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MEASUREMENT OF HbA_{1c} IN PATIENTS WITH CHRONIC RENAL FAILURE

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Abstract

Background—Carbamylated hemoglobin (carbHb) is reported to interfere with measurement and interpretation of HbA_{1c} in diabetic patients with chronic renal failure (CRF). There is also concern that HbA1c may give low results in these patients due to shortened erythrocyte survival.

Methods—We evaluated the effect of carbHb on HbA_{1c} measurements and compared HbA_{1c} with glycated albumin (GA) in patients with and without renal disease to test if CRF causes clinically significant bias in HbA_{1c} results using 11 assay methods. Subjects included those with and without renal failure and diabetes. Each subject's estimated glomerular filtration rate (eGFR) was used to determine the presence and degree of renal disease. A multiple regression model was used to determine if the relationship between HbA_{1c} results obtained from each test method and the comparative method were significantly ($p<0.05$) affected by eGFR. These methods were further evaluated for clinical significance using difference between the eGRF quartiles of >7% at 6 or 9% HbA_{1c}. The relationship between HbA_{1c} and glycated albumin (GA) in patients with and without renal failure was also compared.

Results—Some methods showed small but statistically significant effects of eGFR; none of these differences were clinically significant. If GA is assumed to better reflect glycemic control, then HbA_{1c} was approximately 1.5% HbA_{1c} lower in patients with renal failure.

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Conclusions—Although most methods can measure HbA_{1c} accurately in patients with renal failure, healthcare providers must interpret these test results cautiously in these patients due the propensity for shortened erythrocyte survival in renal failure.

Keywords

carbamylated hemoglobin; HbA_{1c}; chronic renal failure; interference; glycated albumin

1. Introduction

Renal failure is common in patients with diabetes, and HbA_{1c} is widely used as an index of mean blood glucose in these patients. Many factors can affect interpretation of HbA_{1c} measurements in patients with chronic renal failure (CRF). Several reports have suggested that erythrocyte survival is substantially lowered in most patients with CRF; this would be expected to lower HbA_{1c} results [1–3]. Although shortened erythrocyte lifespan would presumably not interfere with the measurement of HbA_{1c}, it could adversely affect the interpretation of HbA_{1c} results.

Carbamylated Hb (carbHb) is formed by non-enzymatic condensation of cyanate with the N-terminal valine of hemoglobin. In chronic renal failure carbHb is increased due to elevated urea, which is dissociated in vivo to yield cyanate ions [4]. A number of old reports have suggested that HbA_{1c} methods, especially those based on charge separation (e.g. ion-exchange HPLC) may have interference from carbHb that would be expected to falsely increase HbA_{1c} results [5–7], but many of these methods are no longer in use. Subsequent reports evaluated newer ion-exchange HPLC assay methods which showed improved separation of the HbA_{1c} fraction from other hemoglobin adducts [8,9] and therefore did not show interference from carbHb.

The present study is twofold; we first evaluated several current HbA_{1c} methods for interference from carbHb in patients with and without renal failure. Although carbHb was not measured directly in the present study, there is a large amount of data showing that this hemoglobin modification is significantly increased in patients with renal failure and the carbamylated fraction (HbA_{1d3}) as well as other measures of carbHb (measurement of valine hydantoin by HPLC) are correlated with plasma creatinine, serum urea and time-averaged urea concentrations [10–12]. Studies have also shown that the amount of carbHb depends upon both the duration and severity of real failure [13–15]. We therefore used eGFR as an indicator of overall renal function in place of direct measurement of carb Hb. We used a boronate affinity chromatography HPLC method as our reference method since this method has been shown to have no interference from carbHb [6,7,16,17].

In addition to the possible method-specific interference of carbHb, CRF, especially end stage renal disease, may also cause changes in erythrocyte lifespan which might alter the interpretation of HbA1c results. Several studies propose the use of glycated albumin (GA) measurement in place of HbA1c as a more accurate assessment of glycemic control in patients with renal disease. One study showed that GA was a better predictor of risk of death and hospitalization in these patients, compared to HbA1c [18]. Serum GA levels were also shown to be better correlated with average glucose (based on 4-point profiles, 3 days per

week for 4 weeks) than HbA1c [19]. In this present study, we investigated the relationship between HbA_{1c} and glycated albumin (GA) in patients with and without renal failure using the same patient samples as for the method-specific carbHb interference study.

2. Methods

We evaluated eight ion-exchange HPLC methods: G7 and G8 (Tosoh Bioscience), Variant II NU, Variant II Turbo, Variant II Turbo 2.0, D-10 and D-10 Dual (Bio-Rad Laboratories), HA-8160 (A. Menarini Diagnostics), two immunoassay methods: Tina-quant HbA1c gen.2 on Integra 800 (Roche Diagnostics) and DCA 2000 (Siemens Healthcare Diagnostics), and one enzymatic method: Direct Enzymatic HbA_{1c} (Diazyme Laboratories) on the Hitachi 917 (Roche Diagnostics). Presumably, hemoglobin species modified by reactants other than glucose and not displaying a cis-1,1-diol group should not interfere with measurement of HbA_{1c} by boronate affinity methods. Published data support this lack of interference of carbHb with boronate affinity methods [6,16,17]. Therefore, we used the boronate affinity ultra^2 HPLC (Trinity Biotech) as our comparative method.

This study was approved by the ethics review committee at $DynaLIFE_{DX}$ in Edmonton, Canada where the samples originated. Whole blood samples (n=120) from subjects normal renal function and subjects in various stages of renal failure were residual samples from routine testing that had been collected in EDTA-containing tubes. The samples were shipped on cold packs to the Diabetes Diagnostic Laboratory at the University of Missouri (Columbia, MO). Several small whole blood aliquots were made from each sample and stored at −70°C until they were shipped on dry ice to various sites for analysis. One aliquot was centrifuged and the plasma was separated and stored at −70°C until analysis of GA. Each patient's eGFR, calculated using the MDRD equation, was used to estimate the degree of renal disease and the level of carbHb. A multiple regression model was used to determine if the relationship between HbA_{1c} results obtained from each test method and the ultra^2 method were significantly ($p<0.05$) affected by eGFR. For those methods' results that were significantly affected by eGFR, results were evaluated for clinical significance by dividing the samples into quartiles based on eGFR results (eGFR ≤11, 11< eGFR ≤ 45, 45< eGFR ≤ 84, eGFR >84). Deming regression was then used to compare the relationships between each method and the ultra^2 for the highest and lowest quartiles; a difference between the quartiles of >7% at 6 or 9% HbA_{1c} was defined as being clinically significant [20]. The relationship between HbA_{1c} (ultra^2 HPLC) and GA was evaluated comparing patients with normal eGFR (eGFR>90 ml/min, no renal disease, n=18) and those with renal failure (eGFR<60 ml/min, n=73). Data analyses were performed using SAS and Excel.

3. Results and Discussion

The D-10, D-10 Dual, DCA 2000, G7 and Direct Enzymatic methods showed very small but statistically significant effects of eGFR; clinical significance was therefore evaluated. The differences in HbA1c from the reference method between the lowest and highest eGFR quartiles is shown in figure 1 as a box plot of the HbA1c simple linear regression residuals for each method. In this way, any inherent calibration bias is removed and only the

difference between the highest and lowest quartiles can be seen. Importantly, none of the methods evaluated showed any clinically significant effects of eGFR.

The relationship between HbA_{1c} and GA is shown in figure 2. The difference in the relationship between the normal and renal failure groups was both statistically (Linear regression, $p<0.0001$) and clinically significant. If we assume that GA is providing an accurate measure of glycemic control in patients with renal failure, HbA_{1c} results are lowered by approximately 1.5% HbA_{1c} in patients with renal failure at critical treatment levels. These results are consistent with the findings of others that have found lower HbA_{1c} results in renal failure when compared to measures of glycated plasma protein or plasma albumin [2,3]. The studies showing that GA is superior to HbA1c use in CRF are somewhat convincing but far from definitive [21]. There are studies showing a linear increase in all-cause mortality with increasing HbA1c levels [22,23] and there is no evidence as yet that physicians can achieve better glycemic control using GA instead of HbA1c in these patients. In addition, as with HbA1c, there may be factors (e.g. proteinuria, altered albumin homeostasis) that interfere with measurement or interpretation of GA. There is ongoing debate about which assay is most useful for monitoring glycemic control in this vulnerable population [24,25].

4. Conclusions

We conclude that most current HbA_{1c} methods can provide valid analytical results for patients with CRF. However, healthcare providers need to be aware of potential interferences when interpreting HbA_{1c} results in clinical settings due to alteration in erythrocyte lifespan in many patients with chronic renal failure which can cause falsely lowered HbA_{1c} results.

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Abbreviations

eGFR	estimated glomerular filtration rate
carbHb	carbamylated hemoglobin

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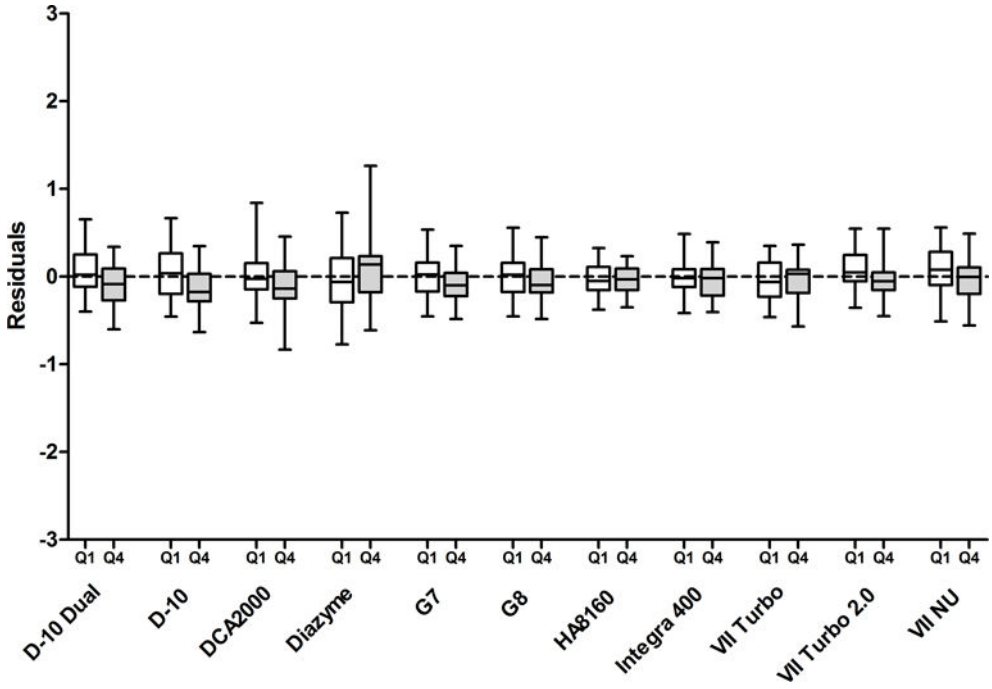

Figure 1.

Box plots of the residuals of the regression of HbA1c compared to the comparative method for the lowest and highest (shaded) eGFR quartiles. The horizontal line within each box is the median of the residuals. The upper and lower limits of each box correspond to the 25th and 75th percentile of the residuals. The highest and lowest whiskers represent the minimal and maximal residuals. Q1, lowest eGFR quartile; Q4, highest eGFR quartile.

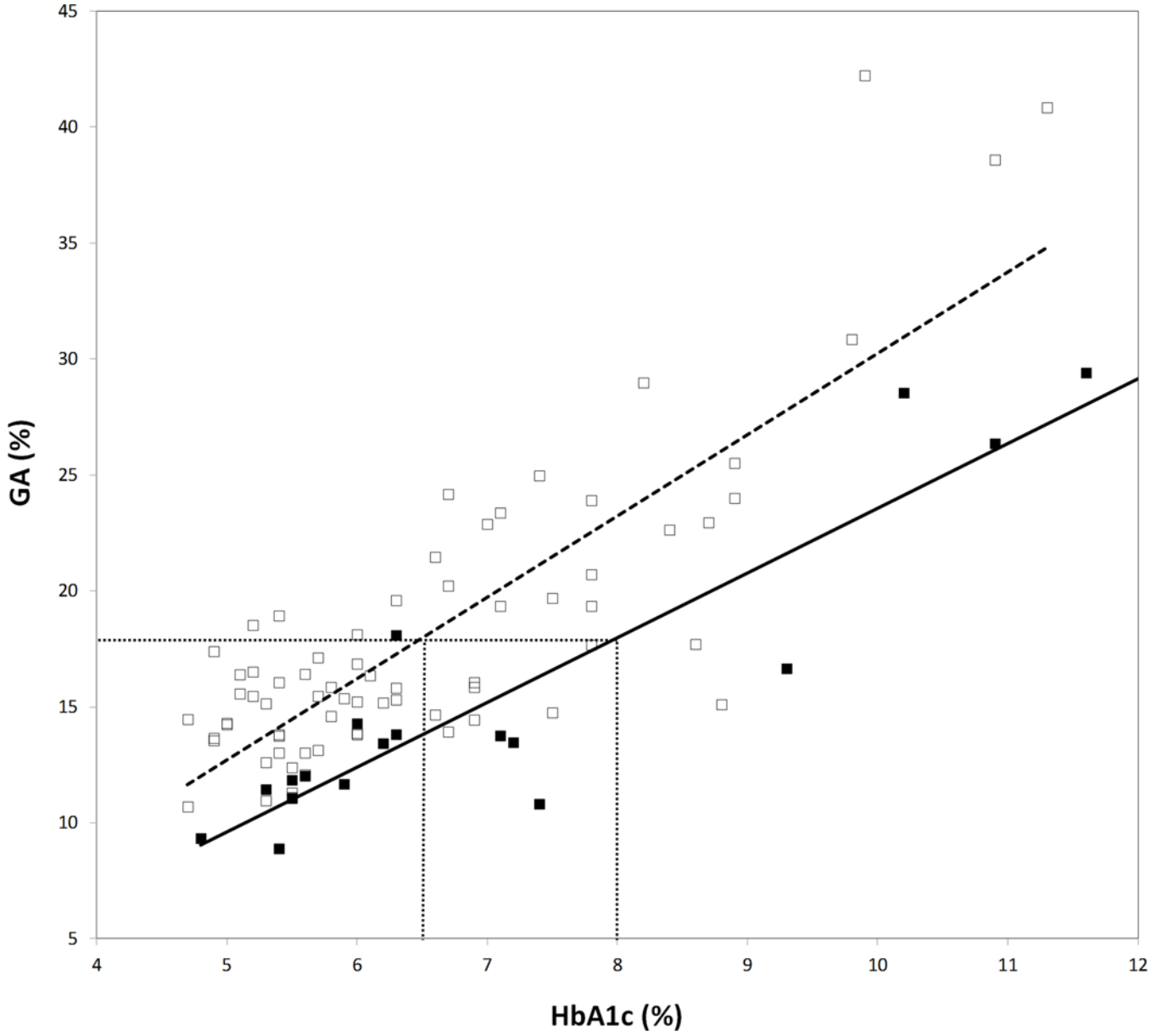

Figure 2.

Relationship between GA and HbA_{1c} in patients with chronic renal failure (□, eGFR <60; —, y=3.51x − 4.88; r^2=0.68) and without renal disease (■, eGFR>90; y=2.79x − 4.34; r^2=0.83). Dotted horizontal and vertical lines indicate the difference in HbA_{1c} between the two groups at a fixed GA.