Asian Journal of Medicine and Biomedicine
eISNN: 2600-8173
https://journal.unisza.edu.my/ajmb

PENERBIT Universiti Sultan Zainal Abidin

Asian Journal of Medicine and Biomedicine, Vol 5:1

Open Access

Comparison of Hba1c Level Measured by HPLC and Capillary Electrophoresis among Patient with High Urea

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Abstract

Utilization of glycated haemoglobin (HbA1c) in diagnosis and monitoring of diabetes mellitus is accepted and validated worldwide. Standardisation between various methods available is no longer an issue. However, knowledge of HbA1c interference by various haemoglobin (Hb) fractions presence in the patient's sample must be taken into account during HbA1c analysis and interpretation. Carbamylated Hb (cHb) is one of Hb fractions, formed when Hb condensed at the N-terminal valine by cyanate derived from spontaneous decomposition of urea which usually raised in patients with renal impairment. This study aimed to compare the level of HbA1c in patient with high urea measured using High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE). After analysis using the laboratory's routine method, or HPLC, the patient's samples with concurrent urea level of >25 mmol/L were re-analyzed within 2 hours using the comparative method or CE. A cut off cHb of 2% on HPLC considered as no interference. The mean level of urea was 31.37±5.09 mmol/L (range 25.2-43.1mmol/L). Out of 68 samples, only 24 cHb were detected by HPLC but only less than 2% and none cHb detected on CE. Correlation between HPLC and CE showed no significant different in HbA1c measurement ($p>0.05$). Therefore, we propose that both HPLC and CE can be used to determine HbA1c level in patient with high urea.

Keywords:
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Received (June 16th, 2020), Accepted (Nov 10th, 2020) & Published (April 30th, 2021)

Cite as: Wan Nor Fazila, W. N., Iwani, A. R., Noor Azlin, C. S., Noorazliyana, S., Julia, O., & Tuan Salwani, T. I. (2021). Comparison of Hba1c Level Measured by HPLC and Capillary Electrophoresis among Patient with High Urea, 5(1), 56-60. *Asian Journal of Medicine and Biomedicine*, *5*(1), 1-9. doi:https://doi.org/10.37231/ajmb.2021.5.1.423

DOI: https://doi.org/10.37231/ajmb.2021.5.1.423

Introduction

HbA1c has been endorsed as a standard of care for diagnosis and monitoring diabetes mellitus (DM), specifically the Type 2 DM (T2DM) by the World Health Organization (WHO), the International Diabetes Federation (IDF) and the American Diabetes Association (ADA).[1, 2] The HbA1c is the product of non-enzymatic reaction of Hb with glucose and its formation is a part of normal physiological state when conditions are favourable. In chronic hyperglycaemic condition such as DM, there is an increased in the average plasma glucose level. The persistent elevated glucose load favor the glycation of glucose molecules to Hb, thus increase the amount of HbA1c in the plasma.[3] Since the average life span of the red blood cell (RBC) is 120 days, HbA1c is viewed as the average blood glucose over the past 120 days. The unique feature of this biomarker is utilized for estimating chronic glycaemia, rather than momentary blood glucose levels.4 Diagnosis of DM is made with HbA1c value of ≥ 6.3% (45 mmol/mol) while cut off level of ≤ 6.5% (48 mmol/mol) is used as a target control in disease monitoring which signals a low risk of developing chronic progressive complications.[5]

An accurate measurement of HbA1c plays a vital role in ensuring optimum and effective monitoring of glycaemic control among diabetic kidney disease (DKD) patients. However, there are many factors influencing the accuracy of HbA1c analytical measurement and they are highly method dependent. One of the factor is the presence of cHb.[6] CHb is formed when Hb condensed at the N-terminal valine by cyanate derived from spontaneous decomposition of urea which is usually found in high level in renal failure patients.[7] Many studies have shown the significant increase of cHb in renal failure patients as compared to normal subjects.[7-9] According to a study, each mmol/L of urea is associated with 0.063% increase in carbamylated hemoglobin.[10]

CHb may interfere with HbA1c measurement in two ways. Firstly, RBC lifespan in hyperuremic states such as in CKD patients is shortened as compared to healthy people.[11] In addition, cHb also may hamper the formation of HbA1c as both carbamylation and glycation competing for the same NH2 sites on Hb molecules.[12] For these reasons, there will be an underproduction of HbA1c that lead to falsely low result making HbA1c insufficient for the assessment of glycaemic balance in patients with uremia. From the analytical point of view, cHb may interfere with HbA1c measurement by co-eluting with HbA1c molecule due to the similarity of their isoelectric points.[13] Thus, interference by cHb is marked with charge-based methods of measuring glycated Hb such as in assay using chromatographic principles such as HPLC as shown in Figure 1.[12,14-16] CE has been shown by a few studies to have better resolution, thus less interfered with cHb.[17] Figure 2 shows peak cHb and HbA1c are well separated on CE. However, emerging studies have shown that there were no differences in HbA1c value between HPLC and other methods such as electrophoresis.[11, 18]

Renal failure is a common consequence in patients with DM. While HbA1c is used widely as an index of mean blood glucose in these patients, there are conflicting results on the effect of cHb to HbA1c measurement. Acknowledging that interference from cHb is method dependant, our study aims to evaluate the outcome in the measurement and interpretation of HbA1c result among diabetic CKD patients with high urea using HPLC and CE.

Methods

Blood Samples

A cross-sectional study was conducted between January until June 2019 at Hospital Universiti Sains Malaysia (USM), Kelantan, Malaysia. The HbA1c samples from DM patients who have blood urea of >25 mmol/L sent to the Endocrine Laboratory, Chemical Pathology Department Hospital USM were traced using the Laboratory Information System (LIS) and recorded. The leftover samples sent for initial HbA1c testing using HPLC were used in this study. The samples were re-analysed for HbA1c on both HPLC and CE within 2 hours apart.

Biochemical Measurement

The blood urea was quantified based on kinetic test using urease and glutamate dehydrogenase on the Architect c8000 biochemistry analyzer. The rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the samples. HbA1c measurements were done on a fully automated system based on the cation-exchange HPLC principle using Bio-Rad D-10 Hemoglobin A1c Program. The analyser automatically mixed, diluted, and injected the samples into the column. Hb fractions were differentiated based on ionic strength with the stationary phase. The mobile phase contains buffers that delivered a gradient of increasing ionic strength to the cartridge. The analyser's software transformed the raw data to a chromatogram which displayed the peaks in the following order according to the chromatographic mobility of the Hb fractions: HbA1a, HbA1b, HbF, LA1c/CHb-1, HbA1c, HbP3, HbA0, and HbA2. HbA1c was expressed as a percentage based on the ratio of HbA1c peak area to the total Hb peak area.

Within 2 hours post-analysis of the samples using HPLC the samples were further analysed on Capillary Electrophoresis method using Sebia Capillarys 2 Flex piercing analyser. Various Hb fractions were separated based on their electrophoretic mobility and electroosmotic flow in an alkaline buffer with a specific pH. This method

has been certified by National Glycohemoglobin Standardization Program (NGSP).

Statistical Analysis

Data entry and analysis were done using Statistical Package for Social Science (SPSS) version 24. Descriptive statistics were used to summarise the socio-demographic characteristics of subjects. Numerical data were expressed as mean ± standard deviation (SD) for normally distributed data or as median ± interquartile range (IQR) for skewed data. Bivariate correlation analyses were conducted using either Pearson correlation (if bivariate normal distribution assumption is not met). Categorical data were expressed as frequency and percentage, while Pearson's Chi-square test was performed to test for correlation. A probability value of $P < 0.05$ was taken as statistically significant.

Results

There was a total of 68 samples with urea level >25mmol/L. Approximately 31 samples were from male patients and 37 samples were from female patients. The mean age of the patients whose blood samples were collected was 57±13 years old. The mean level of urea was 31.37±5.09 mmol/L (range 25.2-43.1mmol/L). The mean of creatinine was 902.79±425.62 µmol/L (range 183-2363umol/L). Out of 68 samples, only 24 cHb were detected by HPLC but less than 2% and none of cHb was detected on CE. Cut off cHb of 2% on HPLC was considered as no interference. Correlation between HPLC and CE showed no significant different in HbA1c measurement ($p>0.05$). The mean values of HbA1c based on HPLC and CE were 6.51 ± 1.19% and 6.48 ± 1.26% respectively.
Conclusion

Discussion

Diabetes Mellitus (DM) is a metabolic disorder caused by insulin insufficiency or insensitivity leads to chronic hyperglycaemia. It can result in various types of vascular complications such as ischaemic heart disease, cerebrovascular accident, nephropathy, retinopathy and peripheral vascular disease.[5] Diabetic kidney disease (DKD) is the most common complications seen in both types of diabetes and is also a recognizable cause of chronic kidney disease (CKD) in both developed and developing countries which contributes to about 40% of the overall CKD cases.[19,20] Although most of the diabetic patients die from cardiovascular diseases and infection, end stage renal failure (ESRF) which is the substantial consequences of DKD is the most devastating and costly complication of DM. It is affecting diabetic patients with much younger age who presented with diverse clinical features and give a major impact on dialysis and transplant needs. Despite modern therapeutics strategies and monitoring, there is great residual risk of DKD onset and progression which exert a considerable challenge to the treating physician in the management of DM in CKD.[20, 21] DKD or diabetic nephropathy is defined as a condition with persistent macro-albuminuria of >300 mg/24 h (or >200 µg/min), or an albumin-to-creatinine ratio (ACR) of >300 mg/g, confirmed in at least 2 of 3 samples and impaired renal function as represented by a high level of serum urea and serum creatinine, in patients with underlying DM.[22,23] Patients with DKD have a higher risk of progression to ESRF as well as a significant increase in cardiovascular (CV) morbidity and mortality.[24] Therefore, it is essential to ensure that these patients are effectively monitored and managed to prevent the DKD complications. It can be achieved by having a regular screening of renal function, the use of an appropriate oral hypoglycemic agent, excellent blood pressure control and more importantly is the optimum glycaemic control.[25] Blood glucose or glycaemic control is a well-known major determinant that influences the onset and progression of nephropathy. It is proposed that a reduction of HbA1c to 7% (53 mmol/mol) will slow down the development of microvascular complications includes DKD in both T1DM and T2DM patients.[26, 27]
Measurement and interpretation of HbA1c in diabetic patients with renal failure is troublesome due to the presence of cHb. Previous studies have shown that cHb interference is highly assay dependent, being the HPLC is the most affected method. In our study, we evaluated 2 current HbA1c methods namely HPLC and CE for interference from cHb in patients with serum urea > 25mmol/L. The formation and amount of cHb are depending on both duration and severity of renal failure which represented by serum urea, plasma creatinine, and time-averaged urea concentrations. The effect of cHb on HbA1c measurement using HPLC assay is also level dependent. A higher level of cHb is seen in those patients with more severe renal impairment.[9, 28, 29] Early studies has described urea level of at least 30 mmol/L or more promotes the formation of cHb, and the level of cHb ≥3.5% is reported to chromatographically interfere with HbA1c measurement.[30, 31] Despite of these drawbacks, a similar HbA1c targets control about 6-7% is still considered as reliable to be used in the estimation of glycaemic control in both patients with and without kidney disease. Meanwhile, HbA1c of >7.5% may overestimate the extent of hyperglycemia in these patients.[32]

In our study, we found that there is no statistically significant difference in HbA1c value measured by HPLC and CE at the urea ranges between 25.2-43.1 mmol/L with cHb of less than 2%. This data was in good agreement with previous studies,[11,18] and supports a study that showed the interference from cHb is only significant when cHb is more than 2%.[14] Although both methods provide valid analytical results for DKD patient up to a certain threshold

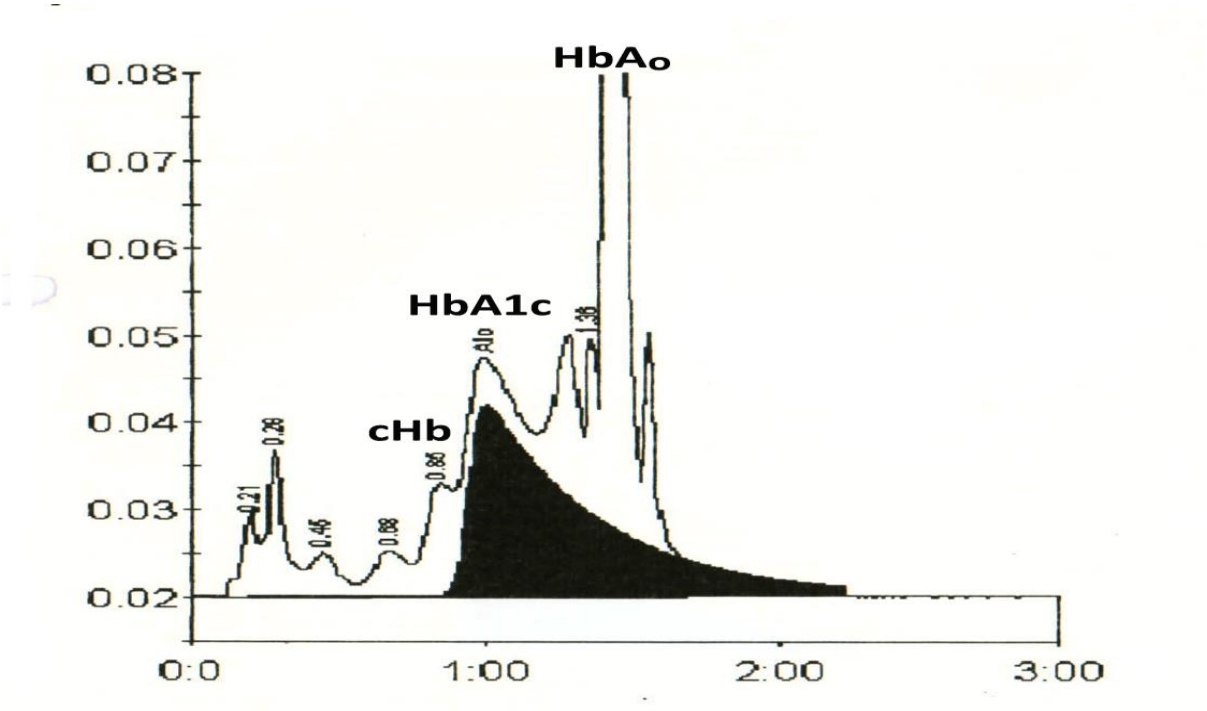

Figure 1: Carbamylated haemoglobin (cHb) and HbA1c peak on HPLC with retention time of 0.85s and 0.88s respectively.

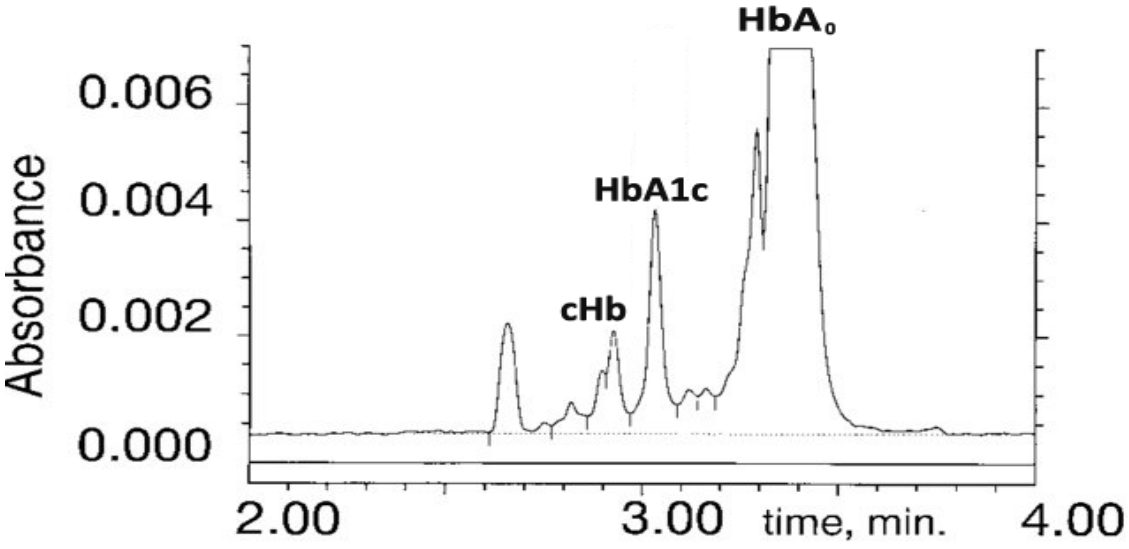

Figure 2: Carbamylated haemoglobin (cHb) and HbA1c peak is better separated on CE compared to HPLC.

of cHb, treating physician should be aware of the other potential interference factors such as anemia, shortens RBC survival and presence of other unidentified Hb adducts such as Hb-advanced glycation end products (Hb-AGE) which can lead to deviation from the true HbA1c result.[33] A careful and vigilance interpretation of HbA1c result is, therefore, must be employed in this vulnerable group of patients. In this study, we did not include the duration of the CKD. This is one of the essential points that deserved to be highlighted because, cHb level not only depending on the mean urea concentration, but the extend of Hb exposure to urea also exerts a significant effect on cHb formation.[28, 34, 35]

Conclusion

In conclusion, this study proposed that urea range between 25.2-43.1mmol/L does not increase the CHb level, thus does not causing significant interference when measured by HPLC. The results obtained from both methods are comparable. The HPLC and CE can be used in patient with high urea. However, duration and severity of uremia and other factors that lead to formation of CHb need to be considered in future study.

Acknowledgment

We would like to thanks to Endocrine laboratory staff, Chemical Pathology Department, Universiti Sains Malaysia and UTAS MAJU SDN BHD for technical and financial support.

Conflict of interest

The authors declare no conflict of interest related to this study.

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