

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 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 HbA_{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 HbA_{1c} was $3.8\% \pm 1.17$ with a range of 1.2–5.4%. There was no correlation between Hb level, daily caloric intake, and the level of HbA_{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 HbA_{1c} ($r = 0.07$), but the family history, smoking, and physical activity were found to be positive statistically significant to HbA_{1c} ($P < 0.05$). The mean of HbA_{1c} in smokers was $3.8\% (\pm 1.4\%)$ and in nonsmokers was $3.1\% (\pm 1.0)$. The mean HbA_{1c} of those with positive family history of DM $4\% (\pm 0.8\%)$ was significantly higher than those with negative family history $3.5\% (\pm 1.3\%)$. The mean of HbA_{1c} in physically active participants was $3.9\% (\pm 0.9)$ and in the nonactive participants was $3.1\% (\pm 1.3)$. **Conclusion:** The results of this study indicate that there is a difference in values of HbA_{1c} between the international values and Sudan, so the normal values for HbA_{1c} in Sudanese need to be established from a large sample.

Key words: HbA_{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 HbA_{1c} should be kept below 7% in all diabetics^[2] and according to the same guidelines, HbA_{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 HbA_{1c} in diabetes was first described in 1969 by Samuel Rahbar.^[4] 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 HbA_{1c} 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 HbA_{1c} over values observed in nondiabetic participants.^[6]

Thus, by the mid-1970s, it was clear that HbA_{1c} is elevated in humans with diabetes mellitus (DM) although the mechanism of this abnormality was not understood. HbA_{1c} 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 HbA_{1c}. In 1989, Simon *et al.* reported the distribution of HbA_{1c} in 3240 healthy adult population. They found an approximately normal distribution of HbA_{1c} is 5.03% (± 0.53).^[9] In England in 2001, Gulliford *et al.* performed a cross-sectional survey and found a mean (standard deviation) HbA_{1c} of 6.34% (± 0.85) in a general population of 9772 nondiabetic, European origin participants aged 16 years and older.^[10] In 2011, Hill *et al.*, in the UK, reported the normal range of HbA_{1c} 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 HbA_{1c} level.

According to a report published in 2009 by an International Expert Committee on the role of HbA_{1c} in the diagnosis of diabetes, HbA_{1c} can be used to diagnose diabetes and that the diagnosis can be made if the HbA_{1c} level is 6.5% or more and HbA_{1c} level below 6% is considered normal.^[12]

HbA_{1c} 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 - HbA_{1c}) - nondiabetic “normal” range being 4–6%.^[13]

Diagnostic criteria for diabetes that made by the WHO report 2011 determined that HbA_{1c} 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 HbA_{1c} was associated with the age has been showed by Pani *et al.* who performed a cross-sectional analysis of HbA_{1c} across age categories in 2473 nondiabetic persons. They stated that their results establish clearly that HbA_{1c} 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 HbA_{1c} with age.^[15] Furthermore, in March 1999, Wiener and Roberts performed a cross-sectional analysis to resolve whether hemoglobin A_{1c} (HbA_{1c}) levels in normal participants increase with age, they measured HbA_{1c} in 399 patients undergoing routine oral glucose tolerance test. None of these groups showed a significant correlation between HbA_{1c} and age; therefore, they cannot see a need for age-specific reference ranges for HbA_{1c}.^[16]

Racial and ethnic differences in HbA_{1c} 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 HbA_{1c} have been also described by Herman in 2009.^[18] Ziemer *et al.* found higher HbA_{1c} 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 HbA_{1c} levels.^[19] In the USA, Boltri *et al.* analyzed data from the 1999 to 2000 National Health and Nutrition Examination Survey and described HbA_{1c} in persons with undiagnosed and diagnosed diabetes by race/ethnicity. They found the Hispanics with undiagnosed diabetes were more likely to have HbA_{1c} $\geq 7\%$ (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 HbA_{1c} in obese persons (defined as BMI >28 kg/m²), but after adjustment for age, the relation between BMI and HbA_{1c} was no longer significant.^[9] Modan *et al.* found no significant correlation between BMI and HbA_{1c},^[21] but in contrast, in the cross-section study conducted by Boeing *et al.*, they found that obesity found to be related with higher HbA_{1c} levels.^[22]

The effect of diet on HbA_{1c} level has been studied by many researchers. Boeing *et al.* also studied the association of diet and other lifestyle factors with HbA_{1c} examined in a nondiabetic adult population and found that there is a direct relation between risk of increase HbA_{1c} and high energy and energy-adjusted saturated fat intakes.^[22] Modan *et al.* found a similar result which is there is no correlation between the intake of any specific food component and

HbA_{1c}.^[21] Furthermore, Harding *et al.* found independent associations between HbA_{1c} concentration across the normal range of HbA_{1c} and both total fat intake and the pattern of dietary fat intake.^[23]

The level of physical activity is known to influence insulin resistance,^[24] and thereby possibly may influence HbA_{1c}. Gulliford *et al.* found a 0.180% lower HbA_{1c} in participants who were vigorously active compared with the inactive participants, and HbA_{1c} gradually decreases with increasing level of physical activity.^[10] However, other studies were done by Modan *et al.* and Boeing *et al.* found no association between the level of physical activity and HbA_{1c}.^[21,22]

Smoking is well known to affect many body systems and thereby possibly may influence HbA_{1c}; Koga *et al.* found no association between smoking and HbA_{1c} levels,^[25] but several studies have documented that smoking is associated with higher HbA_{1c} levels.^[10,21] In another study, Sargeant *et al.* performed a cross-sectional analysis of the association between cigarette smoking and HbA_{1c}, 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 HbA_{1c} levels and number of cigarettes smoked per day and a positive association with total smoking exposure as measured by pack-years.^[26]

Despite this variation, the normal level of HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} analysis. Icteric, lipemic, hemolyzed, or bacterially contaminated sample was not used.

HbA_{1c} 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 glycosylated Hb. When the blood is added to the reagent, the erythrocytes immediately lyses. All Hb precipitates the boronic acid conjugate binds to the cis-diol configuration of glycosylated Hb. The precipitate is evaluated by measuring the blue (glycosylated Hb) and the red (total Hb), color intensity with the NycoCard Reader II, the ratio between them being proportional to the percentage of HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} (*r* = 0.07).

The mean of HbA_{1c} 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 HbA_{1c} - 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 A_{1c} - ($r = 0.32$). 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 HbA_{1c} 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 HbA_{1c} with high statistically significant ($P = 0.001$).

Seven participants were smokers and 13 were not. The mean of HbA_{1c} in smokers was 3.8% (± 1.4) and in nonsmokers was 3.1% (± 1.0). There was a strong association between HbA_{1c} and smoking ($P = 0.000$).

The results showed that there was a significant correlation between physical activity and HbA_{1c} with $P = 0.000$. Ten participants were physically active and the other ten were not. The mean of HbA_{1c} 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^[27] and respiratory function test parameters.^[28] As HbA_{1c} is now used largely for DM control, the normal values of HbA_{1c} in Sudanese have been addressed by this pilot study.

A_{1c} samples were analyzed on one laboratory as differences in results have been observed, and the results outcome showed that the normal mean distribution of HbA_{1c} in Sudanese adult males was 3.8% ($\pm 1.17\%$) and the range was 1.2–5.4% which is lower than in international range (3.6–6.5%).^[9,10,11,13] 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 HbA_{1c} that reported by studies of Ziemer *et al.* and Boltri *et al.*^[19,20]

If the maximum is 5.4% for HbA_{1c} in Sudanese, then the cutoff point of 6.5% which recommended by the WHO for DM diagnosis need to be revised.^[14]

There is a weak statistical correlation between age and HbA_{1c}. Although the mean of HbA_{1c} 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^[16] which showed there is no significant correlation between HbA_{1c} and age and not with Pani *et al.*^[15] study.

There was no correlation between daily caloric intake and the level of HbA_{1c}; these findings were in good agreement with those obtained by Modan *et al.*^[21] and Harding *et al.*^[23] Furthermore, these findings were disagreement with Boeing *et al.*^[22] who was found that there is a direct relation between risk of increase HbA_{1c} 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 HbA_{1c} and BMI. This result agrees with Boeing *et al.*^[22] study that showed the obesity found to be related with higher HbA_{1c} levels and disagrees with studies of Modan *et al.*^[21] and Simon *et al.*^[9] From our study, we can establish normal level of HbA_{1c} (1.2–5.4%) across normal BMI as a guide for whom may study the effect of obesity on the level of HbA_{1c} in Sudanese people in the future.

The high values of HbA_{1c} in participants with family history of DM need further elaboration.

There was a strong association between HbA_{1c} and smoking, the mean of HbA_{1c} in smokers was higher from the mean of in nonsmokers. Many studies proved that the HbA_{1c} levels are increased by smoking^[10,21,26] which in line with our findings, but we disagree with Koga *et al.* who reported that there is no association between HbA_{1c} and smoking.^[25]

In this study, the physical activity level was affected HbA_{1c} significantly ($P = 0.000$). The mean of HbA_{1c} in physically active participants was higher than in nonactive participants. These findings were in agreement with Sardinha *et al.*^[24] and Gulliford *et al.*^[10] in which they reported there was a positive association between physical activity and HbA_{1c}. A negative association between the physical activity and HbA_{1c} was reported by Boeing *et al.*^[22] and Modan *et al.*,^[21] the findings of the current study were disagreement with them.

CONCLUSION

The normal range value for HbA_{1c} in Sudan is between 1.2% and 5.4%.

HbA_{1c} 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 HbA_{1c} between the Sudan and international values, indicating that the normal values for HbA_{1c} 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 HbA_{1c}.

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Conflicts of interest

There are no conflicts of interest.

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