HEMOGLOBIN VARIANTS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract. Measurement of HbA1c levels in diabetic patients is an established procedure for evaluating long-term control of diabetes. Despite its usefulness, conditions that effect hemoglobin concentration, such as hemoglobinopathies give rise to inappropriate HbA1c values. Since information about hemoglobinopathies in the diabetic population in Sri Lanka is limited, a prospective cross-sectional study was carried out among 2,695 diabetic subjects attending the diabetic clinic at Nawaloka Hospital, Sri Lanka. Hemoglobin type and HbA1c were measured by the HPLC method. The results reveal among 2,695 diabetic subjects, 53 (2%) had abnormal hemoglobin types (HbF and HbS). HbA1c concentrations in diabetic patients without Hb abnormalities show a higher correlation with fasting blood glucose than those with hemoglobin abnormalities. This study emphasizes that patients with inappropriate HbA1c values should be investigated for hemoglobinopathies.

Key words: hemoglobin variants, glycosylated hemoglobin, type 2 diabetes mellitus

INTRODUCTION

The measurement of glycosylated hemoglobin (HbA1c) is important for the evaluation and management of patients with diabetes mellitus. HbA1c assesses long-term glycemic control and predicts micro-vascular complications in diabetes (Stratton *et al*, 2000; The ADVANCE Collaborative Group, 2008).

Hemoglobin protein has four subunits, each containing a heme molecule conjugated with a polypeptide (globin). Adult hemoglobin (HbA) contains two types of polypeptides; α and β. In hemoglobin A_2, the β subunit is replaced by a δ subunit. In healthy adults hemoglobin consists of 97% HbA ($\alpha_2\beta_2$), 2.5% HbA_2 ($\alpha_2\delta_2$) and 0.5% HbF ($\alpha_2\gamma_2$). HbA1c measures the glucose attached to the terminal valine in each β chain.

The level of HbA1c in a blood sample provides a glycemic history of the average erythrocyte lifespan (Goldstein *et al*, 2000). There are a large number of hemoglobin variants, causing inaccurate HbA1c results in diabetic patients of various ethnic groups. HbE, HbS and HbC commonly

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and unpredictably interfere with HbA1c (Schnedl *et al*, 1999; Frank *et al*, 2000; Saudek, 2006).

Although over 700 structural hemoglobin variants have been identified, only HbS, HbC and HbE exist in high frequencies in the world. It has been estimated that approximately 7% of the world population are carriers of such disorders. However, the global impact may be substantially larger because hemoglobin variants are reported in approximately one third of diabetic patients throughout the world (Brg *et al*, 2001).

In Sri Lanka, the prevalence of diabetes in 31 to 64 year olds was reported as being 5% (Weerasuriya, 1998). Since information regarding the prevalence of hemoglobin variants among type 2 diabetics in Sri Lanka is lacking, and hemoglobinopathies may affect HbA1c test results, laboratories need to be aware of potential problems. A hemoglobinopathy should be suspected in patients when a HbA1c result varies substantially from other indices of metabolic control (Goldstein *et al*, 2000) such as when: the A1c result does not correlate with results of self blood glucose monitoring, the A1c result is different than expected or radically differs from a previous test result after a change in lab A1c methods, the A1c result is greater than 15%. Therefore, a prospective study was carried out to evaluate the prevalence of hemoglobin variants in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Subjects and sampling

The data for analysis of hemoglobin variants in this study were obtained from 2,695 patients attending the diabetic clinic at Nawaloka Hospital, Colombo, Sri Lanka over a period of 8 months. Ethical clearance for the study was obtained from the Nawaloka Hospital Research and Ethics committee.

Venous blood (3 ml) was taken from each patient and 1.5 ml was transferred into an EDTA containing tube to determine the HbA1c and hematological parameters. A blood film was obtained, red cell indices performed (Hb, MCV, MCH) and hemoglobin typing was carried out. Blood smears were stained with Leishman's stain and examined by a hematologist. The remaining blood was transferred into vials containing sodium fluoride and centrifuged; the plasma was then separated and used to determine the glucose level.

Measurement of HbA1c, plasma glucose and red cell indices

The HbA1c and other hemoglobin variants were measured using the HPLC technique (Hemoglobin Variant Analyzer; Bio-Rad, Hercules, CA) while red cell indices were measured using a Beckman-Coulter automated Hematological analyzer (Gallagher *et al*, 2009). The intra-assay coefficient of variation (CV) was less than 1.5% whilst the inter-assay CV for HbA1c measurement was less than 2.5%. The plasma glucose values were determined using the enzymatic colorimetric method (Randox Laboratories, Antrim, UK) on a Hitachi 911 Chemistry Auto-analyzer.

Statistical analysis

Analysis of data was performed using SPSS and the Student's *t*-test was used to calculate statistical significance. The Pearson correlation was used to assess the correlation between HbA1c and fasting blood glucose (FBS).

RESULTS

Two thousand six hundred ninety-five (2,695) samples were analyzed during a

Table 1
Red blood cell values (Hb, MCV, MCH) and Hb variants in type 2 diabetic patients.

Type of Hb variant	No. of patients	HbF (%)	HbA1c (%)	Hb (g/dl)	MCH (pg)	MCV (fl)
HbS	1	0	4.7	7.0	67.2	20.6
HbF	52	1.8(0.8)	7.0(2.1)	12.2(1.6)	82.7(12.5)	26.1(4.6)

Total sample number: 2695; HbF variant data indicate mean (SD)

three months period. The mean HbA1c was 7.51% (95% CI 7.44-7.59) and the mean blood glucose was 148 mg/dl (95% CI 145-151). The correlation coefficient between blood glucose and HbA1c was 0.67%.

Of the 2,695 samples analyzed, 53 (1.97% of the study population) had evidence of hemoglobin variants. The RBC indices of these diabetic patients are shown in Table 1. A sample from one patient was compatible with hemoglobin S (HbS) suggesting sickle cell disease (HbS; 50.8%). The others variants [52 (98%)] were compatible with the hemoglobin F (HbF). Of these patients, 42 (1.4% of the total sample) had a HbF level greater than 0.9% of their total hemoglobin, which is the normal upper limit of fetal hemoglobin in adults (Pembrey *et al*, 1972) and 10 participants had a HbF level less than 0.9%. Of these 42 patients only two had high HbF levels (10.9% and 22.9%). The mean HbA1c value among diabetic patients with hemoglobin variants was 7.02% compared to 7.52% in patients without a hemoglobin variant (*F* test or *t*-test for unequal variance p=0.044). The correlation between blood glucose and HbA1c was 0.75 among patients without hemoglobin variants and 0.67 among participants with hemoglobin variants.

DISCUSSION

About 1.5% of our sample had abnormal hemoglobin variants. The main type of variant found in our sample was HbF (42/43 or 98%). Synthesis of HbF is reactivated in erythropoetic stress situations, such as hemolysis, bleeding, recovery from bone marrow failure, pregnancy, hematological malignancies, congenital red cell aplasia, aplastic anemia and some myelodysplastic syndromes. In adult life high levels of HbF production are seen only with hemoglobinopathies. In β thalassemia minor, HbF is elevated in 50% of patients, usually to 1-3% of the total hemoglobin and rarely to greater than 5%.

Few attempts have been made to study the prevalence of hemoglobinopathies among diabetics in South Asian countries (De Silva *et al*, 2000; Weatherall and Clegg, 2001). Since heterozygotes have an advantage against malaria, inherited hemoglobin disorders should occur more frequently in malaria endemic regions in South Asia. The structure of hemoglobin variants can alter the stability and function of hemoglobin and adversely affect the accuracy of measured glycosylated hemoglobin in patients with diabetes mellitus (Lahousen *et al*, 2002; Joutovsley *et al*, 2004). The present study was conducted to determine the frequency of hemoglobin variants among type 2 diabetes patients.

Thalassemia is a heterogeneous group of inherited conditions characterized by defects in the synthesis of one or more of the globin chains that form the hemoglo-

bin tetramer. Thalassemia is encountered in every population in the world, but more common in the Mediterranean and in the equatorial regions of Africa and Asia. Gene frequencies for α and β thalassemia on a global basis range from 1 to 80% in areas where malaria is endemic. Some alleles which produce thalassemia in a heterozygous state are hematologically silent, with a normal MCV and HbA_2 (Joutovsley *et al*, 2004). The sickle cell gene is distributed widely throughout Africa, the Middle East and in certain areas of the Indian sub-continent, where carrier frequencies range from 5% to 40% of the population. HbE is predominantly found in the eastern half of the Indian sub-continent and throughout Southeast Asia. The present study reports the prevalence rate of hemoglobinopathies among type 2 diabetes patients is less than 2%, which is low compared to some Asian countries. This study was conducted outside the thalassemic belt, in Sri Lanka, and in an area non-endemic for malaria.

Hemoglobin variants are hematologically and clinically silent, due to the underlying mutation causing no alteration in function, solubility or stability of the hemoglobin molecule. In general, most of these variants are separated by electrophoresis or chromatography, but some may remain undetected (Frank *et al*, 2000; Lahousen *et al*, 2002). Some structural variants are associated with severe clinical problems in the homozygous or even heterozygous state. These may affect the properties of the hemoglobin molecule resulting in changes in hemoglobin solubility, stability or oxygen binding properties. In the present study the structural variants of hemoglobin were identified by HPLC with high resolution (Roberts *et al*, 2000; Camargo and Gross, 2004). Peaks in different hemoglobin variants were identified by elution time. HbF and HbS peaks eluted at approximately 2 and 8 minutes, respectively, whilst HbA1c appeared at 1.8 minutes on HPLC. Alteration of the charge on α and β chains decreases retention time of the non-glycated variant hemoglobin causing it to co-elute with HbA1c, leading to an overestimation of HbA1c (Schnedl *et al*, 2000; Joutovsley *et al*, 2004). A recent review summarizing the effect of HbF on HbA1c concludes the HbF effect on glycated hemoglobin can be variable (Gallagher *et al*, 2009). Measurement of HbA1c using the Boronate affinity method may lead to less interference from hemoglobin variants (Gallagher *et al*, 2009). Hence, subjects with HbF ($\alpha_2\gamma_2$) and HbS trait may have abnormal HbA1c values.

In cases of hemoglobin variants, the results suggest HbA1c concentrations have comparatively poorer correlation with blood glucose in diabetic patients when compared to those without hemoglobin variants (Schnedl *et al*, 1999, 2000).

In summary, our study shows when treating diabetic patients it is essential to have a knowledge of hemoglobin variants, since they influence HbA1c levels and therefore, proper management. It is therefore vital that health care workers in regions where hemoglobin disorders occur in high frequencies, especially in malaria endemic regions, are made aware of this problem when assessing glycemic control in diabetic patients. Diabetic patients with inappropriate HbA1c values should be investigated for the presence of hemoglobin variants.

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