Original Article

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Assessment of Utility of Carbamylated Hemoglobin in Determining the Overall Adequacy of Hemodialysis

Syed Mohd Azhar Hassan, Atul Sajgure, Pavan Wakhare, Nilesh Shinde, John Abraham Tharayil, Nishant Vyas, Tushar Dighe

Department of Nephrology, Dr. D. Y. Patil Medical College, Hospital & Research Centre, Pune, Maharashtra, India.

Abstract

Background: CKD is characterized by progressive and ongoing loss of kidney function of not <3 months duration with or without decrease in glomerular filtration rate (GFR). CKD leads to increase in blood urea and serum creatinine levels. The present study was conducted to assess the utility of Carbamylated Hemoglobin in determining the overall adequacy of Hemodialysis. **Subjects & Methods:** 25 patients with chronic kidney disease divided into two groups and one healthy control group. Carbamylated Hemoglobin (CHb) checked at 0 and at 3 months. Kt/V assessed at 0 and 3 months and average Kt/V was calculated. **Results:** There was non- significant difference in Kt/V, Valine hydantoin absorbance at 570 nm, CHb (ug/gm Hb) and blood flow rate (ml/min) at 8 hours and 12 hours (P> 0.05). The regression equation at 3 months is CHb. = 482.33 + 0.122 age (Yrs) – 0.492 gender + 0.192 duration(months) - 0.624 HD frequency/wks(Hrs) - 0.207 HD vintage(months) + 0.063 BMI + 6.021 Hb.(gm%) + 0.011 BUL(mg/dl) – 0.191 Creatinine(mg/dl) + 0.123 Na(mmol/l) – 1.047 K(mmol/l) + 5.642 Alb.(gm/dl) – 55.12 pH + 0.903 HCO3 - 0.018 iPTH(pg/ml) – 11.54 Kt/V – 96.54 Valine hydantoin absorbance + 0.005 Blood Flow Rate (ml/min). **Conclusion:** Carbamylated haemoglobin had a direct correlation with blood urea nitrogen levels and with serum creatinine levels in chronic kidney disease patients on maintenance haemodialysis.

Keywords: Carbamylated haemoglobin, Serum creatinine, Haemodialysis.

Corresponding Author: Nilesh Shinde, Department of Nephrology, Dr. D. Y. Patil Medical College, Hospital & Research Centre, Pune, Maharashtra, India.
E-mail: med.nephro@dpu.edu.in

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Introduction

CKD is characterized by progressive and ongoing loss of kidney function of not <3 months duration with or without decrease in glomerular filtration rate (GFR)CKD leads to increase in blood urea and serum creatinine levels.[1] Assessment of renal function is one of the most important criteria for evaluation of renal function in patients with chronic kidney disease (CKD) and when on treatment with or without haemodialysis to know the progress of kidney disease with or without progressive loss of kidney function over a period of time.[2]

The measurement of carbamylated Hb (CHb) may be a marker of both uremia and the efficacy of dialysis therapy in CKD. Several methods have been used to quantify carbamylated Hb. One method is ion exchange and evaporation technique followed by gas chromatographic amino acid analysis of an alkaline hydrolysis of valine hydantoin released to quantify carbamylated haemoglobin S. Gas liquid chromatography (GLC) method was used in other studies to measure valine hydantoin released after acid hydrolysis of Hb extracted from whole blood.[3]

Recently, High Performance Liquid Chromatography (HPLC) has been used to quantify CHb based on the release of carbamyl valine from the N-amino acid terminals of the α and β chains of Hb after acid hydrolysis.[4] This method is reliable enough to be used as a diagnostic test to identify those with renal failure and is also cost effective. It may be suggested that carboxy Hb determination is a good index of chronic urea – exposure in patients with CRF and relates to changes in renal dysfunction.[5] The present study was conducted to assess the utility of Carbamylated Hemoglobin in determining the overall adequacy of Hemodialysis.

Subjects and Methods

This study is a case controlled observational follow up study of 25 patients with chronic kidney disease divided into two groups and one healthy control group. 25 Patients on maintenance hemodialysis for > 3 months duration who

presented to the Department of Nephrology, Dr. D.Y. Patil hospital, Pune without any active infection who were willing to give consent were enrolled in the study.

Carbamylated Hemoglobin (CHb) checked at 0 and at 3 months. Kt/V assessed at 0 and 3 months and average Kt/V was calculated. Routine laboratory investigations included Complete Blood Count, Blood Urea, Serum Creatinine, Serum Electrolytes, Serology (HIV, HBsAg, Anti-HCV), Total Proteins, Serum Albumin, Venous blood gas analysis, intact Parathormone. Blood urea and Serum creatinine were measured using standard methodology and the Kt/V Kt/V were calculated using algorithmic estimate of daugirdas. Blood samples were analysed for Hb and an EDTA sample for estimations of CHb was taken with pre-dialysis samples at 0 and at the end of 3 months. Results thus obtained were assessed statistically. P value less than 0.05 was considered significant.

Results

Table 1: Distribution of patients

Gender	No of cases (n=25)	Percentage %
Male	19	76
Female	6	24
Total	25	100

[Table 1] shows that out of 25 patients, there were 19 males and 6 females.

[Table 2] shows that there was non- significant difference in Kt/V, Valine hydantoin absorbance at 570 nm, CHb (ug/gm Hb) and blood flow rate (ml/min) at 8 hours and 12 hours (P> 0.05).

[Table 3] shows that the regression equation at 3 months is CHb. = 482.33 + 0.122 age (Yrs) – 0.492 gender + 0.192 duration(months) - 0.624 HD frequency/wks(Hrs) - 0.207 HD vintage(months) + 0.063 BMI + 6.021 Hb.(gm%) + 0.011 BUL(mg/dl) – 0.191 Creatinine(mg/dl) + 0.123 Na(mmol/l) – 1.047 K(mmol/l) + 5.642 Alb.(gm/dl) – 55.12 pH + 0.903 HCO3 - 0.018 iPTH(pg/ml) – 11.54 Kt/V – 96.54 Valine hydantoin absorbance + 0.005 Blood Flow Rate (ml/min).

Discussion

It is important to know that a patient of CKD has changes in renal function from free haemodialysis state to follow up following patient on regular haemodialysis which also effects glomerular filtration rate. Urea is retained in patients with renal failure along with other metabolites in blood and serves as a marker of nitrogen retention. Under normal conditions it spontaneously undergoes changes under physiological conditions to from ammonia and cyanate. Nitrogenous waste products accumulation occurs in patients with early renal dysfunction and occurs most often before the appearance of other symptoms.[6]

Urea is one of the first nitrogenous waste substance that accumulates in blood in renal diseases and urea levels progressively increase with deteriorating renal function. Assessment of renal function is done by measurement of urea and creatinine levels in serum or plasma for calculation of glomerular filtration rate (GFR) using known prediction equations.[7] Carbamylation is a non-enzymatic post translational modification of proteins involving a reaction of isocyanic acid with the functional groups of amino-acids and proteins. Isocyanic-acid, a reactive chemical form of cyanate is formed as a spontaneous dissolution product of urea and reacts with α and ε amino-acids of several proteins including haemoglobin resulting in carbamylation of proteins like amino-acids, plasma proteins, leucocyte proteins and haemoglobin. The amino-terminal valine of hemoglobin is particularly reactive with isocyanate forming a stable modified haemoglobin termed Carbamylated haemoglobin (Chb).[8]

Evaluation of CHb in patients with various degrees of renal function found that measurement of CHb provides potential clinical value and has been suggested to be useful in differentiating patients with AKI from those with CKD. CHb has any utility as a predictor of patient outcome or is just a surrogate measure of urea will remain unclear until large, longitudinal studies have been performed.[9]

Each alpha amino valine of the four Haemoglobin chains comprising a Hb-molecule may be altered by this reaction. The alpha chains of Hb are known to carbamylate twice as fast in the deoxy compared to the oxy states. The carbamylation of Hb is known to result in conformational changes that results in altered biochemical behaviour of Haemoglobin. Carbamylation of Hb is thus the example of non-enzymatic post translational modification of proteins and is well known for its widely studied reactions and is also found to contribute to uremic toxicity.[10] In a study CHb levels were measured in patients with acute kidney Injury and CKD and compared with those of healthy controls. It was found that both group of patients with renal failure had significantly higher level of CHb as compared to controls. Tarif et al, found a relationship between CHb and both Kt/V and the URR.

Under physiological conditions the concentration of isocyanate is about 1% of urea and in conditions of renal failures, retention of urea along with other metabolites occur and the constant exposure of Hb to increased urea levels results in higher cyanate and increased formation of CHb. Patients of CKD showed significantly higher levels of CHb than those in patients with AKI. Patients with CKD in the earlier studies had significantly higher levels of CHb than those in patients with AKI ($P < 0.05$). Earlier studies have also reported similar finding. Determination of Carbamylated hemoglobin by

Table 2: Comparison of Kt/V, Valine hydantoin absorbance, CHb, blood flow rate at baseline according to HD frequency in study group

At baseline	HD frequency (in hrs)				t Value	P Value
	8Hrs (n=12)		12Hrs (n=13)			
	Mean	SD	Mean	SD		
Kt/V	1.24	.27	1.27	.22	0.33	0.75
Valine hydantoin absorbance at 570 nm	.67	.07	.68	.07	0.44	0.66
CHb (ug/gm Hb)	91.76	25.15	88.37	8.99	0.46	0.65
Blood Flow Rate (ml/min)	327.08	58.83	321.15	70.59	0.23	0.82

Table 3: Comparisonof BMI, HB., Blood Urea, Creatinine, Na, K, Albumin, Ph, HCO3, IPTH, Kt/V, Valine Hydantoin Absorbance, CHB, Blood Flow Rate Between Baseline and 3 months.

Parameter	At baseline		At 3 months		t Value	P Value
	Mean	SD	Mean	SD		
BMI Kg/m2	22.06	3.89	22.07	3.84	0.04	0.97
Hb (gm/dl)	7.62	1.29	7.66	1.21	0.15	0.88
Blood Urea (mg/dl)	114.48	43.58	118.60	47.44	0.38	0.70
Sr Cr (mg/dl)	9.24	2.27	8.95	3.18	0.56	0.58
Serum Na (mmol/l)	141.60	8.53	136.96	6.15	2.39	0.025
Serum K(mmol/l)	4.83	.86	4.41	1.17	1.93	0.065
Serum Albumin(gm/dl)	2.94	.50	3.18	.38	2.67	0.013
pH	7.32	.06	7.32	.05	0.32	0.75
HCO3	17.94	3.95	16.97	4.61	0.88	0.39
iPTH (pg/ml)	184.49	154.36	145.80	86.72	1.85	0.076
Kt/V	1.26	.24	1.22	.22	0.90	0.38
Valine hydantoin absorbance at 570nm	.67	.07	.72	.15	1.66	0.11
CHb (ug/gm Hb)	89.99	18.26	85.72	10.29	1.12	0.27
Blood Flow Rate (ml/min)	324	63.93	325	54.96	0.08	0.93

HPLC (High Performance Liquid Chromatography) was based on the method of Kwan et al.[11]

The acid hydrolysis of CHb results in the formation of valine hydantoin from the modified amino terminal valine. Its quantitation by gas chromatography or high- performance liquid chromatography is a measure of CHb levels. A study by Wynckes et al,[12] showed that patients with chronic kidney disease (CKD) had higher CHb levels than patients with acute kidney injury (AKI). Similarly Stim et al,[13] observed that mean CHb levels were highest in patients with CKD, intermediate in end stage renal disease on haemodialysis and lowest in AKI, when compared to controls relationship of CHb plotted against time at different urea nitrogen levels show that CHb levels increase with increase in urea nitrogen concentration as it increases but the CHb levels seems to plateau after a period of time which could be more than a week. Hb of end stage renal decrease patients (ESRD) had a higher carbamylation rate than normal subjects at all levels of urea – concentrations suggesting that length of exposure to elevated urea concentration and prior carbamylation are important in determining CHb levels.

Conclusion

The present study showed that Carbamylated haemoglobin had a direct correlation with blood urea nitrogen levels and

Table 4: The Regression Equation Chb. On Age, Gender, Duration, Hd Frequency, Hd Vintage, Bmi, Hb., Blood Urea, Creatinine, Na, K, Albumin, Ph, Hco3, Ipth, Kt/V, Valine Hydantoin Absorbance, Blood Flow Rate At 3 Months In Study Group

Parameter	Beta	SE	T Value	P Value
Constant	482.331	366.232	1.317	.236
Age (Yrs)	.122	.121	1.006	.353
Gender	-.492	4.337	-.114	.913
Duration (months)	.192	.203	.946	.381
HD frequency/ wks (Hrs)	-.624	.656	-.951	.378
HD vintage (months)	-.207	.313	-.662	.532
BMI Kg/m2	.063	.581	.108	.917
Hb (gm/dl)	6.021	3.899	1.544	.174
Blood Urea (mg/dl)	.011	.057	.198	.850
Sr Cr (mg/dl)	-.191	.700	-.273	.794
Serum Na (mmol/l)	.123	.258	.475	.651
Serum K(mmol/l)	-1.047	1.312	-.798	.455
9Serum Albumin(gm/dl)	5.642	5.894	.957	.375
pH	-55.118	50.816	-1.085	.320
HCO3	.903	.565	1.597	.161
iPTH (pg/ml)	-.018	.017	-1.074	.324
Kt/V	-11.918	8.647	-1.378	.217
Valine hydantoin absorbance	-96.543	32.547	-2.966	.025
Blood Flow Rate (ml/min)	.005	.027	.168	.872

R-Sq = 95% R-Sq(adj) = 80

with serum creatinine levels in chronic kidney disease patients on maintenance haemodialysis. The BMI in present study remained the same or there was a slight increase owing to dietary improvement. There was non-progressive elevation of blood urea levels, while serum creatinine is independent of CHb levels. Serum sodium, serum potassium and pH are maintained in normal or near normal range. Bicarbonate levels are slightly low while serum Albumin levels show slight improvement owing to improvement in diet. Kt/V and Valine hydantoin levels showed no difference. There was no difference in average Kt/V and Carbamylated hemoglobin at baseline and at 3 months.

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