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RESEARCH ARTICLE

INCIDENCE OF HEMOGLOBIN VARIANTS DETECTED DURING GLYCOSYLATED HEMOGLOBIN ANALYSIS: A SINGLE CENTRE EXPERIENCE

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ABSTRACT:

Haemoglobin A1C (HbA1C) otherwise known as glycosylated haemoglobin is a tool to monitor glycemic control. Various factors like Haemoglobin (Hb) variants, red blood corpuscle lifespan may affect the accuracy of HbA1C determination by high performance liquid chromatography method. The objective of this study was to determine the percentage of Hb variants detected during HPLC method of HbA1C determination in the patient population of our centre. The effectiveness of communication of incidental detection of Hb variants in HbA1C on patient management was also monitored. A study using data of HbA1C results over a period of 10 months of all patients who had their HbA1C measurement at our centre was done. The percentages of incidentally detected Hb variants were determined. Follow up of the patients with incidental detection of Hb variants in HbA1C were examined to determine whether the clinician has evaluated the patient for Hemoglobinopathy and other glycemic control monitors were performed. 0.36% of 5246 subjects were found to have Hb variants. Follow up evaluation after notification of incidentally detected haemoglobin variants during HbA1C analysis revealed 31% were evaluated for hemoglobinopathies and 10.5% were evaluated for alternate glycemic index testing. The poor compliance may be due to lack of understanding the limitations HbA1C measurement by HPLC method or lack of awareness of un-established clinical target values of HbA1C in the presence of Hb variants. It is essential is to formulate a consensus approach to reporting incidental detection of Hb variants in Hb A1C analysis by HPLC method.

KEYWORDS
HBA1C, HPLC, Hb variants, Glycemic control monitors

INTRODUCTION

Hemoglobin A1C [HbA1C] otherwise known as Glycosylated haemoglobin is a tool to monitor glycemic control. HbA1C is produced by non-enzymatic addition of a glucose molecule to the N-terminal valine residue on the β-chain of HbA[1, 2]. Formation of HbA1C depends on both the lifespan of red blood cells and plasma blood concentration[3]. HbA1C can be measured by High performance liquid chromatography[HPLC] and immunoturbidometric methods[2, 3]. Ion exchange HPLC identified HbA1C based on charge differences between HbA1C and other Hemoglobins[Hb]. Immunoassay measures HbA1C by using specific antibodies the N-terminal glycated amino acids of Hb β chain[2].

Various factors like Hb variants, RBC lifespan may affect the accuracy of HbA1C determination by HPLC method.Hb variants are abnormal forms of Hb. A large number of Hb variants have been identified with many being clinically silent. Subjects with Hb variants having shortened RBC lifespan and co-elution of Hb variant with HbA will underestimate the HbA1C result[4].

The laboratory should be aware of the effects of common Hb variants detected on the hplc method used for HbA1C determination. The laboratory should also inform the physician on the presence of these Hb variants and its interferences and clinical implications.

The objective of this study was to determine the percentage of Hb variants detected during HPLC method of HbA1C determination in the patient population of SRL, Chennai. The effectiveness of communication of incidental detection of Hb variants in Hb A1C on patient management was also monitored.

MATERIALS AND METHODS

A study using data of HbA1C results over a period of 10 months [January 2014 to October 2014] of all patients who had their HbA1C measurement at SRL, Chennai was done. The HbA1C reports were investigated to extract all incidentally identified haemoglobin variants from the laboratory information system [LIS].The demographic data were retrieved for all patients who had incidentally identified haemoglobin variants.

Analytical method- HbA1C is measured on Biorad D10 by using cation exchange HPLC method using EDTA ant-coagulation evacuated container bottles. Hb variants including S-window, C-window, variant window and unknown peaks before A0 are identified in the chromotagram. The laboratory routinely reports the HbA1C results along with the presence of Hb variants identified during HbA1C measurement with an additional comment suggesting Hb variant analysis by HPLC method along with fructosamine assay for monitoring glycaemic index. If abnormal peak is found in S-window, additionally sickling test was performed. The clinician is also alerted telephonically about the incidental detection of Hb variants. The criteria for non-reportable HbA1c values are as follows:

HbA1C percentage <3.8% or more than 18.5%

HbF >15%

Any peak in the variant window

Combined area of Variant S and C windows >= 60%

Data were analysed using the Statistical Package for Social Science [SPSS] software version 17.0. The percentage of Hb variant detected were determined by dividing the number of patients with Hb variant and the total number of patients with HbA1c results analysed in our centre. Follow up of the patients with incidental detection of Hb variants in HbA1C were examined to determine whether the clinician has evaluated the patient for Hemoglobinopathy and other glycemic control monitors were performed.

RESULTS

Out of the 5343 results analysed, 97 were from the subjects with more than one HbA1C result and thus, were excluded from the study. 19 out 5246 subjects [0.36%] were found to have Hb variants. Among the 19 variants, 61.1%[11] were found to be having S window and 38.9%[8] were found to be having variant window. S window was seen in 7 African ethnic patients of all the 11 S window detected whereas variant window were seen in 4 South-Indian and 3 North-Indian ethnic patients.

Table 1: Summary of follow up testing post notification of haemoglobin variant incidentally detected during HbA1C testing

Follow up Investigations	**Yes**	**No**
Fructosamine	2	17
Hb variant analysis	6	13

Follow up evaluation after notification of incidentally detected haemoglobin variants during HbA1C analysis revealed 31% were evaluated for hemoglobinopathies and 10.5% were evaluated for alternate glycemic index testing [Table 1].

DISCUSSION

In this study, we report a 0.36% incidental detection of Hb variants in HbA1C analysis by HPLC method. In contrast, 3.1% and 0.03% is the incidental detection of Hb variants in HPLC in Brazil and Korea[5, 6]. The incidence of 0.36% may not represent the true measurement of Indian population, as there is a lot immigrant population resulting from globalization and medical tourism. Seven HbA1C results with incidental Hb variants were non-reportable as the

chromatogram showed a peak in the variant window. Of the entire sickle window detected, except for one all the samples showed positivity in sickling test. This is possibly due to a different Hb variant eluting in the same window as sickle window.

The effect of Hb variant on HbA1C measurement depends on the type of Hb variant detected and the percentage eluted. Though many studies have shown that HbA1C is unaffected in the presence of HbC and HbS traits[7], other haemoglobin variants like HbE, HbS and HbD showed significant difference in HbA1c measurement by immunoassay than HPLC[4, 8]. Hence, overall HbA1C by alternative method like immunoassay or other glycemic index test like fructosamine should be practised in the presence of incidentally detected haemoglobin variants in HPLC method of measurement.

In spite of notification by written and oral mode to the clinician of incidental Hb variant detection in HbA1C measurement, 31% were evaluated for hemoglobinopathy and 10.5% were evaluated for alternative glycemic index testing. This may be due to lack of understanding the limitations HbA1C measurement by HPLC method or lack of awareness of un-established clinical target values of HbA1C in the presence of Hemoglobinopathies[9].

With no international standardized guidelines in reporting HbA1C that includes methodology, effect of haemoglobin variant in HbA1C measurement[10], the need of the hour is to formulate a consensus approach to reporting incidental detection of Hb variants in Hb A1C analysis by HPLC method. Since the current diabetic management is based on the HbA1C and glucose values[10], inaccurate HbA1C results in diabetic patients with Hb variants may hinder the appropriate management of the patient. Hence, a novel HbA1C reporting format including the methodology, reporting of Hb variants when present should be formulated.

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