INTERFERENCES OF HbA1c ANALYSIS IN HOSPITAL UNIVERSITI SAINS MALAYSIA – 3 YEARS STUDY

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ABSTRACT

Haemoglobin A1c (HbA1c) is used to monitor glycaemic control and predict diabetic complications. Measurement of HbA1c can be interfered by haemoglobin (Hb) variant and other Hb derivatives include carbamylated Hb and elevated labile A1c. This study is to determine the percentages and type of interferences during HbA1c analysis and the percentages of non-reportable HbA1c results. This is a cross-sectional study using retrospective data of HbA1c. The HbA1c is measured on Biorad D10 using the ion-exchange high-performance liquid chromatography method. The data were analyzed using descriptive statistics. A total of 26,560 patients were included. The result showed the presence of interferences of 2269 (8.56%). The most common causes of the interferences were the Hb variant (8.48%) followed by carbamylated Hb and labile A1c (0.03% each). The non-reportable HbA1c results were 0.46% with the Hb variant contributed most of the causes. By knowing the presence of interferences particularly the Hb variant, the HbA1c results hopefully are interpreted with caution and correct management can be given to the patients.

Key words: Carbamylated haemoglobin, haemoglobin variants, HbA1c, HPLC, interferences, labile A1c

INTRODUCTION

Haemoglobin A1c (HbA1c) is glycated haemoglobin with glucose attached to the N-terminal valine of the haemoglobin (Hb) β-chain (Burtis et al., 2012). It reflects the plasma glucose concentration of the normal erythrocytes life span of 120 days (Lippi & Targher, 2010). It is used to diagnose diabetes mellitus (DM), monitor glycaemic control, and predict diabetic complications. HbA1c can be quantified by various methods such as ion-exchange chromatography, affinity chromatography, enzymatic, and immunoassays.

Various factors may affect the accuracy of HbA1c results. These factors may interfere with HbA1c measurement during the analytical phase or the interpretation of the HbA1c result. Analytical factors that need to be taken into consideration include the presence of Hb variants such as HbS or HbC trait, high fetal haemoglobin (HbF), chemically modified derivatives of Hb for example carbamylated Hb (carbHb) and elevated labile A1c (LA1c). These factors are highly method dependent and assay selection should be based on characteristics of samples (Nitin, 2010; Hare et al., 2012). In addition, factors affecting the glycation process also may impart a difficulty in HbA1c interpretation. Any condition that shortens or prolonged erythrocytes life span such as iron deficiency anaemia, haemolytic anaemia, or acute blood loss will cause falsely low or high HbA1c levels (Gillery et al., 2000; Sacks et al., 2011).

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Hb variants result from amino acid changes that arise from mutations and/or deletions in the genes that encode α and β chains. Hundreds of Hb variants have been identified, the most common are HbS, HbE, HbC, and HbD (Rhea & Molinaro, 2014). Individuals with homozygous Hb variants usually have reduced erythrocytes lifespan. Therefore, HbA1c is not recommended as a measure of glycaemic control in these patients. However, those with heterozygous diseases, such as HbS, HbC, HbE traits, and silent Hb variants, can potentially affect the accuracy of HbA1c measurement, in which the effect is method-dependent (Sacks et al., 2001). HbF is predominantly high at birth (between 60 & 95%) and reduces near to adult levels during the first year of life. High HbF levels can be found in hereditary persistence of HbF or certain conditions such as leukaemia, thalassemia, and anaemia (Little & Roberts, 2009). CarbHb is found in a patient with renal failure and uraemia. CarbHb is formed by non-enzymatic condensation of cyanate with the N-terminal valine of Hb after dissociation of urea in vivo (Little et al., 2013). LA1c or pre-HbA1c is an unstable Schiff base formed during non-enzymatic glycation of Hb. Although ion-exchange high-performance liquid chromatography (HPLC) can separate the LA1c fraction, an abnormally high level of LA1c may produce error in the HbA1c measurement (Desmons et al., 2017).

Currently, ion-exchange HPLC is the most widely used method for HbA1c measurement. It separates Hb species based on the charges of the molecule (Rhea & Molinaro, 2014). Ion-exchange HPLC is vulnerable to the effect of Hb variant (Chu et al., 2007; Chen et al., 2013). Inaccurate HbA1c results occurred if Hb variant or a derivative could not be separated from HbA or A1c peak (Gallagher et al., 2009). Falsely high or low HbA1c value caused by the presence of a silent Hb variant may lead to over or under-treatment of diabetic patients (Nasir et al., 2010). Although haemoglobinopathy can be detected through inspection of the chromatogram, the interferences of Hb variant and carbHb are variable in ion-exchange assays (Gallagher et al., 2009).

The identification of interferences, especially Hb variants is important for the accuracy of measurement. Therefore, this study is to determine the total and type of interferences during HbA1c analysis.

**MATERIALS AND METHODS**

This is a cross-sectional study using retrospective data of patients’ undergone HbA1c measurement in Endocrinology Laboratory, Hospital USM from January 2017 to December 2019. The HbA1c results and the demographic data were retrieved from the Laboratory Information System (LIS). Only one HbA1c result of the same patients was collected for the analysis. Repeated HbA1c results were excluded from this study. The results were analyzed for reportable HbA1c, presence of interferences, and non-reportable HbA1c. HbA1c is measured on Biorad D10 using the ion-exchange HPLC method. The variant is identified through inspection of the chromatogram during reporting the HbA1c by the presence of abnormal or unknown peaks before or after the A0 peak and the variant window peak after A0 (S-window or C-window). The HbA1c result will not be reportable if there is no HbA1c peak detected, combine areas of variant, S and C windows ≥60% (suspected having homozygous variant), HbF ≥10%, labile HbA1c ≥4%, or the HbA1c value is outside the linearity range (3.8% to 18.5%). Hb analysis was not done for the suspected cases of the Hb variant.

Data analysis was performed using IBM SPSS Software version 26.0. Categorical variables were reported as frequency and percentage while numerical variables were described as mean and standard deviation (SD). The data are normally distributed (checked using a test of normality (Kolmogorov-Smirnov) and histogram with an overlaid normal curve). The percentage of the Hb variant was determined by adding the percentage of Hb variant with the percentage of HbF divided by the total number of HbA1c requests.

\[
\text{Percentage} \ (%) = \frac{\% \text{ of Hb variant} + \% \text{ of HbF}}{\text{Total number of HbA1c}} \quad \text{Equation 1}
\]

**RESULTS**

Total HbA1c requested from January 2017 to December 2019 was 57,623. A total of 26,560 of the results were included after excluding repeated results from the same patients. The demographic characteristics were shown in Table 1. Males’ participants (50.7%) were more than females (49.3%). The majority of the patients were Malay (94.6%). A total of 2269 (8.56%) of the HbA1c results were

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
<th>n (%)</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.2 (13.8)</td>
<td>26560</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13,453 (50.7)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13,107 (49.3)</td>
<td></td>
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<tr>
<td>Race</td>
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</tr>
<tr>
<td>Malay</td>
<td>25,135 (94.6)</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>1,274 (4.80)</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>145 (0.55)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>6 (0.02)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Baseline characteristic of patients (n=26560)
associated with interferences of which 2251 (8.48%) were due to the Hb variant. Others were due to the presence of carbHb and LA1c (0.03% each). The rejected samples, due to clotted and wrong container contributed to 0.05%. A total of 0.46% of the HbA1c results were non-reportable with most of the cases were due to the Hb variant. Among the non-reportable HbA1c results, the Hb variant contributed 4.44% of the causes. In these Hb variants, 39 (1.73%) samples showed no peak during HbA1c analysis, and 31 (1.38%) were HbF. Other causes such as carbHb, LA1c, and HbA1c results of <3.8 or >18.5% respectively, contributed a small percentage to the non-reportable HbA1c results.

**DISCUSSION**

Over 20 years, the prevalence of DM among Malaysians aged more than 30 years old has increased more than two-fold. Currently, based on a multi-ethnic population, an HbA1c cut-off level of 6.3% is used for the diagnosis of DM (Wan Nazaimoon et al., 2013). The recommended HbA1c level in diabetic patients to prevent complications is <6.5%. Small changes in HbA1c measurement cause large changes in HbA1c value since HbA1c is expressed in the percentage of total Hb (Sabariah et al., 2013). Deviation of 1% of HbA1c reflects a change of 1.4 to 1.9 mmol/L in average blood glucose concentration (Nasir et al., 2010).

This study revealed that the presence of interferences of 8.56% and non-reportable HbA1c results of 0.46%. Hb variant was the most common cause of both results. HbF and HbA1c with no peak were considered as Hb variants. Certain Hb variant especially homozygous has no HbA1c peak (0%) because the analyzer could not measure the peak as the chromatograms showed a double peak (Camargo & Gross, 2004). A study has reported that the percentage of Hb variants of 2.3% and 2.2% from these Hb variants were non-reportable (Sabariah et al., 2013). In contrast, our study showed higher percentages of Hb variants (8.48%). This is probably due to the high prevalence of HbE with thalassemia as a result of mixed marriage and migration of Thai population since Kelantan is bordering to Thailand (Thevarajah et al., 2009). The prevalence of thalassemia and haemoglobinopathies are high in Thailand and HbE is the most common structural Hb variant (Nitin, 2010).

Hb variant can cause interference on HbA1c measurement in many ways. The HbA1c results would be falsely low regardless of the methods used if the variant causes reduced erythrocytes life span. Hb variants S, C, D, and E which cause changes in the net charge of Hb interfere with ion-exchange HPLC or electrophoresis method and some Hb variants may co-elute or co-migrate with HbA1c (Little & Sacks, 2009). The HbA1c results become inaccurate when Hb variants or glycated derivate cannot be separated from HbA or HbA1c (Bry et al., 2001; Lorenzo-Medina et al., 2014). When compared to heterozygous which are silent variants, HbA1c is inappropriate for the assessment of glycaemic control in patients with homoyzogous Hb variant such as HbS, HbC, HbSC diseases, or any other conditions with altered erythrocytes survival (Zhang et al., 2018). The method of choice is using serum fructosamine as it is not affected by the Hb variant. Serum fructosamine depends on the glycation of protein and reflects shorter glycaemic status which is 1 to 2 weeks (Schnedl et al., 2001). The presence of Hb variant is suspected and should be assessed if the patient’s home blood glucose monitoring level and HbA1c results were not consistent (Lorenzo-Medina et al., 2014).

Furthermore, this study found only 0.03% of the interferences were from carbHb or LA1c. A study by Desmons et al. (2017) showed a significant interference when LA1c exceeded 4% and carbHb exceeded 2%. This interference may affect the HbA1c level and patient management (Desmons et al., 2017). For the HPLC method, the error from carbHb and LA1c can be minimized by proper inspection of the chromatogram. Hence, the interference from carbHb and LA1c is minimized. Most of the methods especially ion-exchange HPLC can detect carbHb levels, which accurately measures the HbA1c in renal failure patients and LA1c was observed as a distinct peak on the chromatogram (Little et al., 2013; Sivaraman & Patel, 2015).
CONCLUSION

The presence of interferences such as Hb variant during HbA1c interpretation should be avoided to prevent mismanagement of the diabetic patients. Further testing by other methods needs to be performed and Hb variant identification is important for the proper management of patients.

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REFERENCES


