Type of the Paper (Article)

Ability of carbamylated hemoglobin to predict duration and stage of renal diseases

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Abstract

Introduction: Identification of renal disease duration and severity is important for proper management and the prediction of outcomes. Carbamylated hemoglobin (CarHb) is being studied as a novel biomarker in renal diseases.

Aim of the study: To address the ability of CarHb to predict the duration and stage of acute kidney injury (AKI) and chronic kidney disease (CKD).

Subjects and Methods: The study included 62 patients divided into two groups; Group 1 included 29 patients with AKI, and Group 2 included 33 patients with CKD in different stages. The blood CarHb level was assessed for these 62 patients using anti-carbamylated hemoglobin antibody ELISA kits to assess the level of CarHb in U/ml.

Results: There was a positive correlation (r = 0.36) between the CarHb level and duration of AKI in group 1, but this correlation was insignificant (P = 0.09). Also, there was a weak, insignificant correlation between CarHb level and duration of CKD in group 2 (r = -0.1; P = 0.6). In CKD patients, the mean CarHb level in patients presented in stage 3 was 3.7 U/ml, compared to 3.85 U/ml for stage 4 and 3.81 in stage 5; this difference was significant (P = 0.9)

Conclusion: It's very early to describe the role of carbamylated hemoglobin in renal diseases. This study failed to prove a significant correlation between CarHb level and the duration of AKI or CKD. CarHb cannot predict the onset of renal impairment. This study also revealed that AKI and CKD could not be staged based on CarHb level, as there is no significant difference in the mean level between different stages of AKI and CKD.

Keywords: Carbamylated Hemoglobin; Urea Dissociation Products; Renal Impairment; Kidney

1. Introduction

Elevated kidney function is a common presentation in outpatient clinics and emergency rooms. Identification of type and assessment of severity are important for

proper management. In many situations, the duration of renal impairment is difficult to identify by history or traditional investigations. Novel biomarkers are continuously being investigated for this purpose. Hemoglobin carbamylating by isocyanide the product of urea dissociation, may help with this issue [1]. Similar to glycated hemoglobin, carbamylated hemoglobin is expected to give an idea about the duration and severity of renal impairment. The current prospective analytical study sought to investigate the ability of CarHb-level assessment to predict the duration and stages of various types of renal diseases.

2. Subjects and methods

2.1. Subjects

The study included 62 patients divided into two groups according to the KDIGO definitions of acute kidney injury (AKI) and Chronic Kidney Disease (CKD) [2, 3]. Group 1 included 29 patients with AKI, and group 2 included 33 patients with CKD.

Inclusion criteria

Any patient admitted to Fayoum University Hospitals with acute kidney injury or chronic kidney disease.

Exclusion criteria

Patients with normal kidney function, AKI on top of CKDs, end-stage renal disease, or renal replacement therapy were excluded.

2.2. Methods

All the study participants were evaluated by taking a full medical history, undergoing a clinical examination, and reviewing their investigations to assess their renal condition, type of renal disease, and commodities.

Blood samples of 4-5 ml of venous blood were collected to assess carbamylated hemoglobin using a human anti-carbamylated protein antibody ELISA kit.

A volume of 4-5 ml of venous blood was collected from all the subjects into plain tubes and allowed to clot; serum was separated by centrifugation at 3000 rpm for 15 minutes. The separated serum was transferred into appropriately labeled aliquots and stored at -80 °C until further analysis. CarHb was measured using an Ag-coated micro ELISA strip plate specific to CarHb Ab. CarHb concentration is determined by comparing the spectrophotometrically measured optical density of samples to the standard curve (Glory Science).

2.3. Statistical Analysis

Data were collected, and a standard sheet was completed for each subject. Collected data were re-checked for errors, coded to facilitate manipulation, computerized, and statistically analyzed using IBM SPSS (statistical package for social sciences) version 24 (International Business Machines Corp., Armonk, NY, USA). All continuous numerical variables are tested for normality. In the case of non-normally distributed variables, a non-

parametric test was used, or parametric tests after outliers were excluded. Quantitative data were expressed as mean or median for central tendency, standard deviation (SD), and range as measures for dispersion. The independent T-test was used to compare means between two groups unless there were outliers, in which case the non-parametric Mann Whitney U test was used. Qualitative data were expressed as absolute frequencies (numbers) and relative frequencies (percentages). The chi-square test was used to compare proportions and percentages between two or more groups unless there is an expected value less than 5 when comparing two groups, in which case the Fisher exact test is used. A *P-value* was considered significant at a level below 0.05.

3. Results

The current study recruited 62 patients who were admitted to Fayoum University Hospital with different types of renal diseases. According to KDIGO's definitions of AKI and CKD, patients were classified into two groups. Group 1 included patients who met the criteria for the definition of AKI and included 29 patients, representing 47%. Group 2 included patients who met the criteria for the definition of CKD and included 33 patients, representing 53% of the whole study population.

Both groups of the study population were matched in age and sex characteristics with no significant differences. Males represented 48.2% of group 1 (N = 14) and 48.4% of group 2 (N = 16). Females made up 51.8% of group 1 (N = 15) and 51.6% of group 2 (N = 17). These differences in sex distribution were insignificant (*P = 0.7*) (Figure 1). The mean age of the whole study population was 61.43 years. The mean age in Group 1 was 61.93 years, while it was 60.9 years in Group 2; this difference was non-significant (*P = 0.1*) (Figure 2).

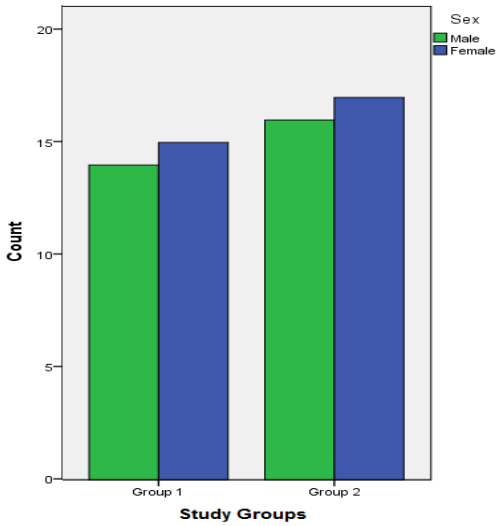

Figure 1: Sex distribution.

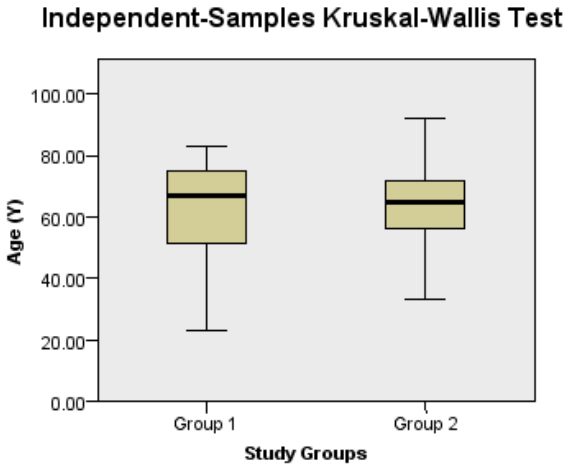

Figure 2: Box blot represents the Mean age of the study population.

Diabetes was prevalent in 42% of the whole study population (N = 26). Diabetic patients represented 41.4% of group 1 and 34.5% of group 2; this difference was not statistically significant (*P = 0.40*). Hypertension was more prevalent than diabetes in our study population (55%; N = 34). Each study group had 17 hypertensive patients, representing 58.6% of group 1 and 45% of group 2, with no significant difference between the two groups (*P = 0.19*) (Table 1).

Table 1: Diabetes & Hypertension prevalence in the study population.

Variables	Group 1	Group 2	Total	P-value
Non-diabetic	17 (58.6%)	19 (65.5%)	36 (58%)	0.413
Diabetic	12 (41.4%)	14 (34.5%)	26 (42%)	
Non-Hypertensive	12 (41.4%)	16 (55%)	28 (45%)	0.191
Hypertensive	17 (58.6%)	17 (45%)	37 (55%)	

Prerenal failure was the most common cause of AKI 10 (34.5%), followed by septic AKI 6 (20.7%), hepatorenal syndrome (HRS) 3 (10.3%), acute interstitial nephritis (AIN) 2 (6.9%), glomerulonephritis (GN) 2 (6.9%), post-renal contrast-induced AKI (CI-AKI) 1 (3.4%), cardiorenal syndrome (CRS) 1 (3.4%), HTN emergency 1 (3.4%), and pancreatitis 1 (3.4%), In CKD, DM and/or HTN represented 45.6%. Other causes included obstructive uropathy (OU) in 2 (6.9%), chronic cardiorenal disease, chronic GN, and multiple myeloma (MM), and in 4 (12.2%) patients, the cause could not be identified (Table 2).

Table 2: Etiology of AKI and CKD in the study population.

Parameters	AKI (Group 1)	CKD (Group 2)
Prerenal	10 (34.5%)	_____
Septic	6 (20.7%)	_____
HRS	3 (10.3%)	_____
AIN	2 (6.9%)	_____
GN	2 (6.9%)	5 (15.2%)
OU	2 (6.9%)	7 (21.2%)
CI AKI	1 (3.4%)	_____
CRS	1 (3.4%)	1 (3%)
HTN emergency	1 (3.4%)	_____
Pancreatitis	1 (3.4%)	_____
DM&HTN	_____	8 (24.2%)
DM	_____	4 (12.1%)
HTN	_____	3 (9.1%)
Unknown	_____	4 (12.1%)
MM	_____	1 (3%)
Total	29 (100%)	33 (100%)

The average duration of AKI from diagnosis to presentation and CarHb assessment was 21.7 19.4 days. In group 2, the mean duration of CKD was 37.5± 44 months. There was a positive correlation (r = 0.36) between the CarHb level and duration of AKI in group 1, but this correlation was insignificant (*P = 0.09*). Also, there was a weak, insignificant correlation between CarHb level and duration of CKD in group 2 (r = -0.1; *P = 0.6*).

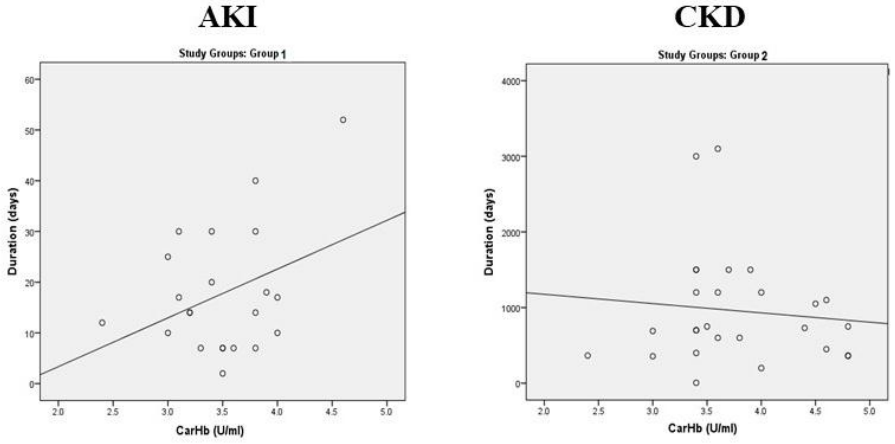

Figure 3: Correlation of CarHb with Duration of AKI and CKD.

Late presentation was noticed in both AKI and CKD. In group 1, 75.9% of patients presented in stage 3, 20.7% in stage 2, and 3.4% in stage 1. In group 2, no patient presented in stages 1 or 2, 42.4% in stage 5, 42.4% in stage 4, and 15.2% in stage 3 (Table 3). There was one patient with AKI who presented in stage 1, and his CarHb level was 3.5 U/ml. In patients with AKI stage 2, the mean CarHb level was 3.5 U/ml, and 3.49 in patients with AKI stage 3. So, there was no significant difference between the mean CarHb level in different stages of AKI (*P = 1*). In CKD patients, the mean CarHb level in patients presented in stage 3 was 3.7 U/ml, compared to 3.85 U/ml for stage 4 and 3.81 in stage 5; this difference was significant (*P = 0.9*) (Table 3, Figure 4).

Table 3: Stages of AKI and CKD on presentation in the study population.

Parameters	**AKI (Group 1)**			**CKD (Group 2)**		
	Frequency	Mean ±SD	95% CI	Frequency	Mean ±SD	95% CI
Stage 1	1 (3.4%)	3.5 ±0	_____	_____		
Stage 2	6 (20.7%)	3.5 ±0.22	_____	_____		
Stage 3	22 (75.9%)	3.49 ±0.52	3.156 - 3.844	5 (15.2%)	3.72 ±0.61	2.963 - 4.477
Stage 4	_____	_____	3.225 - 3.763	14 (42.4%)	3.85 ±0.69	3.409 - 4.291
Stage 5	_____	_____	3.290 - 3.701	14 (42.4%)	3.81 ±0.59	3.454 - 4.161
Total	29 (100%)	3.49 ±0.46	3.290 - 3.701	33 (100%)	3.81 ±0.62	3.580 - 4.040
P-value		1.000			0.9	

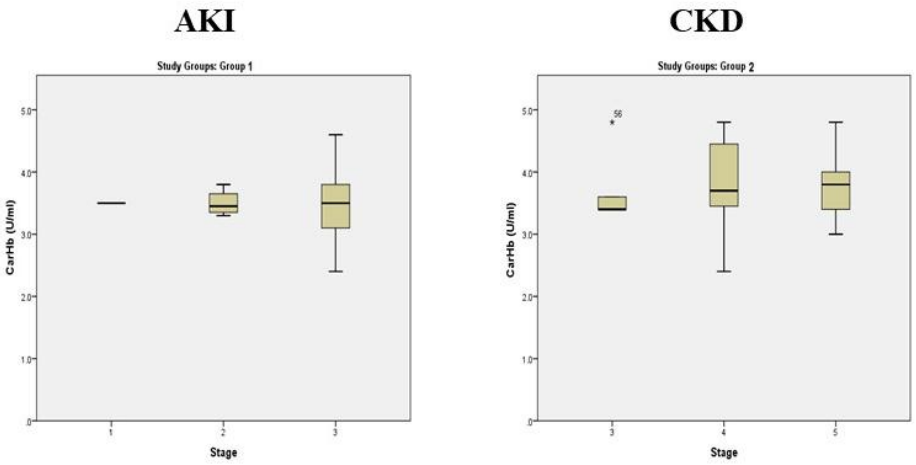

Figure 4: Box blot analysis of the mean CarHb in correlation to different stages of AKI and CKD.

4. Discussion

In outpatient clinics and emergency rooms, renal disease is a common presentation. So, great effort is needed to develop new biomarkers of renal disease to aid in diagnosis and management [4]. Differentiating AKI from CKD is an everyday challenge. After that, identification of the stage of AKI or CKD is usually needed for proper management. Duration and rate of progression of kidney disorders have a great impact on the outcome. This study aimed to test the ability of Carbamylated Hemoglobin (CarHb) level to predict the severity and duration of renal impairment. Identification of such relations may help to predict out-comes and to manage renal disorders.

Our study included 62 patients who were admitted to Fayoum University Hospital with different types of renal diseases. According to KDIGO definitions of acute kidney injury and chronic kidney disease, patients were classified into two groups [2, 3]. Group 1 included patients who met the criteria for the definition of AKI and included 29 patients, representing 47%. Group 2 included patients who met the criteria for the definition of CKD and included 33 patients, representing 53% of the whole study population.

Prerenal failure was the most common cause of AKI (34.5%), followed by septic AKI (20.7%), hepatorenal syndrome, AIN, GN, postrenal, contrast-induced, cardiorenal, HTN emergency, and pancreatitis. Similar causes were observed in a study conducted at Cairo University Hospital; prerenal AKI, drug-induced AKI, obstetric causes, GN, and post-renal AKI were the most common [5].

Our study showed that; CKD, DM, and/or HTN represented 45.6% of cases; other causes included obstructive uropathy, chronic cardiorenal disease, chronic GN, and multiple myeloma; in 4 patients, the cause could not be identified. Similar findings were discovered in the El-Sharkia governorate, Egypt [6]. Different data was published in the Pan African Medical Journal from a study conducted in Cameroon. They found that chronic GN (25.9%) preceded hypertension (22.3%) and diabetes (20.1%) as causes of CKD. The difference in findings could be attributed to population characteristics and the high prevalence of infectious diseases such as AIDS; this was also reflected in the younger age of presentation (16–44.8 years) [7]. The mean duration of AKI from diagnosis to presentation and assessment of CarHb was 21.7 days. In group 2, the mean duration of CKD was 37 months.

CarHb is the result of hemoglobin chain modification by isocyanate. Isocyanate is derived from urea dissociation. Over the past 20 years, CarHb has been investigated as a marker of severity, duration, detection, diagnosis, and follow-up of renal diseases and renal replacement therapy [8].

In our study, we noticed no significant correlation between CarHb and the duration

of renal impairment, neither in AKI (r = 0.361, *P = 0.099*) nor CKD (r = 0.103, *P = 0.61*). Against that, CarHb formation is affected by urea concentration, and the duration of exposure to uremic toxins [9]. Later studies also noted that correlation [10, 11]. The duration of renal illness is usually unknown, especially in renal patients, and is expected to be longer than documented; this inaccuracy in this vital study variable may explain the different findings.

Late presentation was noticed in both AKI and CKD. In group 1, 75.9% of patients presented in stage 3, 20.7% in stage 2, and 3.4% in stage 1. In group 2, no patient pre-sented in stages 1 or 2, 42.4% in stage 5, 42.4% in stage 4, and 15.2% in stage 3. Previous studies showed that all AKI patients were RIFLE-F on presentation [7]; 53.6% of AKI patients presented in stage 3 [12]; and 43.1% in stage 4 [13]. In group 3; no patient presented in stages 1 or 2, 42.4% in stage 5, 42.4% in stage 4, and 15.2% in stage 3. This is a world-wide issue and is associated with worse outcomes [14], increased costs, and complications [15]. The importance of early detection pushed Egyptian authorities to launch a screening program for renal impairment.

In patients with AKI stage 1 and stage 2, the mean CarHb level was 3.5 U/ml, and 3.49 in patients with AKI stage 3. So, there was no significant difference between the mean CarHb level in different stages of AKI (*P = 1*). In CKD patients, the mean CarHb level in patients presented in stage 3 was 3.7 U/ml, compared to 3.85 U/ml for stage 4 and 3.81 U/ml for stage 5; this difference was significant (*P = 0.9*). Earlier studies found a strong relation between CarHb level and the severity of renal impairment [16]. This difference in findings may be due to differences in defining severity. They based their definition on the levels of urea, BUN, and creatinine, while we tested CarHb level in correlation with the stage of AKI or CKD during CarHb level assessment.

Conclusion

Our study concluded that it's very early to describe the role of carbamylated hemoglobin in renal diseases. This study failed to prove a significant correlation between CarHb level and the duration of AKI or CKD. CarHb cannot predict the onset of renal impairment. This study also revealed that AKI and CKD could not be staged based on CarHb level, as there is no significant difference in the mean level between different stages of AKI and CKD.

Ethical Approval Statement: The protocol was approved by the local institutional ethics committee of Fayoum University Hospital, Fayoum, Egypt.

Funding: This research is not funded.

Conflicts of Interest: All authors declare no conflict of interest.

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