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Carbamylated hemoglobin levels in hemodialysis patients in Indian population

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ABSTRACT

Background: Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for > 3 months. The present study was conducted to assess the CarbamylatedHemoglobin levels in patients on Hemodialysis in Indian population and to see its correlation with urea, creatinine, albumin, bicarbonate and their comparison with healthy population.

Materials & Methods: Present study was conducted in Dr. D.Y. Patil Medical College Hospital and Research Center, Pune, Maharashtra in Department of Nephrology who were on maintenance hemodialysis for > 3 months duration without any active infection who were willing to consent and were enrolled in the study. Of 25 patients with chronic kidney disease on maintenance hemodilaysis were divided into two groups and one healthy control group was taken. Group – I: CKD patients on weekly twice haemodialysis. Group – II: CKD patients on weekly thrice haemodialysis. Group – III: Included 10 healthy volunteers as controls.Access details of the patients and the dialysis details of the dialysis sessions at baseline were recorded.CarbamylatedHemoglobin (CHb) checked at baseline. Kt/V assessed at baseline average Kt/V was calculated and compared with healthy controls.

Results: Patients were in age group between 18-20yrs (2patients), 21-30yrs (5patients), 31-40yrs (3patients), 41-50yrs (5patients), 51-60yrs (5patients), 61-70yrs (4patients), 71-80yrs (1patient). There were 19 (76%) and 6 females (24%) and there were 10 healthy controls. The mean age was 44.16 years, BMI was 22.06Kg/m2, Hb was 7.62gm/dl, blood urea was 114.48mg/dl, Sr Cr was 9.24mg/dl, serum Na (mmol/l) was 141.60, serum K(mmol/l) was 4.83, serum albumin(gm/dl) was 2.94, Ph was 7.32, HCO3 was 17.94, iPTH (pg/ml) was 184.49, Kt/V was 1.26, Valinehydantoin absorbance at 570nm was 0.67, CHb (ug/gmHb) was 89.99 and blood flow rate (ml/min) was 324.

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 compared to healthy controls.

Key words: Carbamylated haemoglobin, Chronic kidney disease, Serum creatinine.

Introduction:

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for > 3 months.[1] CKD staging is done based on either GFR (Glomerular filtration rate) or Albuminuria.According to GFR, CKD may be classified as G1 (GFR > 90ml/min/1.73m^2), G2 (GFR 60-89ml/min/1.73m^2), G3a (GFR 45-59ml/min/1.73m^2), G3b (GFR 30-44ml/min/1.73m^2), G4 (GFR 15-29ml/min/1.73m^2), G5 (GFR < 15ml/min/1.73m^2).[2,3]

CKD as per Albuminuria is classified as A1 (AER <30mg/24hrs or ACR <3 mg/mmol), A2 (AER -30-300mg/24hrs or ACR 3-30 mg/mmol), A3 (AER >300mg/24hrs or ACR >30 mg/mmol).CKD leads to increase in blood urea and serum creatinine levels. Rise in the urea levels may be associated with increase in the levels of hemoglobin that combines with urea derivatives to form carbamylated hemoglobin.[4,5]

Carbamylation is a chemical reaction between amino group of an amino acid and isocyanate ion derived from the spontaneous breakdown product of urea. The cyanate ion levels in blood is in equilibrium with urea.[6]Carbamylation of proteins occur when the terminal amino acid attached to the protein interacts with the more reactive isocyanate ion than cyanate ion with which it is in equilibrium, CHb (carbamylatedhemoglobin) is the most common carbamylation reaction of the N-Terminal valine residues of the α and β chains of Hb with isocyanate ion.[7]

Carbamylation of Hb and other proteins occurs commonly in Uremia. For Hb, the most extensively studied protein in CKD, cyanic acid reacts with the terminal valine residues of both α and β chains. The measurement of carbamylatedHb (CHb) may be a marker of both uremia and the efficacy of dialysis therapy in CKD.[8]The present study was conducted to assess the CarbamylatedHemoglobin levels in CKD patients on maintenanceHemodialysis in Indian population.

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Materials & Methods

This study is a case controlled observational study of 25 patients with chronic kidney disease divided into two groups and one healthy control group.

Group – I: CKD patients on weekly twice haemodialysis.

Group – II: CKD patients on weekly thrice haemodialysis.

Group – III: Included 10 healthy volunteers as controls.

Complete history with duration of renal disease, its etiology and complications of CKD if any, presence of Cardiac and Peripheral vascular disease, history of anorexia and weight loss, if any were recorded. Access details of the patients and the dialysis details of the dialysis sessions at baseline were recorded.CarbamylatedHemoglobin (CHb) checked at baseline,Kt/V assessed was calcuated.Laboratory parameters like blood urea, serum creatinine, electrolytes, bicarbonate, albumin and carbamylatedhemoglobin in study group were sampled predialysis and were compared with control group.

Principle

Carbamylated haemoglobin released after hemolysis of the red cells is hydrolysed using hydrochloric acid-glacial acetic acid reagent. The N-terminal valine residues of the α and β chains of haemoglobin are released as carbamylvaline. Carbamylvaline undergoes cyclization to form valinehydantoin which is then extracted with an organic solvent. The amino acid valine released from the hydantoin using acetate-cyanide solution reacts with ninhydrin reagent to produce a colour which is measured spectrophotometrically at 570nm.

Results thus obtained were assessed statistically. P value less than 0.05 was considered significant.

Results

Table 1: Age wise distribution of cases in study group

Age (Yrs)	No of cases (n=25)	Percentage %
<20	2	8
21 – 40	8	32
41 – 60	10	40
61 & above	5	20
Total	25	100

Table 1 shows that patients were in age group between 18-20yrs (2patients), 21-30yrs (5patients), 31-40yrs (3patients), 41-50yrs (5patients), 51-60yrs (5patients), 61-70yrs (4patients), 71-80yrs (1patient).

Graph 1 Comorbidity wise distribution of cases in study group

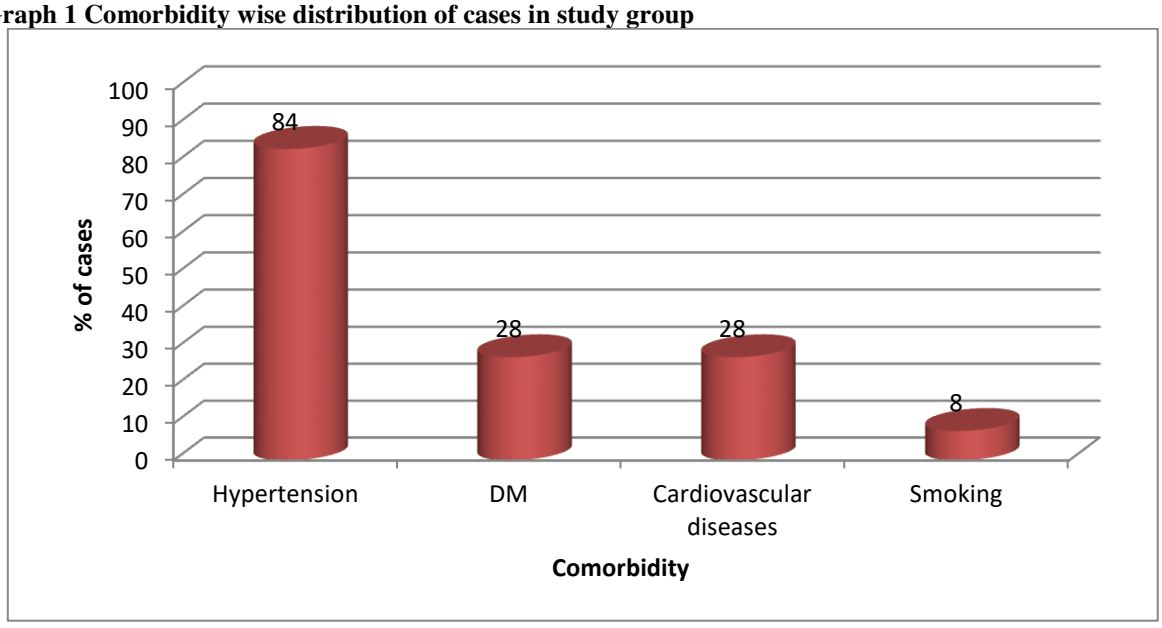

Table 2 Assessment of laboratories parameters in study group.

Parameter	Mean/n	SD
No of cases (n)	25	

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Gender	Male	19 (76%)	
	Female	6 (24%)	
Age (Yrs)		44.16	16.67
BMI Kg/m2		22.06	3.89
Hb (gm/dl)		7.62	1.29
Blood Urea (mg/dl)		114.48	43.58
Sr Cr (mg/dl)		9.24	2.27
Serum Na (mmol/l)		141.60	8.53
Serum K(mmol/l)		4.83	.86
Serum Albumin(gm/dl)		2.94	.50
Ph		7.32	.06
HCO3		17.94	3.95
iPTH (pg/ml)		184.49	154.36
Kt/V		1.26	0.24
Valinehydantoin absorbance at 570nm		0.67	0.07
CHb (ug/gmHb)		89.99	18.26
Blood Flow Rate (ml/min)		324	63.93

Table III shows that there were 19 (76%) and 6 females (24%). The mean age was 44.16 years, BMI was 22.06Kg/m2, Hb was 7.62gm/dl, blood urea was 114.48mg/dl, Sr Cr was 9.24mg/dl, serum Na (mmol/l) was 141.60, serum K(mmol/l) was 4.83, serum albumin(gm/dl) was 2.94, Ph was 7.32, HCO3 was 17.94, iPTH (pg/ml) was 184.49, Kt/V was 1.26, Valinehydantoin absorbance at 570nm was 0.67, CHb (ug/gmHb) was 89.99 and blood flow rate (ml/min) was 324.

Table 3Assessment of laboratories parameters at baseline according to HD (hrs/week) in study group

At baseline	**HD (hrs/week)**				**t Value**	**P Value**
	8Hrs (n=12)		**12Hrs (n=13)**			
	Mean	**SD**	**Mean**	**SD**		
BMI Kg/m2	21.67	3.92	22.43	3.98	0.48	0.63
Hb (gm/dl)	7.69	1.68	7.56	.86	0.25	0.81
Blood Urea (mg/dl)	100.67	30.05	127.23	51.04	1.57	0.13
Sr Cr (mg/dl)	8.87	2.43	9.59	2.15	0.78	0.44
Serum Na (mmol/l)	145.75	8.86	137.77	6.37	2.60	0.016
Serum K(mmol/l)	4.93	.94	4.74	.80	0.55	0.59
Serum Albumin(gm/dl)	3.03	.37	2.86	.60	0.83	0.41
pH	7.32	.04	7.32	.07	0.09	0.93
HCO3	18.22	3.66	17.69	4.33	0.33	0.75
iPTH (pg/ml)	198.39	117.13	171.65	186.29	0.43	0.68
Kt/V	1.24	.27	1.27	.22	0.33	0.75
Valinehydantoin absorbance at 570nm	.67	.07	.68	.07	0.44	0.66
CHb (ug/gmHb)	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 4 shows that there was non- significant difference in laboratories parameters at 8 hours and 12 hours (> 0.05).

Table 4: Gender wise distribution of cases in Control Group

Gender	**No of cases (n=10)**	**Percentage %**
Male	6	60
Female	4	40
Total	10	100

Table5: Descriptive statistics of Control group

Parameter		**Mean/n**	**SD (±)**
No of cases (n)		**10**	
Gender	Male	6 (60%)	

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	Female	4 (40%)	
Age (Yrs)		28.20	±3.55
Hb (gm/dl)		13.69	±2.09
Blood Urea (mg/dl)		13.22	±4.01
Sr Cr (mg/dl)		1.01	±0.13
Serum Na (mmol/l)		138.30	±2.16
Serum K(mmol/l)		3.86	±0.25
Serum Albumin(gm/dl)		4.05	±0.18
CHb (ug/gmHb)		44.69	±3.47

Discussion

Carbamylated haemoglobin is a method of estimation of Urea exposure in a given patient and as a marker of Uremia in chronic kidney disease on maintenance haemodialysis.[9] It is important to know whether CarbamylatedHemoglobin is an index for adequacy of haemodialysis.[10]Carbamylated haemoglobin levels in chronic kidney disease patients cause changes in blood urea nitrogen with dialysis and serve as an index of uremic control. It is also important to know whether urea levels fall and if carbamylated haemoglobin levels also fall and if these two can be correlated over a long-term study.[11]

In the present study we found that in patients on maintenance haemodialysis irrespective of twice per week or thrice per week hemodialysis, the carbamylated haemoglobin levels showed higher levelscompared to healthy population indicating that persistent blood urea elevation in patients on haemodialysis was associated with elevated Carbamylated haemoglobin levels.In the present study, patients on haemodialysis had no significant correlation between serum creatinine and CHb levels while levels of serum sodium, serum potassium and pH were maintained in normal or near normal range while the bicarbonate levels were slightly lower and all parameters were compared with healthy controls.

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[12] resulting in carbamylation of proteins like amino-acids, plasma proteins, leucocyte proteins and haemoglobin.[12] The amino-terminal valine of hemoglobin is particularly reactive with isocyanate forming a stable modified haemoglobin termed Carbamylated haemoglobin (CHb). 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.[13] 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.[14.] Each alpha amino valine of the four Haemoglobin chains comprising aHb-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.[15]Thecarbamylation of Hb is known to result in conformational changes that results in altered biochemical behaviour of Haemoglobin. A study conducted comparing CKD from AKI compared to controls using carbamylatedhemoglobin levels.[16]

Conclusion

The present study showed that Carbamylated haemoglobin had a direct correlation with blood urea nitrogen levels and with serum creatinine levels in chronic kidney disease patients on maintenance haemodialysis compared to healthy controls.

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