015*	0.958
MEAN±SD	39.90±9.67		47.85±9.46		48.70±9.74						

SD:Standard deviation, F:One Way ANOVA test, x2:Chi-Square test. p1:Comparing the three groups, p2:Comparing control and KT/V>1.3, p3:Comparing control and KT/V≤1.3, p4:Comparing KT/V>1.3 and KT/V≤1.3, *:Significant when p-valu<0.05.

There is not a statistically significant distinction in the age distribution between the KT/V>1.3 and KT/V≤1.3 groups in Table 1 based on the chi-square test; however, post-hoc pairwise comparisons show significant age differences between the Control group and both KT/V>1.3 and KT/V≤1.3 groups.

Table 2. Comparison between the three studied groups regarding CBC.

	CONTROL N=20	KT/V>1.3 N=20	KT/V≤1.3 N=20	TEST	P1	PAIRWISE		
						p2	p3	p4
MCHC(%)								
MEDIAN	31.0	28.50	29.0	H=23.33*	<0.001*	<0.001*	<0.001*	0.295
IQR	30.0-32.0	27.0-29.0	28.0-29.50					
MCV(FL)				F=0.198	0.821	NS	NS	NS
MEAN±SD	87.80±4.30	88.07±4.11	88.54±2.70					
PLATELETS(Kμ/L)				F=9.604*	<0.001*	0.002*	0.001*	0.944
MEAN±SD	280.6±64.19	222.8±41.71	217.6±42.08					
HEMATOCRIT(%)								
MEDIAN	39.0	30.10	30.0	H=39.47*	<0.001*	<0.001*	<0.001*	0.821
IQR	38.50-41.0	28.65-32.50	28.25-32.15					
WBCS (Kμ/L)				F=1.003	0.373	NS	NS	NS
MEAN±SD	6.45±1.10	7.02±1.71	7.08±1.74					
RBS(Kμ/L)								
MEDIAN	4.60	3.95	4.10	H=26.63*	<0.001*	<0.001*	<0.001*	0.935
IQR	4.50-5.15	3.80-4.30	3.75-4.30					
HB(G/DL)								
MEDIAN	13.0	9.95	10.05	H=39.64*	<0.001*	<0.001*	<0.001*	0.993
IQR	13.0-14.50	9.60-10.70	9.55-10.65					

SD:Standard deviation, IQR:Interquartile range. F:One Way ANOVA test, H:Kruskal–Wallis test,

p1:Comparing the three groups, p2:Comparing control and KT/V>1.3, p3:Comparing control and KT/V≤1.3, p4:Comparing KT/V>1.3 and KT/V≤1.3, *:Significant when p-value<0.05.

Table 2 shows notable variations in multiple CBC parameters when comparing the Control group to the two KT/V groups, the KT/V groups show lower levels of MCHC, platelet counts, hematocrit, RBCs, and hemoglobin when compared to the Control group, there are no notable differences in MCV and WBC counts across the three groups. no significant difference was observed between the two KT/V groups.

Table 3. Comparison between the two studied groups regarding UR post and urea reduction ratio

	KT/V>1.3 N=20	KT/V≤1.3 N=20	TEST	P
UR POST(MG/DL)				
MEAN±SD	30.45±7.32	41.15±7.98	t=4.419*	<0.001*
URR(%)				
MEAN±SD	0.74±0.03	0.62±0.04	t=11.12*	<0.001*

SD:Standard deviation, t:Student t test.

p:Comparing the two studied groups, *:Significant when p-value<0.05.

In table 3 a higher KT/V group achieves more effective urea removal during dialysis, leading to reduced post-dialysis urea levels and an increased URR. On the other hand, a group with lower KT/V demonstrates less effective urea removal, resulting in elevated post-dialysis urea levels and a reduced URR. These results align with the definition of KT/V.

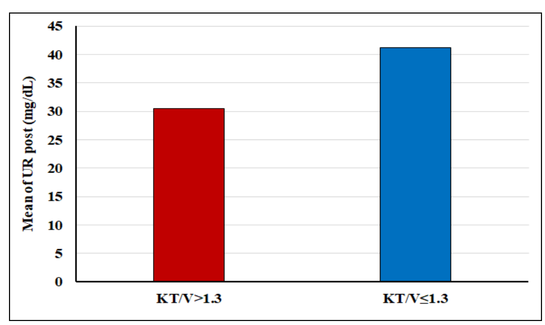

Figure 1. Two groups were compared with respect to UR post.

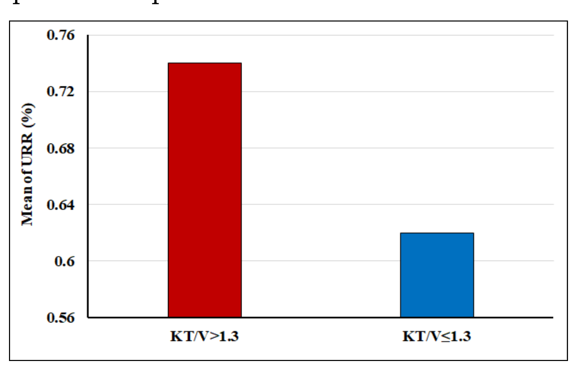

Figure 2. Analysis of the two groups compared with respect to URR.

Table 4. Comparison of GSH levels among the three groups that were investigated

GSH (NG/ML)	CONTROL N=20	KT/V>1.3 N=20	KT/V≤1.3 N=20	TEST	P1	PAIRWISE p2	PAIRWISE p3	PAIRWISE p4
MEAN±SD	2.81±0.58	11.20±2.49	19.71±2.51	F=333.7*	<0.001*	<0.001*	<0.001*	<0.001*

SD:Standard deviation, F:One Way ANOVA test, p1:Comparing the three groups, p2:Comparing control and KT/V>1.3, p3:Comparing control and KT/V≤1.3, p4:Comparing KT/V>1.3 and KT/V≤1.3, *:Significant when p-value<0.05.

Table 4 shows that the control group shows notably lower GSH levels when compared to both the KT/V>1.3 and KT/V≤1.3 groups. The group with KT/V>1.3 shows notably reduced GSH levels when compared to the KT/V≤1.3 group. These findings indicate that the adequacy of dialysis, measured by KT/V, is linked to changes in GSH levels.

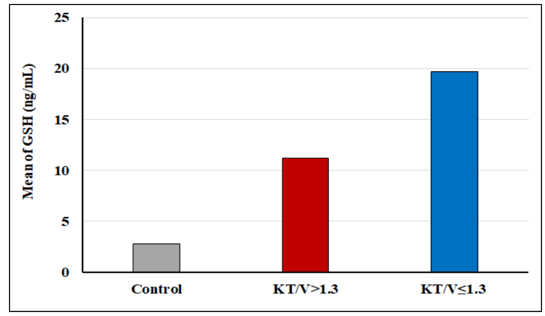

Figure 3. Comparison of the three groups under study in terms of GSH.

Table 5. GSH's ability to distinguish between patients and the control group.

AUC	95% CI	P	CUT OFF	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)	ACCURACY (%)
GSH (NG/ML)								
1.0	1.0-1.0	<0.001*	>3.9	100	100	100	100	100

PPV stands for positive predictive value and NPV for negative predictive value; CI stands for confidence interval; AUC stands for area under the ROC curve. Significant p-value is less than 0.05.

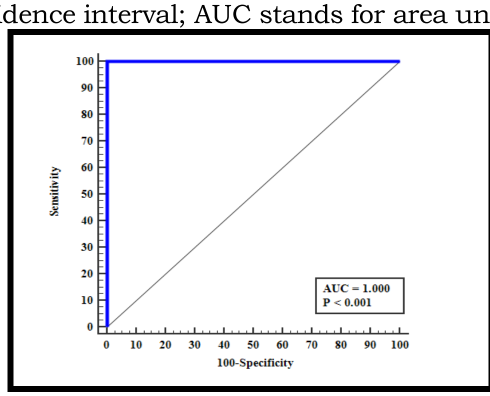

Figure 4. ROC Curve for GSH for discrimination between patients and control group.

Table 5 showed that, GSH levels possess remarkable validity in differentiating between individuals with CKD necessitating dialysis and healthy controls. An AUC of 1.0 signifies flawless differentiation between patients and the control group. GSH levels exhibit high sensitivity and specificity, achieving 100% sensitivity in identifying all persons with CKD necessitating dialysis and 100% specificity in recognizing all healthy individuals within the control group. A PPV of 100% signifies that a positive test confirms a patient with CKD necessitating dialysis, whereas a NPV denotes a healthy control. The ideal cut-off value for distinguishing between patients and controls is determined to be>3.9 ng/mL for GSH. GSH levels exhibit exceptional precision in this situation.

Table 6. Validity of GSH for predicts KT/V≤1.3.

AUC	95% CI	P	CUT OFF	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)	ACCURACY (%)
GSH (NG/ML)								
0.995	0.982-1.0	<0.001*	>14.4	100.0	95.0	95.2	100	97.5

NPV, or negative predictive value; PPV, or positive predictive value; CI, or confidence interval; and AUC, or area under the ROC curve. *: Significant P-value <0.05.

Table 6 shows the efficacy of GSH levels in forecasting insufficient dialysis demonstrated a good predictive accuracy, with an AUC of 0.995. The p-value is below 0.001, signifying a statistically significant correlation between GSH levels and insufficient dialysis. The test exhibits great sensitivity and specificity, demonstrating 100% sensitivity and 95% specificity. It possesses elevated positive and negative predictive values, specifically a 95.2% positive predictive value and a 100% negative predictive value. The overall accuracy is 97.5%, signifying dependable prediction of dialysis based on GSH levels. The findings indicate that monitoring GSH levels could serve as a useful metric for evaluating dialysis adequacy. A GSH level beyond the limit indicates insufficient dialysis, whereas a level below the cutoff indicates sufficient dialysis.

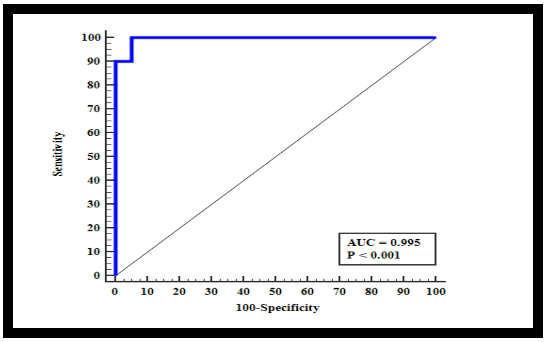

Figure 5. ROC Curve for GSH for predict KT/V≤1.3.

4. Discussion

E-GST has the potential to be a cutting-edge method for comparing the efficacy of different dialysis treatments. The specific toxicity of blood toxins, as well as their number or size, is related to their measurement. In some cases, it can replace or supplement the Kt/V urea technique.[6]

Treatment with hemodialysis is essential for people with end-stage renal disease (ESRD). Sufficient hemodialysis is crucial to improve these patients' quality of life since the elimination of uremic toxins considerably influences their morbidity and death.[7]

No statistically significant variation in age or sex was detected between the two groups, as indicated by a p-value higher than 0.05 in our study.

The results of this match those of Noce et al.,[8] who looked at the E-GST activity of the two groups and discovered that there were no gender or age differences between the traditional HD and HDF groups (P>0.05).

The results showed that the median hemoglobin level was greater in the traditional HD group compared to the HDF group. The median levels of 10.45 and 9.7 mg/dl, respectively, for the remaining CBC values were statistically significant, but the difference was not statistically significant (P>0.05).

Neither group differed significantly from the other with respect to iron study parameters, creatinine levels, or liver function tests (P value>0.05).

Our study revealed no significant differences in the concentrations of phosphorus, potassium, and sodium between the two groups (conventional HD and post-dilutional OL-HDF) (P-value > 0.05). However, a notable difference was observed: patients undergoing HD exhibited a higher median calcium level (9.45 mg/dL) compared to those in conventional HD (8.95 mg/dL). We found no statistically significant difference in hemodialysis adequacy markers (URR and Kt/V) between conventional HD and post-dilutional OL-HDF (P-value > 0.05), indicating comparable effectiveness of hemodialysis, which was also reflected in the EGST level. Our investigation identified a statistically significant distinction (P-value < 0.0001) in E-GST levels among patients on conventional HD, post-dilutional OL-HDF, and the control group, with respective means of 15.73 ng/dL, 15.19 ng/dL, and 2.81 ng/dL.

Noce et al.,8 The comparison of E-GST activity between the control group and all uremic patients (including those undergoing conventional HD and post-dilution OL-HDF) revealed a statistically significant difference (P<0.0001).

Our analysis revealed a significant disparity in E-GST levels between the control group and both the conventional HD and post-dilution OL-HDF groups, with P-values of 0.0001 for each comparison. Nonetheless, no substantial change was noted between standard HD and post-dilution OL-HDF.

The findings of this investigation contradict those of Noce et al.[8], who observed that patients undergoing traditional hemodialysis exhibited significantly elevated E-GST activity levels compared to those receiving post-dilution online hemodiafiltration (10 U/g Hb vs. 8.2 U/g Hb; P-value = 0.003).

Our investigation revealed notable differences between individuals with inadequate dialysis (Kt/V≤1.3) and those with appropriate dialysis (Kt/V >1.3). Inadequate dialysis patients exhibited a diminished URR (62±4% vs. 74.3%, p=0.001), elevated urea levels (41.15±7.98 mg/dl vs. 30.45±7.32 mg/dl, p=0.001), and heightened E-GST (19.71±2.51 ng/ml vs. 11.20±2.49 ng/ml, p=0.0001).

This signifies that E-GST possesses equivalent discriminatory power to other recognized indications, including urea, URR, and Kt/V, in identifying patients undergoing adequate hemodialysis.

Our results are consistent with Yin et al.,[9] In hemodialysis patients, E-GST levels were significantly elevated compared to controls (P<0.05). Furthermore, in cohorts receiving insufficient hemodialysis (Kt/V≤1.3) versus those undergoing appropriate hemodialysis (Kt/V>1.3), E-GST levels were 38.19±4.52 ng/ml compared to 20.32±3.78 ng/ml, P<0.05.

Our research indicates that the control group's GSH levels (2.81±0.58 ng/ml) are significantly lower than those of the KT/V>1.3 (11.20±2.49 ng/ml) and KT/V≤1.3 groups (19.71±2.51 ng/ml). The GSH levels in the cohort with KT/V>1.3 are markedly lower than those in the cohort with KT/V≤1.3. These findings indicate that changes in GSH levels correlate with dialysis adequacy as assessed by KT/V.

Comparing GSH levels between healthy controls and CKD patients undergoing dialysis demonstrates that GSH levels serve as a dependable diagnostic instrument.

GSH levels serve as a highly precise predictor of inadequate dialysis (AUC=0.995, p<0.001), exhibiting a sensitivity of 100%, specificity of 95%, and an accuracy of 97.5%. Dialysis is deemed adequate (≤14.4 ng/mL) when Kt/V exceeds 1.3, and inadequate (>14.4 ng/mL) when Kt/V is less than or equal to 1.3, with a threshold of 14.4 ng/mL distinguishing the two conditions.

Our research demonstrated a substantial correlation between GSH levels and hematological markers in dialysis patients. The Kt/V>1.3 cohort had a positive connection between elevated GSH levels and post-treatment MCV and UR, indicating larger red blood cells and inefficient urea clearance.

4. Conclusion

The amount of E-GST in hemodialysis patients can indicate the efficacy of hemodialysis and the elimination of toxins throughout multiple sessions using various hemodialysis modalities; it is a very sensitive and specific marker.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article

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Conflicts of interest

There are no conflicts of interest.

References

1. Ghimire M, Vaidya S, Upadhyay HP. Prevalence of Newly Diagnosed End-Stage Renal Disease Patients in a Tertiary Hospital of Central Nepal, Chitwan: A Descriptive Cross-sectional Study. JNMA J Nepal Med Assoc. 2021;59(233):61-64.
2. Filipska, A., Bohdan, B., Wieczorek, P. P., & Hudz, N. Chronic kidney disease and dialysis therapy: incidence and prevalence in the world. Pharmacia.2021;68(2):463-470.
3. Faria M, de Pinho MN. Challenges of reducing protein-bound uremic toxin levels in chronic kidney disease and end stage renal disease. Transl Res. 2021;229:115-134.
4. Ding L, Johnston J, Pinsk MN. Monitoring dialysis adequacy: history and current practice. Pediatr Nephrol. 2021;36(8):2265-2277.
5. Churchill BM, Patri P. The Nitty-Gritties of Kt/Vurea Calculations in Hemodialysis and Peritoneal Dialysis. Indian J Nephrol. 2021;31(2):97-110.
6. Bocedi A, Noce A, Rovella V, et al. Erythrocyte glutathione transferase in kidney transplantation: a probe for kidney detoxification efficiency. Cell Death Dis. 2018;9(3):288.
7. Rodriguez RA, Hotchkiss JR, O'Hare AM. Geographic information systems and chronic kidney disease: racial disparities, rural residence and forecasting. J Nephrol. 2013;26(1):3-15.
8. Noce A, Ferrannini M, Fabrini R, et al. Erythrocyte glutathione transferase: a new biomarker for hemodialysis adequacy, overcoming the Kt/V(urea) dogma?. Cell Death Dis. 2012;3(8):e377.
9. Yin R, Qiu H, Zuo H, et al. Detection and adequacy evaluation of erythrocyte glutathione transferase on levels of circulating toxins in hemodialysis patients. Artif Cells Nanomed Biotechnol. 2016;44(5):1228-1231.