Original Article

Glycosylated Hemoglobin (HbA1C) Levels in a Male Kuwaiti Population

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ABSTRACT

Objective: The aim of this study was to determine the reference range of glycosylated hemoglobin in a male Kuwaiti population by using a high performance liquid chromatography (HPLC) based automated instrument.

Subjects and Methods: Blood was collected from 243 male blood donors and the Tectron TOSOH 2.2 automated glycosylated hemoglobin analyzer measured the glycosylated hemoglobin levels.

Results: The mean glycosylated hemoglobin level was 4.8% with a standard deviation of 0.5%. A significant difference was noted between those above and below the age of 40 years but not between Kuwaiti nationals and non-Kuwaitis.

Conclusion: A reference range of 3.8 to 5.8% (mean +/- 2SD) for glycosylated hemoglobin in a male Kuwaiti population has been established using an HPLC based method. This will enable target values for optimal control in diabetics to be established in the Kuwaiti setting.

KEY WORDS: blood donors, glycosylated hemoglobin, high performance liquid

INTRODUCTION

The measurement of glycosylated hemoglobin (GHb) is a well established means of monitoring glycemic control in patients with diabetes mellitus[1]. The risk for development and progression of the chronic complications of diabetes is closely related to the degree of metabolic control[2].

Glycosylated hemoglobins are the products of a non-enzymatic reaction between the free aldehyde group of glucose or other sugars and the unprotonated form of free amino groups of hemoglobin. A labile aldimidine form is generated, in a first relatively fast reaction, which is partially converted into a stable ketoamine form via an Amadori arrangement.

The percentage of glycosylated hemoglobin in human blood depends on the concentration of glucose, the duration of glucose exposure to hemoglobin and the turnover of erythrocytes. Because the average life span of the erythrocyte is 120 days, glycosylated hemoglobin has been accepted as a measurement that reflects the mean daily blood glucose concentration and the degree of carbohydrate imbalance over the preceding two to three months[3]. The interpretation of glycosylated hemoglobin values is based on the assumption of a normal erythrocyte life span. Patients with hemolytic disease or other conditions with shortened erythrocyte survival have a significant reduction in glycosylated hemoglobin.

In normal adults, the principal hemoglobin found is hemoglobin A (97%). HbA2 and HbF are present in small quantities. Using column chromatography, HbAI, formed by the glycation of the alpha and beta chains of HbA, can be separated into at least three minor components, A1a, A1b and A1c[4] .

Three species of glycosylated hemoglobin are currently measured for management of diabetic patients[5]. There is no clinical advantage to measuring a particular glycosylated hemoglobin species. HbA1, HbA1c and total GHb are equally related to the preceding glycemic control[6]. Currently, there are neither internationally approved primary or secondary reference materials, nor a reference method for the measurement of glycosylated hemoglobins. Methods are standardized either internally or against a chosen HPLC method. Hence different values differ considerably between methods and laboratories[7].

The aim of this study was, therefore, to determine the reference range in a male Kuwaiti population for glycated hemoglobin (HbA1c) as measured on the Tectron TOSOH 2.2 analyzer.

METHODS AND MATERIALS

The TOSOH 2.2 analyzer utilises the principles of high performance liquid chromatography (HPLC) and is calibrated against the Diabetes Control and Complications Trial (DCCT) method. It

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uses a cation exchange column and three elution steps with buffers of increasing salt concentration. In this way, the various components of hemoglobin are quickly separated and measured as six fractions corresponding to A1a, A1b, F, Labile A1c, Stable A1c and A0. The separation of the major hemoglobins occurs in 2.2 minutes without the need for any sample preparation. It takes seven minutes to get a first result. The 'within batch' and 'between batch' coefficients of variation (CV) of the method is 1.7% and 0.7%, respectively.

Blood samples were collected into EDTA-containing tubes (Becton Dickinson) from 264 volunteer blood donors. The collection of samples was part of a study for the determination of reference ranges for hematological indices. The samples were stored at 4 ˚C, for a maximum of 48 hours, between collection and hematological analysis and subsequently on the TOSOH analyzer. Samples were analyzed in batches of 30 or 60, in duplicate. The instrument required a two-point calibration.

STATISTICAL ANALYSIS

Statistical analysis and graphical presentation was done using SPSS 7.5 on PC Windows. Significant differences between pairs of means were determined by using the student t-test.

RESULTS

The glycosylated hemoglobin (HbA1c) levels on the 243 blood donors are shown in Table 1. There were only 20 females in the group and, therefore, they were excluded from the analysis. The median age is 31.4 years (range 18-65 years) with 18.5% being above 40 years old. There were 39% Kuwaitis and 61% non-Kuwaitis.

The mean HbA1c level was 4.8% with a standard deviation of 0.5% and individual values showed a normal distribution about this mean (Fig. 1).

All the blood donors had non-fasting blood glucose levels of < 6.5 mmol/l. The minimum number of 120 subjects needed to determine reference values reliably was achieved in the overall group but not in the age and sex groups[8]. Despite this flaw, there is a significant difference ($p<0.01$) between the levels for those above and below 40 years. There was no significant difference between Kuwaitis and non-Kuwaitis.

DISCUSSION

The reference range and the number generated for HbA1C in a given clinical sample are not interchangeable between laboratories. The reference range determined for the Kuwaiti male population from this study is 3.8% to 5.8% (mean +/- 2SD). There is no consensus on either a reference method or a glycosylated hemoglobin standard.

Table 1
Glycosylated hemoglobin (HbA1c) levels in 243 male blood donors in Kuwait

Age	N	Range	Mean	SD
All	243	3.0 - 6.7	4.83	0.50
>40 years	198	3.2 - 6.4	4.78	0.47
<40 years	45	3.0 - 6.7	5.04	0.56

• $p<0.01$ for difference between age groups

Using the TOSOH 2.2 analyzer, HbA1C levels in a male Kuwaiti population have been determined. The method was found to be both easy to use and very reproducible. The method separates the labile HbA1C from the stable HbA1C, thus ensuring the result to reflect only long-term glucose control. The reference range values obtained for the Kuwait population in this study do not differ appreciably from values determined using the same principle (HPLC) in a Caucasian[9] or Nigerian population[10].

Measuring glycosylated hemoglobin, as an assessment of glycemic control, is fixed in the sights of the clinical target setters. The British Diabetic Association offers targets for glycated hemoglobin with the proviso that they may have to be adjusted according to variations in reference ranges of different assays[11]. Other target setters categorize control according to standard deviations of glycated values around the non-diabetic mean[12].

In this study, a reference range for a male Kuwaiti population has been established, using an HPLC based method, thus enabling target values for optimal control to be established in the Kuwaiti setting.

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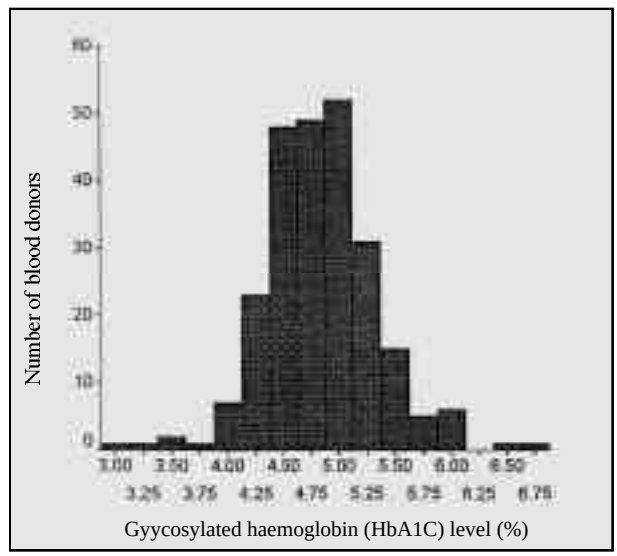

Fig. 1: Distribution of glycosylated hemoglobin (HbA1C) levels in a Kuwaiti population

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