The Role of Carbamylated Hemoglobin in Identifying Acute and Chronic Renal Failure

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Abstract

Carbamylated hemoglobin (CarbHb) levels expressed as valine hydantoin (VH) were measured by high performance liquid chromatography (HPLC) in patients with acute renal failure (ARF, n=35) and chronic renal failure (CRF, n=39). CarbHb levels in CRF patients were approximately 2.5 times of those in ARF ones (121.2 ± 8 *vs* 54.8 ± 6 μgVH/gHb, $p<0.01$). CarbHb levels of 80 μgVH/gHb provided the best statistical values (sensitivity of 89% and specificity of 82%). CarbHb/BUN and Carb/Cr ratios were also effective determinants in differentiation between ARF and CRF. CarbHb/BUN ratio of 1.5 and CarbHb/Cr ratio of 20 were the best statistical cut off points. As such, measurement of CarbHb levels could be a reliable non-invasive method in identifying ARF from CRF patients.

Key word : Carbamylated Hemoglobin, Valine Hydantoin, Acute Renal Failure, Chronic Renal Failure

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Differential diagnosis between acute renal failure (ARF) and chronic renal failure (CRF) is important in the aspects of management and prognosis of patients presenting with high blood urea nitrogen (BUN) and creatinine (Cr) levels[1,2]. Routine investigations including normochronic normocytic anemia, hyperphosphatemia, hypocalcemia, and metabolic acidosis could be detected in both conditions[3]. In clinical practice, demonstration of bilateral small kidneys by X-ray imaging such as ultrasonography is the gold standard diagnosis of CRF. When the kidney sizes determined by X-ray imaging are equivocal, the definite differential diagnosis between ARF and CRF is kidney biopsy which is an invasive procedure and can not be immediately performed in most uremic patients.

Carbamylated hemoglobin (CarbHb) is derived from the nonenzymatic covalent binding of protein in hemoglobin with isocyanic acid, the spontaneous dissociation product of blood urea nitrogen (BUN)[4-10]. Blood CarHb levels increase in patients with renal failure and are positively correlated with the increasing azotemic levels and the longer exposure duration to BUN[11-13]. Patients with CRF have a longer duration of exposure to BUN, when compared with those with ARF, and, thus, can have higher blood CarbHb levels.

The objectives of the present study were 1) to compare blood CarbHb levels between ARF and CRF patients 2) to define the cut off point of CarbHb, CarbHb/BUN ratios, and CarbHb/Cr ratios in identifying the CRF patients from those with ARF.

PATIENTS AND METHOD

Patients

In the present prospective diagnostic study, CarbHb levels were determined in both ARF (n=35) and CRF (n=39) patients who were treated at King Chulalongkorn Memorial Hospital from January 1st to December 31st, 2000. The study was approved by the Ethical Committee, Faculty of Medicine, Chulalongkorn University, Bangkok Thailand. Each patient participating in the study gave informed consent. All patients must be at least 15 years old. Diagnosis of ARF and CRF were based on relevant medical history, laboratory findings, eventual clinical outcome, and X-ray imaging of both kidney sizes. The patients enrolled in the present study had BUN levels ranging from 40-100 mg/dl, the levels that cause a differential diagnosis problem in clinical practice. Exclusion criteria were : a) receiving blood transfusion within 3 months b) having a hemolysis condition c) having upper gastrointestinal bleeding and d) receiving erythropoietin.

Method

Measurement of CarbHb levels

Valine is the amino acid of hemoglobin involved in the production of CarHb. Measuring of CarHb levels involves acid hydrolysis of the hemoglobin which liberates carbamylated valine, this being converted to valine hydantoin (VH). Valine hydantoin is separated by ethyl acetate partition and subsequently measured by high precision liquid chromatography (HPLC) or by gas chromatography. In the present study, determination of CarHb was performed by the HPLC method developed by Kwan et al[6]. In brief, 5-ml of heparinized blood samples were collected by venepuncture into vacutainer tubes (Becton Dickinson, Rutherford, NJ) and were used for analysis. Red blood cells were washed with isotonic sodium chloride and centrifuged at 3,000 g for 10 minutes, then, the supernatant was discarded. The washed samples were stored at -20°C until analysis. A 0.5-ml sample was hydrolyzed at 110°C for 2 hours with 1 ml of 11 mol/L HCl and 1 ml of 17 mol/L acetic acid, followed by cooling in cold water. Two millilitre of 10 mol/L NaOH and 100 µl of the internal standard solution were then added to the hydrolysate. N-carbamyl-D, L-valine (32,16 and 8 mg/L) and N-carbamyl-D-L-norvaline (96 mg/L) (Sigma, St Louis, MO) in HPLC grade water were used as the standard and internal standards, respectively. Valine hydantoin and N-carbamyl-D, L-norvaline were extracted with 5 ml ethylacetate by shaking for 1 minute and were separated by centrifugation (5,000 g, 10 minutes). The resulting extracts were dried under vacuum and reconstituted with 0.5 ml of mobile phase, HPLC-grade water containing 60 ml of HPLC-grade acetonitrile and 1 ml of 17 mol/L acetic acid/L. The injected sample volume was 10 µl, the pump (Gillson HPLC 303 Pump, Gambetta, Villier-le-bel, France) speed was 1.5 ml/min, the detection wavelength was 210 nm, and the sensitivity was 0.05A° full scale. A reverse-phase ODS HPLC column (Novapak, 4.6 × 250 mm; Waters, Bedford, MA) was used. The CarHb concentrations were expressed as micrograms of valine hydantoin per gram of hemoglobin (µg VH/g Hb). In the present study, the intra-and interassay variation coefficients were approximately 5 per cent which were within the acceptable range.

Measurement of basic laboratory

Blood samples were analyzed by regular automated technique for complete blood cell count, blood urea nitrogen, serum creatinine and serum electrolytes.

Statistical analysis

All the results shown in tables and figures were expressed as means ± standard error ($\bar{X} \pm SE$). In comparison between the two groups, the unpaired sample *t*-test was used in the normal distribution data while the nonparametric statistic Mc Nema test was employed in the abnormal distribution data. $P < 0.05$ was considered to be statistically significant. Receiving operation curve (ROC) was established to define the cut off point for CarbHb levels, CarbHb/BUN, and CarbHb/Cr that could identify ARF from CRF patients.

RESULTS

Baseline clinical and laboratory characteristics of patients with ARF and CRF are shown in Table 1. There were no significant differences regarding sex, age, and BUN between the two groups. Patients with ARF had lower serum creatinine levels ($P<0.01$) but higher hemoglobin concentrations than those with CRF ($P<0.01$).

Table 2 illustrates the values of CarbHb levels, CarbHb/BUN ratios, and CarbHb/Cr ratios. It is obvious that patients with CRF had much higher levels of all these three parameters compared with patients with ARF.

To determine the cut off points, the percentage of ARF and CRF patients having different CarbHb levels, CarbHb/BUN ratios and CarbHb/Cr ratios were analysed. (Table 3-5). These values were employed in calculating and plotting the ROC curve of the above three determinants (Fig. 1-3). To assess the clinical usefulness of CarbHb levels, CarbHb/BUN, and Carb/Cr in identifying patients with ARF from CRF, the sensitivity and specificity at various cut off values were determined in both groups. When patients with ARF had blood CarbHb levels below 80 μgVH/gHb, the sensitivity was 89 per cent while the specificity was 82 per cent (Table 3 and Fig. 1). CarbHb/BUN ratios below 1.5 resulted in a sensi-

Table 1. Characteristics of patient groups.

	Patient groups		P-value
	ARF	CRF	
Number	35	39	
Male/Female	16/19	21/18	P > 0.01
Age	52.2 (21 - 78)	51.9 (22 - 80)	P > 0.01
BUN (mg/dl)	62.5 (40 - 100)	63.1 (40 - 100)	P > 0.01
Creatinine (mg/dl)	3.6 (1.2 - 8.9)	5.5 (2.33 - 16.1)	P < 0.01
Hemoglobin (g/dl)	11.5 (9.45 - 14.6)	9.4 (6.42 - 12.8)	P < 0.01

ARF = acute renal failure, CRF = chronic renal failure

Table 2. CarbHb levels, Carb in patients groups.

	Patient groups		P-value
	ARF	CRF	
CarbHb (μgVH/gHb)	54.8 ± 6 (18.11 - 114.87)	121.2 ± 8 (44.37 - 307.23)	P < 0.01
CarbHb/BUN ratios	0.92 ± 0.1 (0.22 - 1.85)	2.05 ± 0.2 (0.6 - 4.05)	P < 0.01
CarbHb/Cr ratios	18.90 ± 2 (4.71 - 63.82)	25.78 ± 2 (7.16 - 65.36)	P < 0.01

Table 3. Number and percentage of ARF and CRF patients at different CarbHb levels.

CarbHb (μgVH/gHb)	Patient groups				Total number	%
	ARF	%	CRF	%		
<60	21	60	3	8	24	32
<70	27	77	4	10	31	42
<80	31	89	7	18	38	51
<90	33	94	10	26	43	58
<100	34	97	13	33	47	64
<110	34	97	19	49	53	72
<120	35	100	23	59	58	78

Table 4. Number and percentage of ARF and CRF patients at different CarbHb/BUN ratios.

CarbHb/BUN ratios	Patient groups				Total number	%
	ARF	%	CRF	%		
<1.0	22	63	7	18	29	39
<1.5	31	89	11	28	42	57
<2.0	35	100	18	46	53	72
<2.5	35	100	29	74	64	86
<3.0	35	100	33	85	68	92
<3.5	35	100	38	97	73	99
<4.0	35	100	39	100	74	100

Table 5. Number and percentage of ARF and CRF patients with different CarbHb/Cr ratios.

CarbHb/Cr ratios	Patient groups				Total number	%
	ARF	%	CRF	%		
<10	7	20	2	5	9	12
<20	25	71	17	44	42	57
<30	31	89	27	69	58	78
<40	32	91	34	87	66	89
<50	33	94	36	92	69	93
<60	34	97	37	95	71	96
<70	35	100	39	100	74	100

tivity of 89 per cent, and specificity of 72 per cent, whereas, CarbHb/Cr ratios lower than 20 had a sensitivity of 71 per cent, and specificity of 56 per cent.

DISCUSSION

In general practice, routine biochemical measurements taken on admission are less helpful in identifying patients with ARF from CRF. The levels of blood urea nitrogen and serum creatinine could not discern the time course of renal failure. Although normochromic normocytic anemia, hypocalcemia, hyperphosphatemia, and metabolic acidosis are more common in CRF patients, these laboratory findings have also been reported in ARF patients. The reliability of ultrasonography is dependent on both the quality of the machine and the experience of the operator. Furthermore, in some CRF patients caused

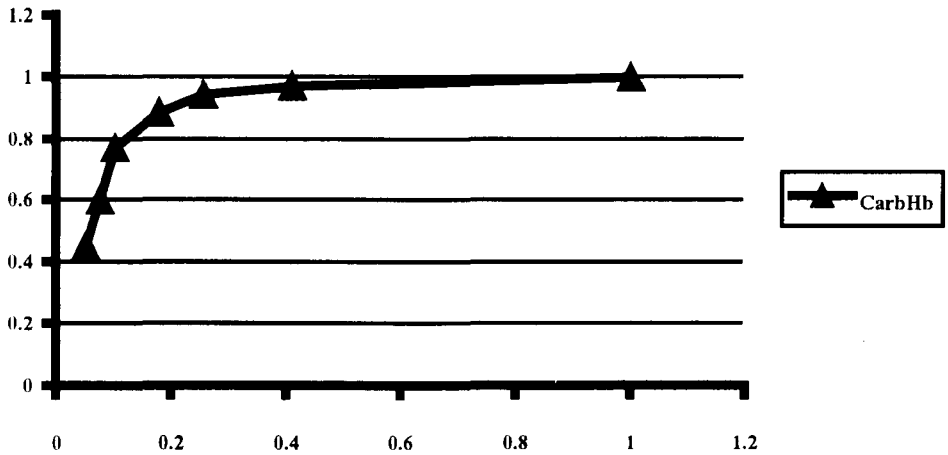

Fig. 1. ROC curve of CarbHb levels in identifying patients with ARF from CRF.

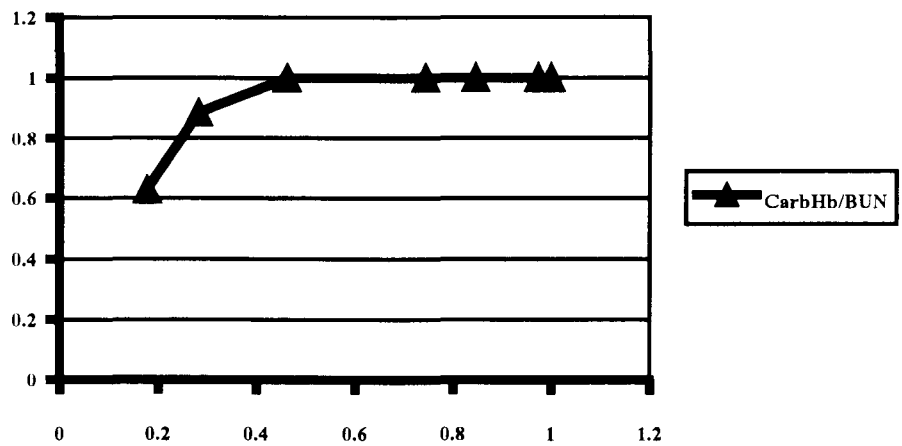

Fig. 2. ROC curve of CarbHb/BUN ratios in identifying patients with ARF from CRF.

by diabetes mellitus, amyloidosis, myeloma, or tumor infiltration, the renal sizes could be well maintained in normal range. Although renal biopsy would provide beneficial details in identifying ARF from CRF patients, the procedure is invasive and could cause serious bleeding complications.

Because of the limitations of the above investigations, it is necessary to develop a new method that could effectively differentiate ARF from CRF. Such a method should also be noninvasive in nature. Measurement of CarbHb, in the form of valine in uremic patients, thus, could achieve both stated objectives.

Indeed, CarbHb levels are affected by various factors. Considering that the life span of red blood cells (RBC) is approximately 120 days, CarbHb levels would be directly correlated with time averaged BUN accumulating during the recent few

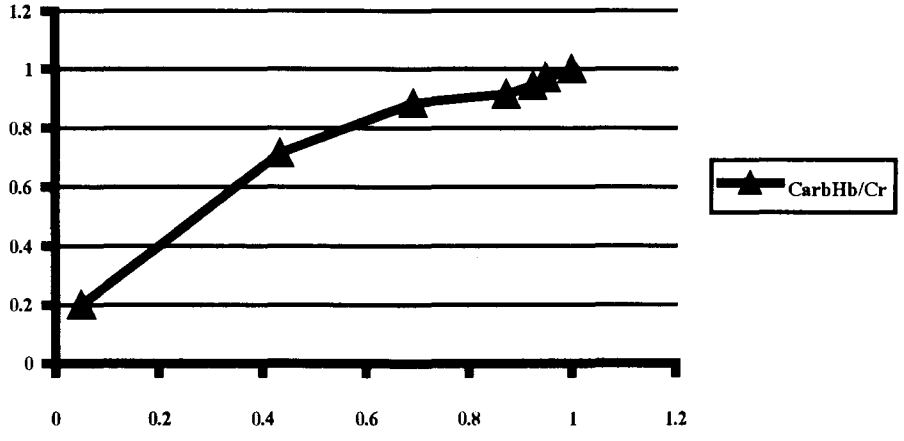

Fig. 3. ROC curve of CarbHb/Cr ratios in identifying patients with ARF from CRF.

months[13]. Hemolytic anemia including thalassemia, and autoimmune hemolytic anemia could decrease CarbHb levels. The shortened life span of RBC observed in the uremic state, however, does not affect CarbHb levels at any concentrations of BUN. This is the result of adequately persistent high levels of the time averaged BUN. Furthermore, the levels of CarbHb were expressed per gram of hemoglobin. Prior blood transfusion within 2-3 months could result in lowered CarbHb concentrations. Age, sex, blood glucose levels[5,6], and modalities of dialysis[14,15], however, do not alter the levels of CarbHb.

The results in the present study have shown that CarbHb levels in CRF patients are more than two times those of ARF patients (Table 2). CarbHb, thus, would be a beneficially non-invasive tool in identifying ARF from CRF. To obtain the best cut off point of CarbHb levels from the ROC curve, one would observe that CarbHb at the level of 115 μgVH/gHb could provide a sensitivity of 100 per cent but a specificity of only 41 per cent. In contradistinction, when the CarbHb concentrations are 80 μgVH/gHb, the sensitivity is 89 per cent while the specificity rises up to 82 per cent, both figures of which are satisfactory. (Table 3 and Fig. 1)

In two earlier studies, the reported cut off point values of CarbHb were 190 and 160 μgVH/gHb[1,4]. The disparity of the values obtained from various studies might be caused by many factors[1,2,4,7]. 1) The present study enrolled patients having BUN in the range of 40-100 mg/dl, whereas, in the previous studies, there was no limitation of BUN levels[1,2,4,7]. 2) Percentage of coefficient variation in the current study was approximately 5 per cent which is in acceptable range while there were no available data of such determinants in the former reports[1,2,4,7]. 3) There might be differences in the accuracy of extraction technique and quality of HPLC instruments among various studies[1,2,4,7].

CarbHb/BUN and CarbHb/Cr ratios could also be accurately alternative parameters. From Table 4 and Fig. 2, CarbHb/BUN at the value of 2.0 has a sensitivity of 100 per cent but the specificity of only 54 per cent. The most appropriate cut off point of CarbHb/BUN ratio is 1.5 which could yield a sensitivity of 89 per cent and specificity of 72 per cent. On the other hand, CarbHb/Cr ratio of 20 is the best statistical cut off point with sensitivity and specificity of 71 per cent and 56 per cent, respectively. It is obvious that CarbHb/BUN is a more reliable parameter than CarbHb/Cr ratio. This stems from the fact that the average values of BUN are not different between ARF and CRF patients, whereas, the average levels of Cr are higher in the CRF group. (Table 1)

From the data in Table 1, it can be seen that ARF patients had significantly lower average serum creatinine levels but higher average hemoglobin concentrations than those with CRF. It seems likely that the average values of both parameters could have differentiation ability between ARF and CRF states. Unfortunately, when one considers in detail of each individual data, there is overlapping of the values from both patient groups.

There are still some limitations in the measurement of CarbHb levels. To circumvent the effect of hemolysis, the collected blood samples should be analyzed within 5-7 days. The processes of measurement are complicated, labor-intensive, time-consuming, and expensive. Furthermore, only a limited number of medical centers have HPLC instruments. In the future, more simple methods for determining CarbHb could be developed, leading to the nationwide use of CarbHb in identifying ARF from CRF.

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บทบาทของคาร์บามัยเลทเด็ด ฮีโมโกลบิน ในการแยกภาวะไตวายเฉียบพลัน และไตวายเรื้อรัง

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ได้ทำการศึกษาวัดระดับ คาร์บามัยเลทเด็ด ฮีโมโกลบินในเลือด ซึ่งถูกสะกัดเป็นสารวาลีน ไฮแดนโตอิน โดยวิธี ไฮเพอร์ฟอร์แมนซ์ลิควิดโครมาโตกราฟฟี่ พบว่าระดับวาลีน ไฮแดนโตอิน ในผู้ป่วยไตวายเรื้อรัง (39 ราย) มีค่าสูงเป็นประมาณ 2.5 เท่าของผู้ป่วยไตวายเฉียบพลัน (35 ราย) ค่าที่เป็นจุดตัดในการแยกภาวะไตวายเรื้อรัง จากไตวายเฉียบพลัน ได้แก่ 1) ระดับวาลีน ไฮแดนโตอิน 80 ไมโครกรัม/กรัม ฮีโมโกลบิน 2) อัตราส่วนระหว่างวาลีน ไฮแดนโตอิน และระดับยูเรียไนโตรเจน ในเลือดสูงกว่า 1.5 และ 3) อัตราส่วนระหว่างวาลีน ไฮแดนโตอิน และระดับครีเอตินินในเลือดสูงกว่า 20

คำสำคัญ : คาร์บามัยเลทเด็ด ฮีโมโกลบิน, วาลีน ไฮแดนโตอิน, ไตวายเฉียบพลัน, ไตวายเรื้อรัง

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