Original Article

A clinical comparative study on carbamylated haemoglobin as a surrogate marker to differentiate acute kidney injury from chronic kidney disease

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Abstract

Introduction: Carbamylated haemoglobin is the result of reaction of isocyanate with N-terminal valine residues of the α and β chains of haemoglobin. Carbamylated haemoglobin concentration is dependent on the degree and duration of uraemia and thus may potentially serve as a marker to differentiate acute kidney injury (AKI) and chronic kidney disease (CKD). **Methods:** A hospital-based prospective clinical comparative study was conducted in an urban tertiary medical care centre. Carbamylated haemoglobin was estimated in a total of 60 patients, 30 each of chronic kidney disease and acute kidney injury. The comparison of the carbamylated haemoglobin levels among the CKD and AKI groups was done using Mann–Whitney test. The mean value of carbamylated haemoglobin among the CKD group was 240.71 ± 75.64 μgVH/g, whereas among the AKI group, it was 67.15 ± 17.05 μgVH/g. These values are statistically significant with $P < 0.001$. **Results:** Carbamylated haemoglobin values were elevated in relation to renal dysfunction, and it significantly correlated with chronicity of kidney disease. Mean CarHb among the CKD group was significantly high in comparison to the AKI group with statistical significance, with a P value of <0.001. **Conclusion:** It was significantly attributed in this study that carbamylated haemoglobin >100 μgVH/g is diagnostic of CKD and a value <100 μgVH/g is diagnostic of AKI. Thus, in this study, it can be concluded that carbamylated haemoglobin is a useful marker to differentiate AKI from CKD.

Keywords: Acute kidney injury, carbamylated haemoglobin, chronic kidney disease, uremia

Introduction

Renal function is one of the most important parameters to be assessed in patients to identify renal failure with either acute kidney injury (AKI) or chronic kidney disease (CKD). AKI is a heterogeneous syndrome defined by rapid (hours to days) decline in the glomerular filtration rate (GFR), resulting in the retention of metabolic waste products, including urea and creatinine, and dysregulation of fluid, electrolyte, and acid–base homeostasis.[1] CKD is defined as an irreversible and usually progressive decline in renal function and is generally measured by a reduction in GFR.[2]

The 2015 Global Burden of Disease Study reported an enormous increase in global life expectancy between 1980 and 2015, from 61.7 years to 71.8 years.[3] Much of this improvement is attributable to declining mortality as a result of communicable, maternal, neonatal, and nutritional diseases. With an aging population, CKD has become one of most common non-communicable diseases in the world and a leading cause of mortality. Half of the people in the United States are expected to develop CKD

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during their lifetime.[4] Associated with huge health system costs, particularly in the advanced stages, which includes kidney failure treated with dialysis and kidney transplantation, CKD has come into focus as a common, morbid, and often preventable disease.[5]

The incidence of AKI in unselected hospitalised patients is between 0.4% and 18%, depending on the definition used, and accounts for 1% to 4% of all hospital admissions.[6] Several large studies suggest that the incidence of AKI in hospitalised patients has increased by approximately 13% per year over the past 3 decades.[7] In these studies, incidence was identified by diagnostic codes that are highly specific for AKI (97%) but are relatively insensitive (35% sensitivity), and thus, these studies likely underestimate the true incidence.[8] There is limited information about community-acquired AKI; there is significant etiologic and geographical variation in the reported incidence and risk factors for AKI in the community settings. An increase has been observed in the incidence of severe AKI [requiring renal replacement therapy (RRT)] between 2000 and 2009, and a doubling has been observed in the number of deaths attributable to AKI.[9] The increased incidence is likely related to increasing patient age and a higher burden of co-morbidity, including a higher prevalence of CKD. AKI survivors had a prolonged hospital length of stay and a greater requirement for post-hospitalisation care, thus incurring significantly higher health care costs.[10,11] Urea is one of the many metabolites that accumulate in renal failure. In solution, urea exists as an equilibrium pair with cyanate.[12,13] Carbamylated haemoglobin is the result of reaction of isocyanate with N-terminal valine residues of α and β chains of haemoglobin.[14] The degree of carbamylation can be assessed by measuring the amount of valine hydantoin formed after acid hydrolysis of haemoglobin.[15] Carbamylated haemoglobin (CarHb) is a simple, useful, and reproducible index for measuring the carbamylation reaction *in vivo*. Carbamylated haemoglobin concentration is dependent on the degree and duration of uraemia[16] and thus may potentially serve as a marker to differentiate AKI and CKD.

Materials and Methods

A hospital-based prospective clinical comparative study was conducted in an urban tertiary medical care centre for a period of 18 months after obtaining due clearance from the Institutional Ethics Committee.

The sample size was estimated using the GPower software v. 3.1.9.2. Considering the effect size to be measured (d) at 80% for two-tailed hypothesis, the power of the study at 85%, and the margin of the error at 5%, the total sample size needed came up to 60. Simple random sampling was performed. Therefore, the study included 30 patients with AKI and 30 patients with CKD.

Inclusion criteria

1. Evidence of renal dysfunction in adult patients (age $\geq$ 18).
2. Patients diagnosed with AKI.
3. Patients diagnosed with CKD.

Exclusion criteria

1. Patients not willing to consent
2. Patients on haemodialysis.

Baseline data including age and sex, detailed medical history including previous history of co-morbidities, medications and risk factors, clinical examination, previous renal function tests, and current relevant investigations were collected and recorded. Informed consent was taken from the patients. A pre-structured and pre-tested proforma was used to collect the data. Patients were classified as AKI and CKD. Carbamylated haemoglobin levels were estimated and recorded. In this study, the Rosen method for measurement of CHB was adapted for a modified colorimetric analysis of CHB.[17]

The haemoglobin concentration of blood samples in each of the EDTA bottles was estimated. Whole blood was washed three times with physiological saline and diluted 1 in 100 (0.1 ml blood with 9.9 ml distilled water) to obtain a haemoglobin haemolysate. This dilution gives approximately 0.09 gHb to 0.15 gHb range. To 1.0 ml of the haemolysate was added 0.5 ml acid mixture (10 ml conc. hydrochloric acid/1.0 ml glacial acetic acid) and incubated at 37 ^{0}C for 30 minutes. n-Butanol–acetic acid mixture (1.0 ml) was added to the haemolysate to extract the valine hydantoin formed. To each test tube marked blank, test, and standard were added 1.0 ml distilled water, 1.0 ml extracted valine hydantoin, and 1.0 ml each of the valine standards (LS and HS), respectively. Acetate cyanide solution (0.5 ml) was then added to all the tubes, followed by 0.5 ml ninhydrin solution and tubes placed in boiling distilled water for 20 minutes. The dilute isopropanol (0.5 ml) was immediately added to all the tubes. The contents of the tubes were allowed to cool to room temperature and then read spectrophotometrically at 570 nm using a 1 cm cuvette after setting to zero with the blank reagent. The concentration of valine was calculated using the absorbance readings obtained. The results were expressed as valine hydantoin equivalent of carbamylated haemoglobin.[18]

The study subjects once included in the study were subjected to a series of investigations like haemoglobin, ultrasonography – kidney, urethra, bladder (USG-KUB), renal function tests, and serum electrolytes.

Statistical analysis

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis of all the explanatory parameters will be done using frequency and proportions for categorical variables, whereas mean and standard deviation were used for continuous variables.

Independent Student *t*-test was used to compare the mean Hb (gm%) level between two study groups. Chi-square test was used to compare the age, gender distribution, and USG-KUB findings between the two study groups. Mann–Whitney test was

used to compare the mean values of different study parameters at the time of admission and discharge between two study groups. Similarly, the comparison of mean carbamylated Hb levels between two study groups was done using the same test. Wilcoxon signed rank test was used to compare the mean values of different study parameters between the time of admission and discharge in CKD and AKI groups. The level of significance (*P* value) was set at $P < 0.05$.

Results

A total of 60 study subjects, 30 in each group of AKI and CKD, were included in the study as per the inclusion and exclusion criteria.

Among the CKD group, the age varied from as low as 20 years of age to as high as 97 years of age. Among this diverse age group, 73.4% (n = 22) belonged to age group >50 years of age with 40% being above 70 years of age (n = 12).

Among the AKI group, the age varied from as low as 25 years of age to as high as 83 years of age. Among this diverse age group, 33.3% (n = 10) belonged to the age group of >50 years of age with 13.3% (n = 4) being above 70 years of age [Table 1].

Among the CKD group, the majority of patients, that is, 40% (n = 12), belonged to the age group of >70 years in contrast to the AKI group, where the majority, that is, 30% (n = 9), belonged to the age group of 30–40 years.

The age distribution was subjected to statistical analysis by Chi-square test and is of statistical significance, $P = 0.007$.

Among the CKD group, 80% (n = 24) of study subjects were male in contrast to AKI group, where 60% (n = 18) were male. On statistical analysis, the *P* value was 0.09 [Table 2].

Haemoglobin values among the CKD group varied from 6.2 g% to 14.2 g% with a mean of 10.66 ± 1.78 g%. Among the AKI group, haemoglobin varied from 9.6 g% to 14.8 g% with a mean value of 12.43 ± 1.44 g%. The statistical analysis conducted by the Student *t*-test was significant with a *P* value <0.001 [Table 3].

USG-KUB was done for all patients to know the structural changes in the kidney of both groups.

Among the CKD group, it was noticed that about 53.3% (n = 16) had shrunken kidneys, 20% (n = 6) had nephropathy changes, and 26.7% (n = 8) had normal kidneys in contrast to all subjects in the AKI group, that is, 100% (n = 30), with a normal kidney size and structure on USG supporting the diagnosis of the study subjects [Table 4].

Renal function tests done at admission and at discharge for both the groups of study subjects were recorded and analysed. Renal function tests of the study subjects were analysed using Mann–Whitney test.

At admission, a mean blood urea level of 82.87 ± 41.86 g/dL was identified among the CKD group in contrast to 107 ± 33.75 g/dL among the AKI group. Among the CKD group, a mean serum creatinine level of 4.83 ± 2.09 g/dL was recorded, whereas among the AKI group, a mean value of 2.5 ± 0.66 g/dL was recorded. The mean estimated glomerular filtration rate (eGFR) value among the CKD group was 16.58 ± 6.39 mL/min/1.73 m2, and that among the AKI group was 34.55 ± 14.04 mL/min/1.73 m2. The statistical analysis performed by Mann–Whitney test proved statistical significance of all three investigations, that is, blood urea, serum creatinine, and eGFR values with *P* values of 0.004, <0.001, and <0.001, respectively [Table 5].

At discharge, the mean blood urea level among the CKD group was 70.57 ± 36.79 g/dL in contrast to 32.37 ± 6.8 1 g/dL among the AKI group. On analysis, the mean serum creatinine level at discharge was 3.68 ± 1.62 g/dL among the CKD group and 1.02 ± 0.2 1 g/dL among the AKI group. eGFR values at discharge were summoned

Table 1: Comparison of age distribution between two study groups using Chi-square test

Age	CKD group		AKI group		χ^2 value	*P*
	N	%	*n*	%		
20-30 yrs.	1	3.3%	4	13.3%	15.800	0.007*
31-40 yrs.	0	0.0%	9	30.0%		
41-50 yrs.	7	23.3%	7	23.3%		
51-60 yrs.	5	16.7%	3	10.0%		
61-70 yrs.	5	16.7%	3	10.0%		
> 70 yrs.	12	40.0%	4	13.3%		

Table 2: Comparison of gender distribution between two study groups using Chi-square test

Gender	CKD group		AKI group		χ^2 value	*P*
	n	%	*n*	%		
Males	24	80.0%	18	60.0%	2.857	0.09
Females	6	20.0%	12	40.0%	2.857	0.09

Table 3: Comparison of mean Hb (gm%) levels between two study groups using independent Student *t* test

Group	*n*	Mean	SD	Mean Diff	*P*
CKD group	30	10.66	1.78	-1.77	<0.001*
AKI group	30	12.43	1. 44		

Table 4: Comparison of USG-KUB findings between two study groups using Chi-square test

USG-KUB	CKD group		AKI group		χ^2 value	*P*
	n	%	*n*	%		
Shrunken kidneys	16	53.3%	0	0.0%	34.737	<0.001*
Nephropathy	6	20.0%	0	0.0%		
Normal kidneys	8	26.7%		100.0%		

to analysis, and the mean value of eGFR among the CKD group was 22.18 ± 9.0 1 mL/min/1.73 m2 and that among the AKI group was 81.56 ± 26.18 mL/min/1.73 m2. The statistical analysis performed by Mann–Whitney test proved statistical significance of all three investigations, that is, blood urea, serum creatinine, and eGFR values with *P* values of <0.001 [Table 6].

When renal function tests were compared between at admission and at discharge in the study groups separately, it was observed that among the CKD group, there was improvement in all the three parameters, that is, blood urea, serum creatinine, and eGFR, but not normalised characteristic of CKD, whereas in the AKI group, all these parameters were normalised. This analysis was done using the Wilcoxon signed rank test.

Among the CKD group, the mean blood urea level at admission was 82.87 ± 41.86 g/dL and that at discharge was 70.57 ± 36.79 g/dL. This value was statistically significant, $P = 0.04$. The mean of serum creatinine levels among the CKD group at admission was 4.83 ± 2.09 g/dL, and that at discharge was 3.68 ± 1.62 g/dL, which was statistically significant with a *P* value of <0.001. eGFR values at admission and discharge were subjected to statistical analysis. It was observed that the mean values were 16.58 ± 6.39 mL/min/m2 and 22.18 ± 9.01 mL/min/m2, respectively, with a *P* value of < 0.001 [Table 7].

Among the AKI group, the mean blood urea level at admission was 107 ± 33.75 g/dL and that at discharge was 32.37 ± 6.81 g/dL. This value was statistically significant, $P < 0.001$. The mean of serum creatinine levels among the AKI group at admission was 2.50 ± 0.66 g/dL and that at discharge was 1.02 ± 0.21 g/dL, which was statistically significant with a *P* value of <0.001. eGFR values at admission and discharge were subjected to statistical analysis. It was observed that the mean values were 34.55 ± 14.04 mL/min/m2 and 81.56 ± 26.18 mL/min/m2, respectively, with a *P* value of <0.001 [Table 8].

The comparison of the carbamylated haemoglobin levels among the CKD and AKI groups was done using Mann–Whitney test.

The mean value of carbamylated haemoglobin among the CKD group was 240.71 ± 75.64 μgVH/g, whereas that among the AKI group was 67.15 ± 17.05 μgVH/g. These values are statistically significant with $P < 0.001$ [Table 9].

These values are well diversified, and it can be observed that the carbamylated haemoglobin value is higher among the CKD group in comparison to the AKI group. Thus, it can be derived that a carbamylated haemoglobin value >100 μgVH/g is diagnostic of CKD and a carbamylated haemoglobin value of <100 μgVH/g is diagnostic of AKI.

Table 5: Comparison of mean values of different study parameters at time of admission between two study groups using Mann–Whitney test

Parameters	Group	N	Mean	SD	Mean Diff	P
B. Urea	CKD group	30	82.87	41.86	-24.13	0.004*
	AKI group	30	107.00	33.75		
S. Creatinine	CKD group	30	4.83	2.09	2.33	<0.001*
	AKI group	30	2.50	0.66		
eGFR	CKD group	30	16.58	6.39	-17.97	<0.001*
	AKI group	30	34.55	14.04		

Table 6: Comparison of mean values of different study parameters at time of discharge between two study groups using Mann–Whitney test

Parameters	Group	N	Mean	SD	Mean Diff	P
B. Urea	CKD group	30	70.57	36.79	38.20	<0.001*
	AKI group	30	32.37	6.81		
S. Creatinine	CKD group	30	3.68	1.62	2.67	<0.001*
	AKI group	30	1.02	0.21		
eGFR	CKD group	30	22.18	9.01	-59.38	<0.001*
	AKI group	30	81.56	26.18		

Table 7: Comparison of mean values of different study parameters between time of admission and discharge in CKD group using Wilcoxon signed rank test

Parameters	Time	N	Mean	SD	Mean Diff	P
B. Urea	Admission	30	82.87	41.86	12.30	0.04*
	Discharge	30	70.57	36.79		
S. Creatinine	Admission	30	4.83	2.09	1.15	<0.001*
	Discharge	30	3.68	1.62		
eGFR	Admission	30	16.58	6.39	-5.60	<0.001*
	Discharge	30	22.18	9.01		

Table 8: Comparison of mean values of different study parameters between time of admission and discharge in AKI group using Wilcoxon signed rank test

Parameters	Time	N	Mean	SD	Mean Diff	P
B. Urea	Admission	30	107.00	33.75	74.63	<0.001*
	Discharge	30	32.37	6.81		
S. Creatinine	Admission	30	2.50	0.66	1.48	<0.001*
	Discharge	30	1.02	0.21		
eGFR	Admission	30	34.55	14.04	-47.01	<0.001*
	Discharge	30	81.56	26.18		

Table 9: Comparison of mean carbamylated Hb levels between two study groups using Mann–Whitney test

Group	N	Mean	SD	Mean Diff	P
CKD group	30	240.71	75.64	173.57	<0.001*
AKI group	30	67.15	17.05		

Discussion

AKI is defined as an abrupt decline in glomerular filtration rate sufficient to decrease the elimination of nitrogenous waste products and other uremic toxins.[19] AKI can be treated with non-dialytic therapy alone or RRT if necessary. CKD is defined as abnormalities of kidney structure or function, present for more

than 3 months, with implications of health.[20] Risk factors of CKD include hypertension, diabetes, advanced age, and persistent or recurring AKI.[21] Thus, utmost treatment and initiation of RRT for AKI at optimal time become the mainstay for prevention of progression to CKD. Thus, differentiating AKI from CKD becomes a necessity. This study aimed to correlate renal failure with carbamylated haemoglobin and as a marker to differentiate AKI and CKD as it is simple and non-invasive. A reliable marker that can prompt treatment of AKI can prevent progression to CKD, thereby reducing the overall disease burden.

Carbamylated haemoglobin concentration is dependent on the degree and duration of uraemia. This was supported by a study conducted by Stim J *et al.*,[16] who, in their comparative study of 84 subjects, found that carbamylated haemoglobin formation is dependent on urea concentration and length of exposure to urea. They concluded that carbamylated haemoglobin may be a useful index of the duration and degree of exposure to high blood urea levels in patients with renal failure and may potentially serve as an index of the adequacy of dialysis.

Several methods have been used to quantify carbamylated haemoglobin. Manning *et al.*[22] used the 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. The gas liquid chromatographic method was used in other studies to measure valine hydantoin released after acid hydrolysis of haemoglobin extracted from whole blood.[23] High-performance liquid chromatography has been used to quantify CHB based on the release of carbamyl valine from the N-amino acid terminals of the alpha and beta chains of haemoglobin after acid hydrolysis.[24,25]

In this study, a colorimetric method for measurement of carbamylated haemoglobin was used with the help of a spectrophotometer. This method of estimating carbamylated haemoglobin is substantiated by a study done by Okaka EI *et al.*,[18] which aimed at developing a colorimetric method for the measurement of carbamylated haemoglobin in patients with renal failure. They found that there was a significant correlation between carbamylated haemoglobin and urea, creatinine, and haemoglobin concentration. They found that at a carbamylated haemoglobin cut-off level of 100 μgVH/g haemoglobin, the sensitivity and specificity of this test to identify persons with CKD from those with normal renal function were 95% and 80%, respectively, and thus, carbamylated haemoglobin levels can be determined with reasonable accuracy using a colorimetric method.

At the end of the study, the mean value of carbamylated haemoglobin among the CKD group was 240.71 ± 75.64 μgVH/g, whereas that among the AKI group was 67.15 ± 17.05 μgVH/g. These values are statistically significant with $P < 0.001$. These values are well diversified, and it can be observed that the carbamylated haemoglobin value is higher among the CKD group in comparison to the AKI group. Thus, it can be derived that a carbamylated haemoglobin value >100 μgVH/g is diagnostic of CKD and a carbamylated haemoglobin value of <100 μgVH/g is diagnostic of AKI.

The results of our study are comparable to that of a study by Naresh *et al.*[26] In their comparative study of 40 subjects with 20 controls, they evaluated the diagnostic performance and usefulness of carbamylated haemoglobin in the differentiation of AKI from CKD. They found that the levels were high in both AKI and CKD, but patients with CKD had significantly high levels of carbamylated haemoglobin compared to patients with AKI. They concluded that carbamylated haemoglobin can be helpful in differentiating AKI from CKD as both of them present in a similar manner.

The result of this study was also substantiated by Wynckel A *et al.*,[14] who studied CarHb levels in 28 patients with AKI and 13 patients with CKD. They found that the levels <80 mcg CV/g haemoglobin is seen in AKI and the levels will help in differentiating ARF from CKD.

Our study did have its limitations. The sample size of the study with only 30 patients in each group is small and prompts the need for larger study groups. Our study only included CKD and AKI patients without comparison with healthy controls. Further, our study did not include longitudinal follow-up of patients to assess for the impact of CarHb levels on future morbidity and mortality.

Conclusion

In this study, carbamylated haemoglobin was estimated in a total of 60 study subjects, 30 each of CKD and AKI, respectively. Carbamylated haemoglobin values were elevated in relation to renal dysfunction, and it significantly correlated with chronicity of kidney disease. Mean CarHb among the CKD group was significantly high in comparison to the AKI group with statistical significance, with a P value of <0.001.

It was significantly attributed in this study that carbamylated haemoglobin >100 μgVH/g is diagnostic of CKD and a value <100 μgVH/g is diagnostic of AKI.

Thus, in this study, it can be concluded that carbamylated haemoglobin is a useful marker to differentiate AKI from CKD.

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Conflicts of interest

There are no conflicts of interest.

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