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SYMPOSIUM

Influence of Variables on Hemoglobin A1c Values and Nonheterogeneity of Hemoglobin A1c Reference Ranges

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Abstract

Introduction:

Hemoglobin A1c (HbA1c) values are influenced by analytical interferences such as HbF and hemoglobin variants and clinical factors such as increased red cell turnover. Although less well-known, demographic factors such as race, age, and sex also influence HbA1c values.

The HbA1c reference range should be homogenous in the United States based on the use of National Glycohemoglobin Standardization Program certified methods and the recommendations in the National Academy of Clinical Biochemistry guidelines.

Methods:

Data on age, race, sex, HbA1c, and glucose values were extracted from the National Health and Nutrition Examination study for a 3 year period. A search for reference range data for laboratories in the United States was performed using the Google search engine.

Results:

Extracted data agree with published data on the influence of age, sex, and smoking status on HbA1c values. There is substantial heterogeneity in HbA1c reference ranges in laboratories in the United States.

Conclusion:

Age, sex, and smoking status influence HbA1c values. Despite standardization of HbA1c methods and published recommendations, there is wide heterogeneity in HbA1c reference ranges in the United States.

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Abbreviations: (HbA1c) hemoglobin A1c, (NACB) National Academy of Clinical Biochemistry, (NGSP) National Glycohemoglobin Standardization Program, (NHANES) National Health and Nutrition Examination Survey

Keywords: age, HbA1c, race, reference range, smoking status, variables

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Introduction

Variables that influence hemoglobin A1c (HbA1c) values may be classified as analytical or clinical. The presence of HbF, for example, is a variable that influences the analytical measurement of HbA1c,[1] especially in immunoassay methods, and is therefore classified as an analytical variable. The two clinical variables that are most often identified as influencing HbA1c results are red cell survival and blood glucose concentration. In patients with hemolytic anemia or in those with β thalassemia trait, HbA1c may be lower than expected based on the glucose concentration due to the decreased red cell survival. However, there are several other variables that can influence the HbA1c value, including race,[2–5] smoking status,[6] renal function[7–9] of the patient, age,[10,11] and the season.[12,13]

The reference range may influence interpretation of HbA1c results, although it is not really an analytical or clinical variable. The National Academy of Clinical Biochemistry (NACB) recommends that the HbA1c reference range "should not deviate significantly (e.g., >0.5%) from the 4–6% range."[14] We wished to investigate if laboratories follow this recommendation.

This article is a review of the influence of some clinical variables (e.g., age, sex, and smoking status) on the HbA1c value and investigation of HbA1c reference ranges of laboratories in the United States.

Methods and Materials

A research associate was assigned the task of investigating the HbA1c reference ranges of laboratories in the United States, using the Google search engine with a query on HbA1c testing. These reference ranges were then plotted graphically in Gaussian curve format (**Figure 1**) with the NACB recommended range in black.

Data on smoking status, age, and race was extracted from the National Health and Nutrition Examination Survey (NHANES) database for the period 1999 to 2002, inclusive, into a Microsoft Excel file, and the HbA1c data for males is plotted graphically in **Figure 2** and females in **Figure 3** for race and age using the 50th, 90th, 95th, and 97.5th percentiles. For the studies on age, only HbA1c values on patients whose glucose was less than 6.0 mmol/liter were included. For smoking status, shown in **Figure 4**, the smokers were divided into everyday smokers, most-day smokers, and nonsmokers.

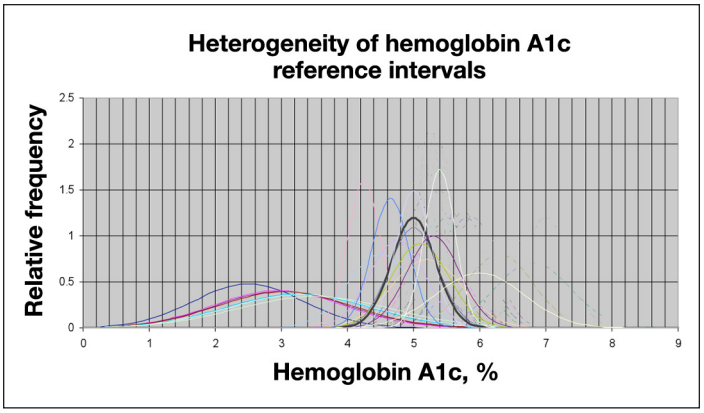

Figure 1. Hemoglobin A1c reference ranges for laboratories in the United States. The NACB-recommended reference range is in black.

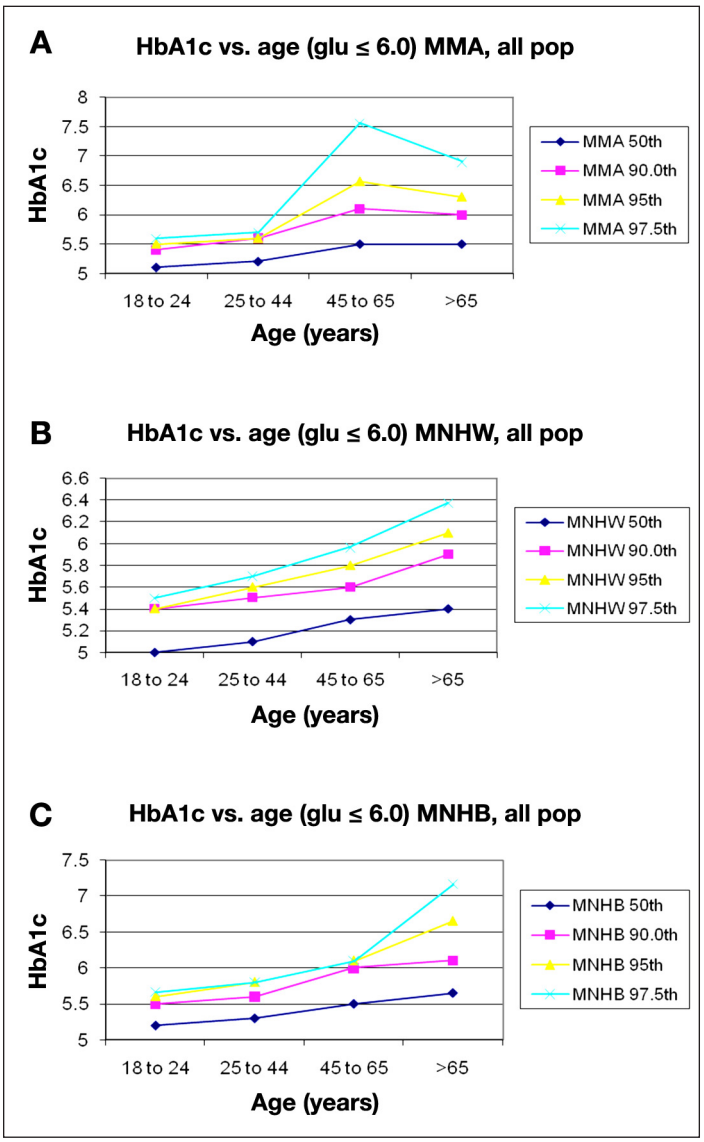

Figure 2. Hemoglobin A1c value (y axis) plotted against age (x axis) for males using different population percentiles in the **(A)** Mexican American (MMA), **(B)** non-Hispanic white (MNHW), and **(C)** non-Hispanic black (MNHB) groups from data in the 1999 to 2002 NHANES study, with fasting glucose values less than or equal to 6.0 mmol/liter.

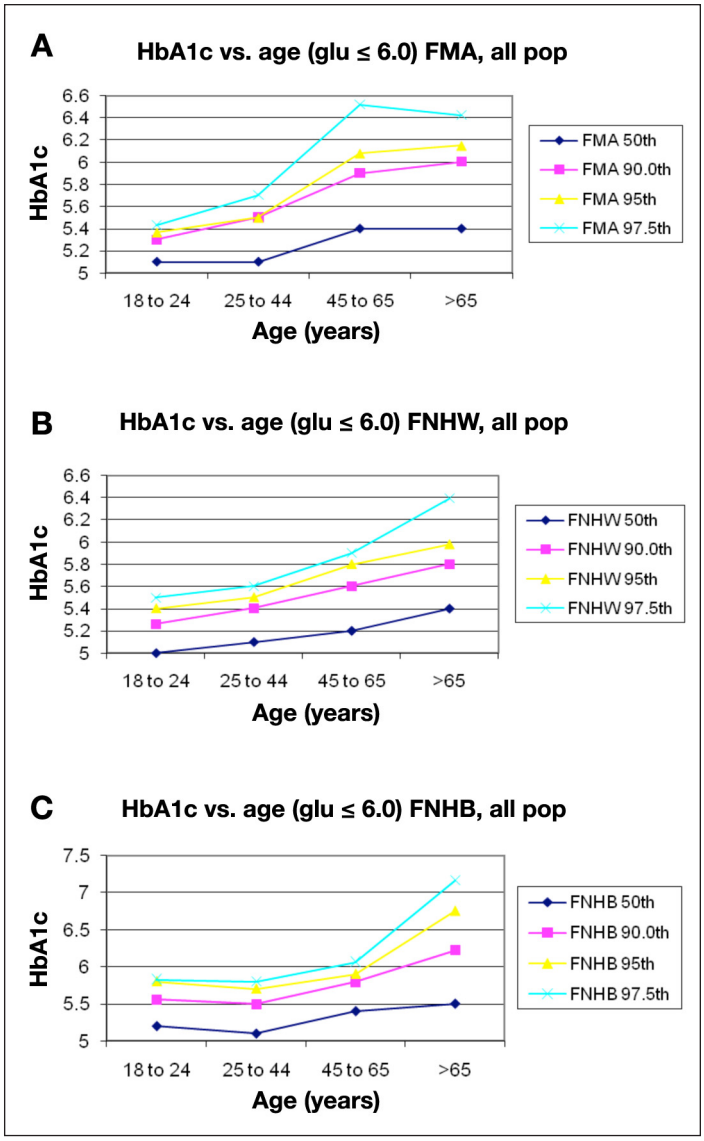

Figure 3. Hemoglobin A1c value (y axis) plotted against age (x axis) for females using different population percentiles in the **(A)** Mexican American (FMA), **(B)** non-Hispanic white (FNHW), and **(C)** non-Hispanic black (FNHB) groups from data in the 1999 to 2002 NHANES study, with fasting glucose values less than or equal to 6.0 mmol/liter.

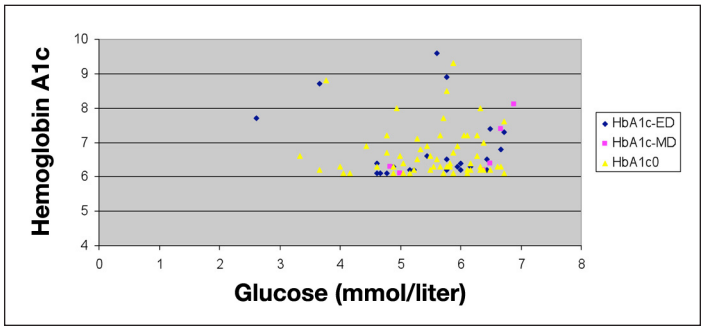

Figure 4. Hemoglobin A1c levels in everyday smokers (ED), most-day smokers (MD), and nonsmokers (0) from the NHANES 1999–2002 data on patients with fasting glucose values less than 7.1 mmol/liter.

Only patients with a fasting glucose values less than 7.1 mmol/liter were included in this study.

Results

Figure 1 shows the heterogeneity of HbA1c reference ranges in laboratories in the United States with the reference range for an individual laboratory plotted as a Gaussian curve in a unique color with the NACB recommended HbA1c reference range plotted in black. Hemoglobin A1c reference ranges were found for 104 laboratories. The lowest and highest HbA1c values used for the lower limit of the reference range was 0 (used by 13 laboratories) and 6 (used by one laboratory), respectively. The lowest and highest HbA1c values used 5 (2 laboratories) and 8.5 (one laboratory), respectively, for the upper limit of the reference range. Four laboratories used an HbA1c value greater than 7 for the upper limit of the reference range, and one used a value of 8.

In both **Figure 2** (male) and **Figure 3** (female), there is a slight progression in the HbA1c value with age. The data from **Figures 2** and **3** are summarized in **Tables 1** and **2**. In the male Mexican American, the 90th percentile HbA1c goes from 5.4 at ages 18 to 24 years old to 6.0 at ages >65 years (difference 0.6), whereas the non-Hispanic white male goes from 5.4 to 5.9 (difference 0.5) and the non-Hispanic black male from 5.5 to 6.2 (difference 0.7). In females, the 90th percentile in Mexican Americans goes from 5.1 at ages 18 to 24 years to 6.0 at >65 years of age (difference 0.9), whereas the non-Hispanic white female goes from 5.3 to 5.8 (difference 0.5) and non-Hispanic black female values go from 5.6 to 6.3 (difference 0.7). This may be related to the increased incidence of type 2 diabetes in aging populations.

The difference in 90th percentiles between the 18 to 24 and >65 age groups change with race, with non-Hispanic

Table 1.
Data from the NHANES Study (1999 – 2002), with 90th Percentile Limits for HbA1c Values for Different Races and Ages in Males

Race	Age (years)		Difference
	18–24	>65	
Mexican American	5.4	6.0	0.6
Non-Hispanic white	5.4	5.9	0.5
Non-Hispanic black	5.5	6.1	0.6

whites having the lowest difference and non-Hispanic blacks having the highest difference.

In **Figure 4**, the everyday smokers show an increase in HbA1c levels over the most-day smokers and nonsmokers for the same fasting glucose concentration. Patients with fasting glucose values greater than 7.1 mmol/liter were excluded from this study.

Discussion

The wide range of HbA1c reference ranges from laboratories in the United States causes some concern. Despite the fact that, according to the College of American Pathologists glycohemoglobin survey, the overwhelming majority of laboratories use HbA1c methods certified by the National Glycohemoglobin Standardization Program (NGSP) and the recommendation of the NACB on the HbA1c reference range,[14] there appears to be a substantial variation in the HbA1c reference range in laboratories in the United States. It is somewhat disconcerting to see some laboratories using 0 for the lower end of the HbA1c reference range, as well as some laboratories using an HbA1c reference range with a midpoint of 2.5 or 3.2, both of which are well below the NACB recommended reference range. Also, there are some laboratories reporting an upper end of the HbA1c reference range above 7.0%, which is the target treatment value of the American Diabetes Association.

One possible explanation for the lower reference range is the use of the International Federation of Clinical Chemistry HbA1c calibrator values. The HbA1c values generated by this calibrator are about 2% lower than those generated by NGSP-calibrated methods.[15] The reporting of HbA1c results using different calibrator systems can cause confusion. The consensus statement recommendations on standardization of HbA1c measurements[16] will probably not be adopted in the United States, so the very real possibility exists that there will be multiple HbA1c reporting systems in the world, despite the consensus statement.

However, there is no justification for the use of 0 as the lower reference limit for HbA1c. Similarly, the use of an upper end of the HbA1c reference range above 8.0% causes concern, as this is well above the American Diabetes Association treatment goal of 7%.[17]

The slight increase in mean HbA1c value with age, seen in **Figures 2** and **3** and **Tables 1** and **2** may be related to the increased incidence of diabetes, especially type 2, in the aging population. The difference is not sufficient to introduce age-specific or racial-specific reference ranges. Our findings agree with those of other authors.[10,11]

Table 2.
Data from the NHANES study (1999 –2002), with 90th Percentile Limits for HbA1c Values for Different Races and Ages in Females

Race	Age (years)		Difference
	18–24	>65	
Mexican American	5.1	6.0	0.9
Non-Hispanic white	5.3	5.8	0.5
Non-Hispanic black	5.6	6.3	0.7

For different races, there is a difference in the 90th percentile HbA1c value in the 18 to 24 age group, with this difference most noticeable in the female data, where the value goes from 5.1 in Mexican American female to 5.6 in the non-Hispanic black female group. In females >65 years, the difference in the 90th percentile HbA1c values goes from 5.8 in non-Hispanic white females to 6.3 in non-Hispanic black females. For females, the change with age is most in the Mexican American female group and least in the non-Hispanic white female group. For males, the differences between the two age groups between races is not as pronounced, and the difference is similar for all three ethnic groups.

Our data on the influence of race on HbA1c values are the same as that of Herman and colleagues[3] who found that whites had the lowest HbA1c level, with Hispanics having higher HbA1c levels and blacks having the highest HbA1c level. Our data on HbA1c in Hispanic populations is similar to that of Kirk and associates.[2] The reasons for the influence of race on HbA1c is unknown, but one that both laboratory personnel and physicians should know. The incidence of diabetes in different races in the United States varies.[18] The influence of race on HbA1c values maybe important in relating the patient's HbA1c value to the target treatment goals, a problem noted by others in a slightly different context (that of slightly different HbA1c values obtained by different analytical methods).[19]

Our data on the effect of smoking agrees with that of Nilsson and coworkers[6] in that smoking increases the HbA1c value. The cause for this increase is unknown but may be due to the increased passage of glucose across the red cell membrane into the cell, increasing the HbA1c.

Conclusion

The variation of the HbA1c reference range in laboratories reporting HbA1c, despite well-published recommendations, causes some concern, and there is also a potential problem of different laboratories using different HbA1c reference ranges based on different HbA1c calibrators. The consensus statement that was meant to harmonize HbA1c reporting worldwide may end up causing wider disparity. Unfortunately, there is no simple solution.

Laboratorians and clinicians should be aware of the fact that HbA1c values increase with age and smoking. The influence of race on HbA1c values is important in relating target treatment goals for HbA1c to individuals of different races.

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