Erythrocyte Glutathione Transferase is A Sensitive Marker of Hemodialysis Adequacy

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ABSTRACT

Background: Erythrocyte glutathione transferase (e-GST) is a non-dialyzable dimeric protein in red cells. It binds and sequesters a variety of small or large toxic compounds.

Objective: This study verifies whether e-GST can assess hemodialysis (HD) adequacy in different techniques or is complementary to the Kt/V urea parameter.

Patients and Methods: This is a pilot cross-sectional study included 20 end-stage renal diseases (ESRD) patients on conventional HD, 20 ESRD patients on hemodiafiltration (HDF) for at least 6 months, and 20 healthy controls. Serum e-GST was measured for all patients and controls.

Results: Serum e-GST can predict inadequate dialysis at cut off value >14 ng/ml with area under curve (AUC) 0.871, sensitivity 95%, specificity 60%, PPV 70.4% and NPV 92.3%. e-GST was significantly high in patients on conventional HD and HDF (mean ±SD 18.35±5.61 ng/ml, 15.20±4.40 ng/ml) respectively compared with control subjects (mean ±SD 2.80±1.36 ng/ml) P-value <0.0001. Post hoc analysis showed a significant difference between control and both conventional HD and HDF patients (P <0.0001, 0.0001) respectively while no significant differences between conventional HD and HDF patients' P-value (0.061). Patients were redistributed according to kt/v. Patients with kt/v ≤1.3 have significantly higher e-GST (mean ±SD 20.05±4.35 ng/ml) compared with kt/v >1.3 (mean ±SD 13.5±3.82 ng/ml) p-value 0.0001. The patients who have elevated e-GST have increased odds of inadequate dialysis (odds ratio: 28.5). In Conventional HD and HDF, e-GST was negatively correlated with kt/v and URR (P<0.0001).

Conclusion: Erythrocyte glutathione transferase is a highly sensitive marker for hemodialysis adequacy in different modalities and didn't need any calculations for interpretation.

Keywords: Erythrocyte glutathione transferase, Hemodialysis, Adequacy.

INTRODUCTION

End-stage renal disease (ESRD) is a major public health problem worldwide [(1)]. An ideal dialysis therapy should remove all toxins (small, middle molecules, and protein-bound solutes) However, only small toxins are easily removed by all dialysis techniques [(2)]. Assessment of solute removal during dialysis has always been based on urea removal in a single hemodialysis session despite the adverse effect of middle molecules and protein-bound solutes on patient survival so it should not be used as the sole indicator of dialysis adequacy [(3)].

The identification of new clinical indicators able to reveal the degree of blood purification from small as well as large toxins in a wide range of dialysis sessions will be of medical interest. Glutathione-S-transferases is a dimeric protein composed of 2 identical subunits of about 25 kDa. Glutathione-S-transferases represent a superfamily of enzymes involved in cell protection and detoxification, prominent function of these enzymes is the conjugation of glutathione (GSH) to toxic hydrophobic compounds provided by an electrophilic center. This reaction facilitates toxin inactivation and renal elimination. Red blood cells express almost exclusively a single GST isoenzyme, GST-P1which represents more than 95% of the erythrocyte GST (e-GST) pool. Over-expression of e-GST has been found in uremic patients under maintenance hemodialysis (MHD)[(4)].

Erythrocyte glutathione transferase (e-GST) is a non-dialyzable enzyme compartmentalized in the red cells devoted to cell protection by promoting the conjugation of glutathione with toxins of very different shapes also via binding and sequestering small or large toxic compounds and peptides [(5)], e-GST may be considered a sort of ideal long-term biomarker that should provide a measure of circulating toxins in the period not limited to a single day or dialysis session but extended up to multiple dialytic sessions within 1–2 months of the life span of circulating erythrocytes [(6)].

The study aims to verify whether e-GST can assess hemodialysis adequacy in different techniques or is complementary to the Kt/V urea parameter.

MATERIALS AND METHODS

This pilot cross-sectional study was carried out on 20 ESRD patients on conventional HD, 20 ESRD patients on HDF matched as regard age and sex between 18 and 60 years old on regular hemodialysis 3 sessions /week, each session 4 hours for at least 6 months and 20 healthy controls with normal renal function.

Excluding patients with hyperbilirubinemia and Chronic liver disease. Then patients were redistributed according to Kt/V into 2 groups: The adequate dialysis group: (Kt/V > 1.3) and the inadequate dialysis group: (Kt/V ≤ 1.3). All patients were subjected to detailed history taking, clinical examination, complete blood count (CBC), Chemistry:

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(AST, ALT, total bilirubin, direct bilirubin Serum creatinine), iPTH, Iron study (serum iron, TIBC, Ferritin), pre-dialysis urea, post-dialysis urea & single pool Kt/V urea and urea reduction ratio (URR) was calculated.

All controls were subjected to serum creatinine, total & direct bilirubin, and HBA1C. Serum Erythrocyte glutathione transferase was measured by enzyme-linked immunosorbent assay (ELISA) technique for all patients before mid hemodialysis session and controls.

Ethical consent:

Approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Every patient signed informed written consent for the acceptance of participation in the study. This work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Serum Erythrocyte glutathione transferase measurement:

Overall 4ml of venous blood samples was withdrawn Serum was collected by serum separator tube, the serum was allowed to clot for 10-20 minutes at room temperature then Centrifugation was done (at 2000-3000 RPM) for 20 minutes. Then supernatants were collected and stored at -20 °C. Serum e-GST was measured by ELISA kit based on double-antibody sandwich enzyme-linked immunosorbent assay technology. www.bt-laboratory.com | 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China

Statistical analysis:

Data were collected, revised, coded, and entered into the statistical package for the social science, version 20 (SPSS Inc., Chicago, Illinois, USA). The qualitative data were presented as numbers and percentages, whereas quantitative data were presented as mean with standard deviation (SD). Comparison between two groups with qualitative data was done by using the $\chi 2$ Test. Comparison between two groups with quantitative data was done by two-tailed independent t-test when the distribution of the data was found parametric. Mann–Whitney test was used with the nonparametric data. Comparison between three groups with quantitative data was done by ANOVA with post-Hoc Tukey HSD Test when the distribution of the data was found parametric. Kruskal-Wallis test was used with the nonparametric data. Spearman correlation coefficients were used to assess the correlations. P-value < 0.05 was considered significant.

RESULTS

Table (1) showed the demographic and laboratory parameters for both patients on conventional HD and HDF. Serum e-GST can predict inadequate dialysis at cut off value >14 ng/ml with area under curve (AUC) 0.871, sensitivity 95%, specificity 60%, PPV 70.4% and NPV 92.3% **(Figure 1)**.

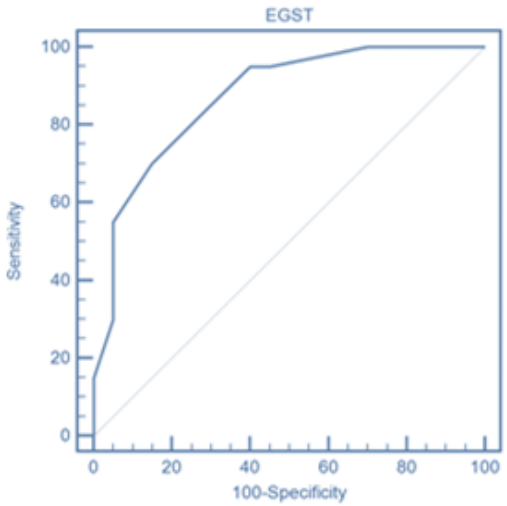

Figure (1): ROC curve of E-GST to predict inadequate dialysis (kt/V≤1.3).

Table (1): Demographic and laboratory data of patients on conventional hemodialysis and HDF.

	Group A Conventional HD N = 20	Group B HDF N = 20	P-value	Sig.
Age (years)	47.15 ± 14.34	55.25 ± 15.39	0.093	NS
Hypertension (N/%)	11 (55.0%)	18 (90.0%)	0.013	S
Filter size (m^2)	1.58 ± 0.14	2.11 ± 0.04	0.0001	HS
Dialysate flow (ml/min)	505 ± 82.56	530 ± 92.34	0.372	NS
Pump flow (ml/min)	286 ± 36.48	316 ± 37.89	0.015	S
Epo dose (IU/wk)	7750 ± 3087.61	5466.67 ± 2669.05	0.036	S
HGB (gm/dl)	10.38 ± 1.3	11.44 ± 0.99	0.006	HS
Na (mmol / litre)	136.4 ± 5.47	140.65 ± 3.1	0.004	HS
Total bilirubin (mg/dl)	0.5±0.11	0.3 ± 0.061	0.036	S
ERI	10 ± 2.11	5.10 ± 1.12	0.011	S
iPTH (pg/dl)	374 ± 88.91	171.5 ± 39.41	0.002	HS
URR (%)	67.85 ± 10.65	70 ± 6.44	0.446	NS
Kt/V	1.36 ± 0.44	1.4 ± 0.23	0.754	NS

• Chi-square test,* Independent t-test was used and # Mann Whitney test were used

HDF: hemodiafiltration, EPO: erythropoietin, HGB: hemoglobin, ERI: erythropoietin resistance index iPTH: intact parathyroid hormone

e-GST was significantly high in patients on conventional HD and HDF (mean ±SD 18.35±5.61 ng/ml, 15.20±4.40 ng/ml) respectively compared with control subjects (mean ±SD 2.80±1.36 ng/ml) P-value <0.0001. Post hoc analysis showed a significant difference between control and both conventional and HDF patients (P <0.0001, 0.0001) respectively while no significant differences between conventional HD and HDF P-value (0.061). Patients were redistributed according to kt/v. Patients with kt/v ≤1.3 have significantly lower URR and higher e-GST (mean ±SD 62.07±6.28 %, 20.05±4.35 ng/ml) respectively compared with kt/v >1.3 (mean ±SD 75.78±4.36 %, 13.5±3.82 ng/ml) respectively p-value 0.0001, 0.0001 respectively**.** The prevalence of e-GST level in patients with inadequate dialysis (Kt/V ≤ 1.3) was 95 %(19 patients) while it was 40% (8 patients) in patients with Kt/V > 1.3 with a P-value (0.001). The patients who have elevated e-GST have increased odds of inadequate dialysis (odds ratio: 28.5) **(Table 2)**.

Table (2): Logistic regression analysis of e-GST for inadequate dialysis (Kt/V <1.3)

	B	S.E.	Wald	P-value	Odds ratio (OR)	95% C.I. for OR	
						Lower	Upper
EGST(ng/ml)	3.350	1.123	8.899	0.003	28.500	3.155	257.444

e-GST: erythrocyte glutathione transferase.

In this study, there was a significant correlation **(Table 3).** In Conventional HD and HDF e-GST was negatively correlated with kt/v and URR (kt/v r=-0.746, -0.790 P<0.0001, 0.0001) respectively (URR r= -0.714, -0.771 P<0.0001, 0.0001) respectively.

Table (3): Correlation of e-GST in conventional HD, HDF & control subjects

	e-GST								
	HDF group			Conventional group			Control group		
	r	P-value	Sig	R	P-value	Sig	R	P-value	Sig
Age (years)	-0.316	0.175	NS	0.255	0.277	NS	0.155	0.513	NS
Total bilirubin(mg/dl)	0.058	0.807	NS	0.439	0.053	NS	0.555*	0.011	S
Direct bilirubin(mg/dl)	0.121	0.611	NS	0.522*	0.018	S	-0.207	0.382	NS
Creatinine (mg/dl)	0.062	0.796	NS	0.253	0.283	NS	0.679**	0.001	S
UREA 1(mg/dl)	0.519*	0.019	S	0.153	0.520	NS			
UREA 2(mg/dl)	0.088	0.712	NS	0.525*	0.017	S			
URR(%)	-0.771**	≤0.001	HS	-0.714**	≤0.001	HS			
Kt/V	-0.790**	≤0.001	HS	-0.746**	≤0.001	HS			
Duration Of HD (months)	0.274	0.242	NS	-0.447*	0.048	S			
Dialysate flow (ml/min)	-0.468*	0.037	S	0.111	0.643	NS			
HGB(gm/dl)	0.315	0.176	NS	0.199	0.400	NS			

* Spearman correlation coefficients were used

HGB: hemoglobin, URR: urea reduction ratio, e-GST: erythrocyte glutathione transferase, HD: hemodialysis, HDF: hemodiafiltration.

DISCUSSION

Adequate hemodialysis is highly required to improve the quality of life [7]. e-GST may be an innovative tool able to measure the efficiency of multiple dialysis sessions [3].

In this study, we verified whether e-GST is a novel biomarker of hemodialysis adequacy in different dialysis techniques (standard bicarbonate hemodialysis (HD) and (post dilutional OL-HDF) or complementary to the Kt/V urea. The difference in this study is that we assess the e-GST level, not activity as activity was studied before by **Noce *et al.*** [4].

Serum e-GST can predict inadequate dialysis at a cut-off value >14 ng/ml with the area under the curve (AUC) 0.871. No available study assesses e-GST level values to detect inadequate dialysis to compare with it. e-GST was significantly high in patients with conventional HD and HDF compared with control subjects while no significant differences between conventional HD and HDF patients. This expresses that the convection method has no effect on the e-GST level by its removal. e-GST has a large molecular mass and is non-dialyzable so its level is related to the adequacy of removal of uremic toxins.[5]

This is agreed with **Noce *et al.*** [4] on comparing the e-GST activities of the control group versus all uremic patients(conventional HD, post dilutional OL-HDF) there was a significant statistical difference (P<0.0001), but disagreed with **Noce *et al.*** [4] who demonstrate a significant difference in e-GST activity in patients on conventional HD in comparison with post dilutional OL-HDF (10U/g Hb, 8.2 U/g Hb) respectively (P-value =0.003).

This returns to **Noce *et al.*** [4] study showed significant statistical differences as regard markers of hemodialysis adequacy (pre-dialysis BUN& Kt/V) between conventional HD& post dilutional OL-HDF (P-value=0.0001,0.0002) respectively that was reflected on EGST activity. In our study, there was no significant statistical difference between conventional HD & post-dilutional OL-HDF as regards markers of hemodialysis adequacy that was also reflected on the e-GST level. This means that e-GST has the same power to express dialysis adequacy as URR, Kt/V Urea, Also it is a mirror of URR & Kt/V, the more removal of the uremic toxin the less oxidative stress and less e-GST activity. In healthy subjects, the intra-cellular level of e-GST remains virtually constant during childhood and adult life [7] increasing only in two pathological conditions, that is hyperbilirubinemia and uremia[8,9]. No other pathologies have been reported to induce e-GST hyper-activity. The activity of e-GST increases from 5.8±0.4 U/gHb in healthy subjects to 10.2±0.5 U/gHb in maintenance hemodialysis (MHD) patients[9, 10].This hyperactivity represents a defense reply against systemic toxicity of the uremic condition.

When the Patients were redistributed according to kt/v. The prevalence of e-GST levels in patients with inadequate dialysis was 95% (19 patients). The patients who have elevated e-GST have increased odds of inadequate dialysis (odds ratio: 28.5). No available study assessed e-GST prevalence and odds ratio to detect inadequate dialysis to compare with it. Patients with kt/v ≤1.3 have significantly lower URR and higher e-GST compared with kt/v >1.3. This means that e-GST can differentiate whether they receive adequate hemodialysis or not, it is like URR and Kt/V that is agreed with **Yin *et al.*** [11] who showed that The level of e-GST was significantly higher in patients on hemodialysis in comparison to the control group (P - value< 0.05), also e-GST levels in the inadequate hemodialysis group (Kt/V ≤ 1.3) was significantly higher than in the adequate hemodialysis group (Kt/V > 1.3) (Mean±SD (38.19 ± 4.52) ng/ml, (20.32 ± 3.78) ng/ml respectively (P-value < 0.05) but they did not study e-GST prevalence in both groups as we did in our study.

This is inconsistent with **Noce *et al.*** [4] who found no significant statistical difference between patients with Kt/V ≤ 1.3& patients with Kt/V >1.3 as regard e-GST activity (9.7±0.7 U/gHb, 8.7±0.4 U/gHb) respectively (P value=0.156).

In our study, Conventional HD and HDF e-GST was negatively correlated with kt/v and URR. This is inconsistent with **Noce *et al.*** [4] who did not find a correlation between e-GST activity and Kt/V in all uremic patients (conventional HD and post dilutional OL-HDF) (r =0.0378, P=0.05). In our study e-GST positively correlated with urea 1 In HDF patients, while with urea 2 In conventional HD patients. This is consistent with **Noce *et al.*** [4] e-GST activity has a significant positive correlation with BUN predialysis and BUN postdialysis in all uremic patients (r =0.1906, P< 0.001), (r=0.0822; P=0.003) respectively. In control e-GST and positively correlated with serum creatinine. This is agreed with **Dessì *et al.*** [12], e-GST activity positively correlated with the progression of CKD in four stages 1,2,3,4 according to "KDIGO" classification, the activity was (7.4±0.5, 8±1, 9.5±0.6, 12±1 U/gHb) respectively.

CONCLUSIONS

Erythrocyte glutathione transferase is a promising marker for assessing the adequacy of different hemodialysis modalities. it is highly sensitive and easily measured and didn't need any calculations for interpretation. e-GST positive patients had increased odds of inadequate dialysis 28.5 times more than a negative one.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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