Reference values for hemoglobin $A_{1c}$ in males living in Khartoum State: Pilot study 2016

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Abstract

Objective: The objective of this study was to establish the normal values of hemoglobin $A_{1c}$ in Sudanese healthy adult males living in Khartoum State and compare the result with the international values. Materials and Methods: A cross-sectional study was conducted during 2016 in Khartoum State on adult males of ages between 35 and 45 years and not known to be diabetic or suffering from any chronic illness. Twenty participants were assessed by a questionnaire covering age, family history of diabetes mellitus (DM), physical activity, daily caloric intake and smoking history, and body mass index (BMI) was calculated by measuring weight and body height by weight and height measuring devices (scale). A sample of 3 ml venous blood was taken for fasting blood glucose to exclude DM and measurement of Hb$A_{1c}$ using modified ELISA reader known as Nycocard© machine. Correlations between the variables were estimated and $P < 0.05$ was considered statistically significant.

Results: The mean of Hb$A_{1c}$ was 3.8% ± 1.17 with a range of 1.2–5.4%. There was no correlation between Hb level, daily caloric intake, and the level of Hb$A_{1c}$ ($r = 0$). There was intermediate correlation between body mass index and Hb$A_{1c}$ ($r = 0.32$). All the participants in this study had normal body mass index (18.5–25) with mean (23.6). There was weak statistical correlation between age and Hb$A_{1c}$ ($r = 0.07$), but the family history, smoking, and physical activity were found to be positive statistically significant to Hb$A_{1c}$ ($P < 0.05$). The mean of Hb$A_{1c}$ in smokers was 3.8% (±1.4%) and in nonsmokers was 3.1% (±1.0). The mean Hb$A_{1c}$ of those with positive family history of DM 4% (±0.8%) was significantly higher than those with negative family history 3.5% (±1.3%). The mean of Hb$A_{1c}$ in physically active participants was 3.9% (±0.9) and in the nonactive participants was 3.1% (±1.3).

Conclusion: The results of this study indicate that there is a difference in values of Hb$A_{1c}$ between the international values and Sudan, so the normal values for Hb$A_{1c}$ in Sudanese need to be established from a large sample.

Key words: Hb$A_{1c}$, Khartoum, reference values, Sudanese

INTRODUCTION

Hb$A_{1c}$, haemoglobin $A_{1c}$ is formed by the glycosylation of hemoglobin (Hb). Its value represents the glycemic status of a person over the last 2–3 months.[1]

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According to the American Diabetes Association Guidelines 2007, the value of Hb$A_{1c}$ should be kept below 7% in all diabetics[2] and according to the same guidelines, Hb$A_{1c}$ is now referred to as $A_{1c}$.

Hemoglobin $A_{1c}$ was first separated from other forms of Hb by Huisman et al. and Meyering in 1958 using a chromatographic column.[3]

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The increase in the level of HbA1c in diabetes was first described in 1969 by Samuel Rahbar.[6] Another cross-section study was conducted later by Rahbar et al. at Tehran University found a similar abnormality in 57 diabetic patients.[5] After that discovery, numerous small studies were conducted correlating it to glucose measurements resulting in the idea that HbA1c could be used as an objective measure of glycemic control. In a larger study of diabetic patients, Trivelli et al. found a 2-fold increase of HbA1c over values observed in nondiabetic participants.[4]

Thus, by the mid-1970s, it was clear that HbA1c is elevated in humans with diabetes mellitus (DM) although the mechanism of this abnormality was not understood. HbA1c was introduced into clinical use in the 1980s and subsequently has become an important test in clinical practice.[7]

The term “glycosylated” was used initially, but it has been pointed out that this term strictly refers to glycosides. Therefore, the Joint Commission on Biochemical Nomenclature has proposed that the term “glycation” is appropriate for any reaction that links sugar to a protein, or in the particular case of a reaction with Hb, the term “glycated hemoglobin.”[8]

Different studies have shown variation in the normal ranges of HbA1c. In 1989, Simon et al. reported the distribution of HbA1c in 3240 healthy adult population. They found an approximately normal distribution of HbA1c is 5.03% (±0.53).[9] In England in 2001, Gulliford et al. performed a cross-sectional survey and found a mean (standard deviation) HbA1c of 6.34% (±0.85) in a general population of 9772 nondiabetic, European origin participants aged 16 years and older. In 2011, Hill et al., in the UK, reported the normal range of HbA1c for nondiabetic people found to be between 3.6% and 5.5% on 78 nondiabetic participants.[11]

These variations could be due to different factors which can affect HbA1c level.

According to a report published in 2009 by an International Expert Committee on the role of HbA1c in the diagnosis of diabetes, HbA1c can be used to diagnose diabetes and that the diagnosis can be made if the HbA1c level is 6.5% or more and HbA1c level below 6% is considered normal.[12]

HbA1c results in the UK have usually been aligned to the assay used in the Diabetes Control and Complications Trial (DCCT), expressed as a percentage (DCCT - HbA1c) - nondiabetic “normal” range being 4–6%.[13]

Diagnostic criteria for diabetes that made by the WHO report 2011 determined that HbA1c of 6.5% was considered as the cutoff point for diagnosing diabetes. A value <6.5% does not exclude diabetes diagnosed using glucose tests.[14]

Whether HbA1c was associated with the age has been showed by Pani et al. who performed a cross-sectional analysis of HbA1c across age categories in 2473 nondiabetic persons. They stated that their results establish clearly that HbA1c increases with age, even after multivariate adjustments for sex, fasting, and 2 h postload glucose and suggested that nonglycemic factors may contribute to the relationship of HbA1c with age.[15] Furthermore, in March 1999, Wiener and Roberts performed a cross-sectional analysis to resolve whether hemoglobin A1c (HbA1c) levels in normal participants increase with age, they measured HbA1c in 399 patients undergoing routine oral glucose tolerance test. None of these groups showed a significant correlation between HbA1c and age; therefore, they cannot see a need for age-specific reference ranges for HbA1c.[16]

Racial and ethnic differences in HbA1c have been recognized for many years but have generally been attributed to differences in access to medical care or quality of care.[17] Race- and ethnicity-related differences in HbA1c have been also described by Herman in 2009.[18] Ziemer et al. found higher HbA1c levels in non European origin persons than in white persons across the full spectrum of glycemic after adjustments for plasma glucose and other characteristics known to correlate with HbA1c levels.[19] In the USA, Boltri et al. analyzed data from the 1999 to 2000 National Health and Nutrition Examination Survey and described HbA1c in persons with undiagnosed and diagnosed diabetes by race/ethnicity. They found the Hispanics with undiagnosed diabetes were more likely to have HbA1c ≥7% (60.5%) which is more than blacks (39.3%) and whites (37.8%).[20]

High body mass index (BMI) is known as an important risk factor for diabetes, with higher BMI causing insulin resistance and thereby higher levels of glycemia. Simon et al. found a higher level of HbA1c in obese persons (defined as BMI ≥28 kg/m²), but after adjustment for age, the relation between BMI and HbA1c was no longer significant.[9] Modan et al. found no significant correlation between BMI and HbA1c.[21] but in contrast, in the cross-section study conducted by Boeing et al., they found that obesity found to be related with higher HbA1c levels.[22]

The effect of diet on HbA1c level has been studied by many researchers. Boeing et al. also studied the association of diet and other lifestyle factors with HbA1c examined in a nondiabetic adult population and found that there is a direct relation between risk of increase HbA1c and high energy and energy-adjusted saturated fat intakes.[23] Modan et al. found a similar result which is there is no correlation between the intake of any specific food component and
HbA1c. Furthermore, Harding et al. found independent associations between HbA1c concentration across the normal range of HbA1c and both total fat intake and the pattern of dietary fat intake.

The level of physical activity is known to influence insulin resistance, and thereby possibly may influence HbA1c. Gulliford et al. found a 0.180% lower HbA1c in participants who were vigorously active compared with the inactive participants, and HbA1c gradually decreases with increasing level of physical activity. However, other studies were done by Modan et al. and Boeing et al. found no association between the level of physical activity and HbA1c.

Smoking is well known to affect many body systems and thereby possibly may influence HbA1c; Koga et al. found no association between smoking and HbA1c levels but several studies have documented that smoking is associated with higher HbA1c levels. In another study, Sargeant et al. performed a cross-sectional analysis of the association between cigarette smoking and HbA1c, a marker of long-term glucose homeostasis in 2704 men and 3385 women, aged 45–74 years showed that there was a dose-response relationship between HbA1c levels and number of cigarettes smoked per day and a positive association with total smoking exposure as measured by pack-years.

Despite this variation, the normal level of HbA1c was generally taken as less than 6%, and these values were applied to diabetics patients in Sudan, could there be a difference in the normal level of HbA1c in Sudanese population is clearly a valid question. In this pilot study, the normal level in healthy adult males is investigated.

**MATERIALS AND METHODS**

This is an analytical, cross-sectional community-based study performed in Khartoum state capital of Sudan, during 2016 in Sudanese healthy volunteer males above 35 years who were not diabetics or with high fasting blood glucose (FBG), not hypertensive, not anemic, and have no endocrine or renal problems or any chronic illness, participants with BMI >25 kg/m² were also excluded from the study.

Ethical approval of this study was obtained from the National Ribat University.

The objectives of the study were explained to all individuals participating in the study. Twenty volunteers were included after taking their informed consent.

Questionnaire interviews with all participants were done covering information about age, family history of DM, daily caloric intake, smoking, alcohol history, and physical activity.

Physical examination of the blood pressure, height, weight, and calculation of BMI was done.

Five milliliters of venous blood was collected by the standard procedure from each participant under complete aseptic conditions. About 2.5 ml was placed in fluoride oxalate containers and then used for FBG measurement. The other 2.5 ml was placed in ethylenediaminetetraacetic acid container and used for Hb and HbA1c analysis. Icteric, lipemic, hemolyzed, or bacterially contaminated sample was not used.

HbA1c level was measured using modified ELISA reader known as NycoCard Reader. The kit of NycoCard Reader contains test devices with a porous membrane filter; test tubes prefilled with reagent contain a blue boronic acid conjugate that binds cis-diols of glycated Hb. When the blood is added to the reagent, the erythrocytes immediately lyse. All Hb precipitates the boronic acid conjugate binds to the cis-diol configuration of glycated Hb. The precipitate is evaluated by measuring the blue (glycated Hb) and the red (total Hb), color intensity with the NycoCard Reader II, the ratio between them being proportional to the percentage of HbA1c in the sample.

All the data collected in this study were analyzed using the Statistical Package for Social Sciences (SPSS) computer program version 20 (t-test for mean and P value for significance).

Correlations between the variables were estimated by the correlations coefficient of determination (r) (R = 0: No correlation, 0 > R < 0.25: Weak correlation, 0.25 ≤ R < 0.75: Intermediate correlation, 0.75 ≤ R < 1: Strong correlation, and R = 1: Strong correlation).

P < 0.05 was considered statistically significant.

**RESULTS**

The study covered twenty healthy adult male from Khartoum State, ten of them were between 35 and 40 years old and the other ten were between 40 and 45 with a mean of 38 years.

The mean of HbA1c was 3.8% (±1.17) and a range of 1.2–5.4%. Minimum value was 1.2% and maximum value was 5.4%.

There was a weak statistical correlation between age and HbA1c (r = 0.07).
The mean of HbA₁c in the group of age range between 35 and 40 years old was 3.5% (±1.4) and in the group of age range above 40 years was 4.1% (±0.8).

There was no correlation between Hb level, daily caloric intake, and the level of HbA₁c - correlation coefficient = 0. The mean of Hb concentration was 14 g/dl – all participants showed normal Hb concentration.

There was intermediate correlation between body mass index and Hb A₁c - (r = 0.32). All the participants in this study had normal body mass index (18.5-25) with mean (23.6).

Nine participants showed positive family history of DM, the mean HbA₁c of them 4% (±0.8) was significantly higher than those with negative family history 3.5% (±1.3). Family history was found to be strongly correlated with HbA₁c with high statistically significant (P = 0.001).

Seven participants were smokers and 13 were not. The mean of HbA₁c in smokers was 3.8% (±1.4) and in nonsmokers was 3.1% (±1.0). There was a strong association between HbA₁c and smoking (P = 0.000).

The results showed that there was a significant correlation between physical activity and HbA₁c with P = 0.000. Ten participants were physically active and the other ten were not. The mean of HbA₁c in physically active participants was 3.9% (±0.9) and in the nonactive was 3.1% (±1.3).

**DISCUSSION**

The normal values used in Sudanese laboratories are mainly derived from European studies. Differences in the normal values in Sudan have been documented in some hematological values and respiratory function test parameters. As HbA₁c is now used largely for DM control, the normal values of HbA₁c in Sudanese have been addressed by this pilot study.

HbA₁c samples were analyzed on one laboratory as differences in results have been observed, and the results outcome showed that the normal mean distribution of HbA₁c in Sudanese adult males was 3.8% (±1.17%) and the range was 1.2–5.4% which is lower than in international range (3.6–6.5%).

These differences most probably due to ethnic and genetic differences because all these comparable studies were performed in Western countries, and it is agreement with race- and ethnicity-related differences in HbA₁c that reported by studies of Ziemer et al. and Boltri et al. If the maximum is 5.4% for HbA₁c in Sudanese, then the cutoff point of 6.5% which recommended by the WHO for DM diagnosis need to be revised.

There is a weak statistical correlation between age and HbA₁c. Although the mean of HbA₁c in the group of age between 35 and 40 years old was 3.5% is less than in the age group of above 40 years (4.1%). The participants of the first group of age are more physically active (8 participants) than the participants of the second group of age (2 participants), and this weak correlation could be due to the differences in physical activity or due to small sample size and for the small age group range. These findings were in agreement with the study of Wiener and Roberts, which showed there is no significant correlation between HbA₁c and age and not with Pani et al. study.

There was no correlation between daily caloric intake and the level of HbA₁c; these findings were in good agreement with those obtained by Modan et al. and Harding et al. Furthermore, these findings were disagreement with Boeing et al. who was found that there is a direct relation between risk of increase HbA₁c and high energy and energy-adjusted saturated fat intakes.

The entire participant in this study had normal BMI (18.5–25) with mean (23.6) – correlation coefficient showed the intermediate relation between HbA₁c and BMI. This result agrees with Boeing et al. study that showed the obesity found to be related with higher HbA₁c levels and disagrees with studies of Modan et al. and Simon et al. From our study, we can establish normal level of HbA₁c (1.2–5.4%) across normal BMI as a guide for whom may study the effect of obesity on the level of HbA₁c in Sudanese people in the future.

The high values of HbA₁c in participants with family history of DM need further elaboration.

There was a strong association between HbA₁c and smoking, the mean of HbA₁c in smokers was higher from the mean of in nonsmokers. Many studies proved that the HbA₁c levels are increased by smoking, which in line with our findings, but we disagree with Koga et al. who reported that there is no association between HbA₁c and smoking. In this study, the physical activity level was affected HbA₁c significantly (P = 0.000). The mean of HbA₁c in physically active participants was higher than in nonactive participants. These findings were in agreement with Sardinha et al. and Gulliford et al. in which they reported there was a positive association between physical activity and HbA₁c. A negative association between the physical activity and HbA₁c was reported by Boeing et al. and Modan et al. the findings of the current study were disagreement with them.
CONCLUSION

The normal range value for HbA1c in Sudan is between 1.2% and 5.4%.

HbA1c is increased by smoking and decreased with exercise and physical activity. In conclusion, the results of this study confirm that there are difference values of HbA1c between the Sudan and international values, indicating that the normal values for HbA1c in Sudanese need to be revised and should be lower than those currently used. Large-scale studies to establish all normal Sudanese physiological values are needed and, especially for HbA1c.

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Conflicts of interest

There are no conflicts of interest.

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