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Percentage of Haemoglobin Variants Detected during HbA1c Analysis in Hospital Kuala Lumpur

[1]S Intan Nureslyna*, [1]MN Sabariah, [2]CR Lim, [2]WS Wan Nor Syafiqah, [2]DR Chen, [2]SY Choy & [3]O Nor'Ashikin

[1]Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia

[2]Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia

[3]Department of Pathology, Hospital Kuala Lumpur. Jalan Pahang, 50586 Kuala Lumpur, Wilayah Persekutuan Malaysia

ABSTRACT

HbA1c is an established index of glycaemic control and correlates strongly with risk of chronic diabetic complications. However, the accuracy of HbA1c measurement can be affected by many factors, among which is the presence of haemoglobin (Hb) variants. The aim of the study was to determine the percentage of Hb variant detected during HbA1c monitoring in Hospital Kuala Lumpur. The study also analysed non-reportable HbA1c results in the presence of Hb variants. A cross-sectional study using retrospective data of HbA1c results over five months' period was analysed on Biorad Variant II Turbo, a high performance liquid chromatography (HPLC) assay. The Hb variants were grouped either as HbS, HbC, others (Hb variant apart from HbS or C), and a combination of HbS or C with Others. A total of 11,904 patients were included. Only 2.3% (273) had Hb variants; HbS trait (10.3%), others (89%), and the combination of HbS trait with others (0.7%). No patient with HbC variant or its combination was found. Only 2.2% of those with Hb variant had non-reportable HbA1c. Although the percentage of Hb variants detected during HbA1c analysis and non-reportable HbA1c results were low, their presence should be noted.

Keywords: HbA1c, Haemoglobin variant, HPLC, Biorad Variant II Turbo

INTRODUCTION

Haemoglobin (Hb) A1c (HbA1c) is an established index of glycaemic control in patients with diabetes mellitus (DM), reflecting average glucose over the preceding 2-3 months[1,2]. HbA1c concentration correlates strongly with the risk of chronic diabetic complications[3,4] and it is an important component in management of patients with DM[2,5].

HbA1c is produced by non-enzymatic addition of a glucose molecule to the N-terminal valine residue on the β-chain of Hb A. It constitutes a major portion of glycated Hb. Formation of HbA1c depends both on the lifespan of red blood cell (RBC) and plasma glucose concentration. Thus, conditions affecting RBC turnover (such as haemolytic anaemia and massive blood loss) will adversely affect HbA1c formation[6].

Hb variants are abnormal forms of Hb. They arise from point mutation either in α, β, δ or γ Hb chains. More than 1000 Hb variants have been identified with half being clinically silent[7]. The common Hb variants are HbS, HbE, HbC, and HbD. Individuals with homozygous Hb variant usually have reduced RBC lifespan and therefore, HbA1c is not recommended as a measure of glycaemic control in these patients. However, those with heterozygous diseases, such as HbS, HbC and HbE traits, as well as other clinically silent Hb variants, can potentially affect the accuracy of HbA1c measurement, the effect of which is HbA1c method dependent[6]. Meanwhile, the presence of elevated HbF can also affect HbA1c measurement.

High performance liquid chromatography (HPLC) and immunoassays are the two most common methods used in clinical laboratories[1, 6]. Ion-exchange HPLC identifies HbA1c based on charge differences between HbA1c and other Hb[6]. Hb variants that cannot be separated from HbA or HbA1c will produce spuriously increased or decreased HbA1c results by ion-exchange HPLC[6]. Immunoassay measures HbA1c by using specific antibodies towards the N-terminal glycated amino acids of Hb β chain. Most immunoassays avoid spurious results for several Hb variants, but interference can occur depending on the antibody and assay used. The presence of Hb variant cannot be detected when using an immunoassay method for HbA1c[6].

*Corresponding author: *nureslyna@.upm.edu.my*

The laboratory should be aware of the effects of common Hb variant on the HbA1c method used and physicians should be alerted on the presence of these interferences. Incorrect reporting of HbA1c result can lead to mismanagement of diabetes as the results are used for treatment decision. Therefore, the objective of this study was to determine the percentage of Hb variant detected through HbA1c monitoring and its interrelated factors in the patient population of Hospital Kuala Lumpur (HKL).

MATERIALS AND METHODS

A cross-sectional study using retrospective data of HbA1c results over a five-month's period of all patients who had their HbA1c measurement at the Chemical Pathology Laboratory of HKL. The HbA1c reports and demographic data were retrieved from the laboratory information system (LIS). Only one HbA1c result per patient was included in the study. Meanwhile, repeated HbA1c result from the same patient was excluded.

HbA1c is measured on Biorad Variant II Turbo by using the HPLC method. Hb variant is identified if the chromatogram, which is reviewed during reporting, showed abnormal peaks such as unknown peaks after or before the A0, variant window peak after A0, S-window or C-window. The laboratory routinely reports the presence of Hb variant discovered during HbA1c measurement with an additional comment such as 'Hb variant detected. Suggest Hb analysis for confirmation'. If the abnormal peak is found either in S- or C-window, the comment states that HbS or HbC variant respectively was detected.

Thus, only Hb variants could be sub-classified to HbS, HbC, others (Hb variants apart from HbS or HbC), a combination of HbS with others and a combination of HbC with others. No additional confirmatory testing, such as Hb analysis of samples containing Hb variant, was performed.

The Hb variants were also sub-classified into those HbA1c results that could and could not be reported as a result of interferences. The criteria for non-reportable HbA1c are as follows:

i. The percentage of HbF >5%, or
ii. No HbA1c detected, or
iii. HbA1c peak area percentage < 4.1 % or more than >16.8%.

Data were analysed using the Statistical Package for Social Sciences (SPSS) software version 19.0. The percentage of Hb variant detected was determined by dividing the number of patients with Hb variant and the total number of patients with HbA1c result sent to HKL during that time period. From those with Hb variant, a Chi-Square test was used to analyse the association between gender and ethnicity with the type of Hb variants.

The study was approved by the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, UPM, and also the ethical committee of the Ministry of Health, Malaysia.

RESULTS

Out of 13,139 HbA1c results analysed, 1,235 results were from the patients with more than one HbA1c result and thus, were excluded. Only 2.3% (273) of 11,904 patients were noted to have Hb variants. The demographic characteristics of all the patients and patients with Hb variants are presented in Tables 1 and 2, respectively. The majority of patients were Malays (50.7%) and there were more females (53.2%) compared to males (46.8%). Similarly, there was a higher percentage of Malays with Hb variants. A significant association was found between Hb variants and ethnicity ($p<0.05$). The percentage of Malays who had Hb variants was 4%, followed by Indians (0.4%), and Chinese (0.2%).

The majority of Hb variants were the other variants (89%), followed by HbS (10.3%), and the combination of HbS with others (0.7%). There were no patients with HbC or its combination. For non-reportable HbA1c, six (2.2%) patients with Hb variants had HbA1c values of > 16.8%, while none had HbF > 5% or HbA1c < 4.1%.

DISCUSSION

DM is a worldwide health issue with prevalence increasing throughout the years. It was estimated that 171 million had DM in the year 2000 and this is projected to increase to 366 million in the year 2030[8]. In Malaysia, the prevalence of diabetes had doubled from 6.3% in 1986 to 11.6% in 2006[9]. These patients are at risk of developing chronic complications which are associated with significant mortality and morbidity. Good glycaemic control reduces development of these complications with HbA1c widely used as a measure of glycaemic control. The normal HbA1c in those without DM is 4-6%. For diabetic patients, the recommended HbA1c is < 6.5%. Since HbA1c is measured as the percentage of total Hb, even small deviations in measurement lead to large changes in HbA1c value.

Table 1. Demographic profile of the patients with HbA1c Measurement from January 2010 to May 2010 in Hospital Kuala Lumpur

Profile of patients	Number of patients (%)
Gender	
Male	5,566 (46.8)
Female	6,338 (53.2)
Ethnicity	
Malay	6,031 (50.7)
Chinese	2,479 (20.8)
Indian	3,055 (25.7)
Others	339 (2.8)

Table 2. Types of Hb variant and its association with gender and ethnicity

Patients profile	n	HbS	Types of Variant Others	HbS with Others	X^2	*p*-value
Gender						
Male	130	17 (13.2%)	113 (86.9%)	-	3.865*	0.145
Female	143	11 (7.7%)	130 (90.9%)	2 (1.4%)		
Ethnicity						
Malay	243	19 (7.8%)	222 (91.4%)	2 (0.8%)		
Chinese	6	1 (16.7%)	5 (83.3%)	-	36.152*	0.0
Indian	11	7 (63.6%)	4 (36.4%)	-		
Others	13	1 (7.7%)	12 (89.0%)	-		

* Chi-Square test

In this paper, we report a percentage of Hb variants of 2.3%. In other parts of the world, the reported Hb variant detected during HbA1c analysis varies from as low as 0.03% in Korea, to as high as 3.1% in Brazil, depending on the method used and the population studied[10,11]. The co-inheritance of two or more Hb variants although uncommon can also be detected during HbA1c analysis. Six HbA1c results noted to have Hb variant were non-reportable, as the HbA1c results were > 16.8%, and this is possibly due to the effect of Hb variant. In these patients, it is advisable that another alternative method for HbA1c be used to better reflect the patients' glycaemic control.

The effect of Hb variant on HbA1c measurement depends on the type of Hb variant and the HbA1c method used. The effects of these variants on several commercially available HbA1c assays have been described over the years, particularly ones looking at common variants such as HbS, HbC HbE and HbD traits[12-16]. Nonetheless, no significant differences in the HbA1c results were noted in several commercial HbA1c assays including the Biorad Variant II turbo assay used in our study when compared to a boronate affinity method (CLC 330, *Primus Diagnostics*) in the presence of HbC or HbS traits[15]. CLC 330 has previously been shown to be unaffected by the presence of HbC and HbS traits[18]. In contrast, the presence of HbD and HbE trait gave higher HbA1c result when measured by Variant II Turbo[17].

Reporting of a falsely high or falsely low HbA1c result may lead to over-treatment or under-treatment of diabetes, respectively; both of which may lead to further complications. The laboratory should report the presence of Hb variant when it is detected, as recommended by the National Association of Clinical Biochemistry (NACB) guidelines 2002. However in 2009, only 39% of laboratories routinely reported the presence of Hb variants whilst some only reported when the variant had caused interferences to the test[19].

A possible interference by Hb variant should be suspected if the HbA1c value does not correlate with the patient's clinical presentation or when the HbA1c result is >15%[1]. In addition, efforts should also be made to identify the Hb variant, while alternative HbA1c methods that are free of the interference should be used[1]. For situations in which the HbA1c values are unreliable, measurements of other glycated proteins such as fructosamine will provide another alternative.

In the current work, we did not perform further confirmatory tests for Hb variants in those noted to have Hb variants during the HbA1c analysis, nor did we compare the effects of these variants on an alternative HbA1c method, which might limit the findings of this study.

CONCLUSION

Hb variants were detected during routine HbA1c analysis in 2.3% of the patients. Only six of these variants were non-reportable with the current HbA1c method used. Although the percentage of Hb variants detected was low, their presence should be noted. Clinicians should be notified of the Hb variants as these variants may alter the actual HbA1c values, and thus, not represent the true glycaemic control of patients with diabetes.

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