Normal Values of Hemoglobin A_{1c} among Women in Khartoum State: (A Pilot Study, 2016)

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Abstract: _Introduction: Currently no local studies have been conducted to investigate the normal reference range of HbA_{1C} in Sudanese women, and the normal reference range of HbA_{1C} is obtained from international data.These data were largely based on western population which is different from Sudan in their environment, ethnic groups, nutritional habitsand body mass index.Most of the studies about HbA_{1C}conducted in Sudan generally or Khartoum state specifically were concentratingon abnormal HbA_{1C}rather than the normal values. Objectives: The research aimed to measure the normal values of haemoglobin A_{1C} in Sudanese women living in Khartoum State during the period from May 2016 to July 2016,and to compare the normal values of HbA_{1C} in Sudanese women to that found in international references andto determine the relation between HbA_{1C} level and Hb level in non diabetic Sudanese women in Khartoum state. Methods: A cross sectional, community based study was conducted in Khartoum state during May 2016 to July 2016 among healthy Sudanese adults, non-diabetic female volunteers above 30 years old. Twenty women were included as a pilot. After their consent they were assessed via a questionnaire covering age, occupation, chronic illness, menstrual cycle, pregnancy, number of kids, exercise, daily caloric intake for the last three days and smoking history. Body mass index (BMI) was calculated using BMI formula based on metric unit by measuring weight and height. BMI= weight (kg)/ height^2(m^2). Arterial blood pressure was checked for each volunteer to exclude hypertension. Blood samples were analyzed for FBG (to exclude DM), Hb level and glycosylated hemoglobin level by colorimetric reaction using machine TWIN $_{AIC\ analyzer}$. Correlations between the variables were estimated and P value less than 0.05 was considered statistically significant. Results: The mean of HbA_{1c} was found to be 3.43 % +1.17 with a range of (1.4% - 5.3 %). There was no correlation between Normal FBG, Hb level, daily caloric intake, age and the level of HbA_{1c}. The mean of the BMI was 25.09 with a maximum of 31.9 and a minimum of 16.46 with no correlation between HbA_{1c} and BMI. The mean of HbA_{1c} in physically active participants was 3.9 % (+0.9)and in the non active participants was 3.1 % (+1.3). Conclusion: According to this study the normal values of HbA_{1c} in Sudanese women is found to be different from the international values._

Keywords: HbA$_{1c,}$ Hb, FBG, BMI, DM.

1. Introduction

The term glycosylation refers to the covalent bonding of blood glucose to the red blood cells. Normally, only a small percentage of blood glucose, usually between 4.5%- 6%, is covalently linked to the red blood cells in hemoglobin of the non diabetic population.

This value is commonly referred to as glycosylated hemoglobin or more specifically HbA$_{1C}$. The quantification for HbA$_{1C}$ is based on the total accumulation and reaction between glucose and red blood cells over their life span of 90-120 days. Apparently, short term or hourly elevations in blood sugar levels do not seem to acutely affect the total HbA$_{1C}$ value. This may be due to the suspected slow rate in which glucose and hemoglobin combine[1].

In 1958, HbA$_{1C}$ was isolated from other types of Hb for the first time by Huisman &Meyering using chromatography [2].In 1968,SamuaelRahbar mentioned that HbA$_{1C}$ is increased in diabetes [3].Rahbar at Tehran university found the same abnormality in 57 diabetic patients in a cross sectional study [4]. The reactions leading to its formation were characterized by Bunn and his coworkers in 1975 [5] .The use of HbA$_{1C}$ for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and coworkers [6] .

HbA$_{1c}$ was introduced into clinical use in the 1980s and subsequently has become an important test in Clinical practice [7].

In 1989 a study was conducted by Simon on 3240 healthy, non-diabetic adult. According to his study, the normal HbA$_{1C}$ was found to be (5.3) %. He found that there is no gender variation of HbA$_{1C}$although levels in females tend to increase particularly at the age of menopause.The use of the oral contraception made no difference [8].

The synthesis rate of HbA$_{1C}$ is positively related to the glucose concentration of red blood cells and HbA$_{1C}$ can reflect the mean blood glucose level within the past 8 to 12 weeks. HbA$_{1C}$ can continue in the whole lifespan of red blood cells (120 days)[9].

HbA$_{1C}$ level is not affected by acute changes in blood glucose and the interval time between prior meal and HbA$_{1C}$ measurement. This is why it is considered stable, not influenced by the time of blood collection, fasting status and use of insulin [9].

In addition to measuring glucose in blood, HbA$_{1C}$ detection is an established tool in the assessment of glycemic control [9]. The concentration of HbA$_{1C}$ depends on the average plasma glucose level over the period of erythrocyte life span [10]. By measuring glycosylated hemoglobin, clinicians are

able to get an overall picture about the average of blood sugar levels over this period. The higher the HbA_{1C}, the greater the risk of developing diabetes-related complications [11].

In the 59th annual meeting of Diabetes Association of the USA, HbA_{1C} detection was recommended as the gold standard for evaluating the glucose control. In non-pregnant women,normal $HbA_{1C}$is considered to be between 4.7-6.3%. However, in pregnant women HbA_{1C} might be lower. Pregnant women are younger and the fasting blood glucose increases over age. Thus, relatively older, healthy non-pregnant women may have higher HbA_{1C}. The lifespan of red blood cells is reduced in pregnant women (including those with diabetes mellitus), resulting in reduction in HbA_{1C} [12].

In recognition of the importance and reliability of HbA_{1C}, the American Diabetes Association Standards of Medical Care in Diabetes considered levels ≥ 48 mmol/mol (≥6.5%) as another criterion for the diagnosis of diabetes[13]. On the other hand and because of the association of higher levels of $HbA_{1C}$with cardiovascular disease, nephropathy and retinopathy [14], the American Diabetes Association (ADA) recommended that the value of HbA_{1C} should be kept below 7% in all diabetics [15].

Although the HbA_{1C} reference values for the general population in Western countries are well established, values for healthy Sudanese including women are not clearly defined. The majority of studies about HbA_{1C}that wereconducted in Sudan generally or Khartoum state specifically were considering HbA_{1C} outside the normal physiological conditions such as HbA_{1C} in pregnancy, diabetes mellitus, anaemia or patient with abnormal Hb structure.In these studies, the judgment of any deviation from the normal values was based on the international reference and not on solid local figures that were derived from local studies putting in consideration the possibility of differences, simply because of lack of such studies.

In 1989, a study was conducted about Glycosylated Hemoglobin Levels in Sudanese Sickle Cell Anemia Patients in Khartoum state. Glycosylated hemoglobin was measured, by a colorimetric method in 49 patients with sickle cell anemia attending Khartoum Teaching Hospital. The level obtained (4.9% ± 1.3) was significantly lower than the control value (5.6% ± 0.2; p less than 0.0025) [16].

Pregnancy is one of the factorsthat affect HbA_{1C}. Generally it is believed that HbA_{1C} is lower in pregnant females compared to non pregnant. In a study conducted in Khartoum, Sudan the mean concentration of the HbA_{1C} in pregnant women was (4.407±1.044) % in first trimester, (4.797±0.621) % in second trimester, (4.823±0.616) % in third trimester and (5.660 ±0.461%) in control group (P value was significant). The study also showed that the mean concentration of HbA_{1C} of the first trimester is lower than that of the second and third trimesters, but there was no significant difference between the mean concentration of the second and third trimester [17] .Compared to the international values, HbA_{1C} in pregnant Sudanese women was lower during all trimesters in non-diabetic.

O'Kane et al. proposed that the reference range of HbA_{1C} is 4.1-5.9% in pregnant women without DM, and in the first, second and third trimesters, the HbA_{1C} was 5.1%, 4.9% and 5.0%, respectively [18]. Mosca et al. conducted study in Italy and found that HbA_{1C} reference intervalis 4.0%-5.5% for pregnant non-diabetic women and 4.8%-6.2% for non-pregnant controls. The HbA_{1C} results for non-diabetic pregnant women at different gestational periods were 3.8-5.5% at 15-24 weeks, 4.0-5.5% at 25-27 weeks, and a small but significant increase in HbA_{1C} values at 28-36 weeks, 4.4-5.5% [19].

Study conducted in postmenopausal women with type 2 diabetes mellitus showed that glycosylated hemoglobin decreases significantly after estrogen replacement therapy (ERT) (-3%, P=.03)[20].

Normal level of HbA_{1c} (in non diabetics) can be affected by a number of physiological variables. Among these, the proposed and well studied factors include age, race, BMI and exercise.

In March 1999K.Wiener and N.B.Roberts performed a cross-sectional analysis to resolve if HbA_{1c}levels in normal subjects increase with age. They did not found significant correlation between HbA_{1c} and age[21]. On other hand a cross-sectional analysis of HbA_{1c} across age categories in non-diabetic participants from 2001 to 2004 showed a positive association between age and HbA_{1c}[22].

Considering race, a Meta-Analysis study on 391 articles published during 1993 to 2005 showed that the higher HbA_{1C} observed among African Americans compared with non-hispanic whites [23].This observation was supported also by Ziemerwho found a higher HbA_{1c} levels in black population compared to the whites[24].

The body mass index(BMI) is a useful tool to estimate a healthy body weight. According to the WHO, normal BMI for adult is (18.5-25)kg/m^2. Obesity (>30) kg/m^2 is a risk factor for diabetes due to insulin resistance. A Japanese study in 2006 showed a significant positive correlation between BMI and levels of HbA_{1c} in non-diabetic subject[25]. This is not supported byModan et al. who found no significant correlation between BMI and HbA_{1c} [26].

Exercise is known to decrease levels of glucose in the blood and to improve symptoms of diabetes,thus wasproposed to affect HbA_{1c}. A study on 32 African –American diabetic women found significant differences between exercisers and non-exercisers [27]. This finding was confirmed by a study of two groups with type 2 diabetes in Jordan. Over a period of 10 weeks, there was a significant reduction in post-exercise blood glucose and HbA_{1c}, however the grater reduction was noted in the resistance exercise group than the group received treadmill exercise [28] .

Despite this variation, the normal level of HbA_{1c} was generally taken as less than 6% and these values were applied to diabetic patients in Sudan. Normal level of HbA_{1C} reflects the long term mean blood glucose. The sensitivity of HbA_{1C} in the diagnosis of DM is relatively low when compared with OGTT, and thus normal HbA_{1C} may not be

used to exclude DM or pre-DM. Thus, OGTT is still a golden standard in the diagnosis of DM. Some problems with OGTT are reproducibility of glucose measurements, relating to pre analytical and analytical variables, the different population characteristics and different OGTT and HbA_{1C} thresholds in different countries. Therefore, normal OGTT result does not always exclude DM and false-negative rate of the OGTT cannot be accurately calculated. At the same time, published data raise questions about a possible role for HbA_{1C} in high risk women with anon-diagnostic OGTT.

The correlation coefficient between HbA_{1C} and blood glucose is 0.80. Generally, HbA_{1C} of 6% is equivalent to blood glucose of 135 mg/dl (7.5 mmol/l). An elevation of HbA_{1C} by 1% reflects an increase of blood glucose by 35 mg/dl (1.95 mmol/l) [29].

HbA_{1C} is determined by FBG and postprandial blood glucose. When HbA_{1C} is < 7.3%, postprandial blood glucose contributes more influence on HbA_{1C}. When HbA_{1C} is 7.3-8.4%, FBG and postprandial blood glucose have same contribution on HbA_{1C}. When HbA_{1C} is > 8.5%, FBG contributes more on HbA_{1C}.

HbA_{1C} is expressed in % in NGSP (National Glycohemoglobin Standarization Program and mmol/mol in IFCC (Inernational federation for clinical chemistry).

HbA_{1C} can indicate people with prediabetes or diabetes as follows:
- Normal: Below 42 mmol/mol (6.0%).
- Prediabetes: 42 to 47 mmol /mol(6.0% to 6.4%).
- Diabetes: 48 mmol(6.5%)**.**
Due to the documented racial difference this study aimed at investigating the normal values of HbA_{1C} for Sudanese women to be used as a local reference.

2. Methods

A cross-sectional, analytic community based study among twenty healthy Sudanese adult, non-diabetic female volunteers above 30 years old was performed in Khartoum state, capital of Sudan during May 2016 to July 2016. Ethical Approval of this study was obtained from the National Ribat University NRU. All volunteers were not diabetics, not pregnants, not smokers or known to have any type of haemoglobinoapathy. Informed consent was taken from each volunteer, after a clear explanation about the objectives of the study.

The participants were assessed via a questionnaire covering age, occupation, chronic illness, menstrual history, pregnancy, parity, exercise, daily calorie intake for the last three days and smoking history.

After an overnight, each volunteer was assessed clinically by physical examination. Arterial blood pressure was determined using the auscultaotry method. Weight was measured by a sensitive balance in Kg and height by a metric scale in meters.BMI was calculated using the formula: weight (kg)/ height (m^2).

Five ml of venous blood were collected by the standard procedure from each participant under complete aseptic conditions.2.5 ml were placed in fluoride oxalate containers, and then used for FBG measurement. The other 2.5 ml were placed in EDTA container and used for Hb and HbA_{1c} analysis.

HbA_{1C} level was measured using TWIN A_{1C} analyzer.The calibration is used radio frequency technology. 2 ml of lytic reagent was added to 20 μl of blood and was incubated for 10 min to produce Hemolysate blood, from which 5μl was mixed with 375μl of first reagent. The mixture was located in reading location for processing. After 2 minutes, 75 μL of second reagent was added and the result was recorded.

All the data collected in this study was analyzed using the SPSS (Statistical Package for Social Sciences) 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 , 1= strong correlation).

P value < 0.05 was considered statistically significant.

3. Results

A total of 20 eligible females from Khartoum state were studied. Of these, 15 were between 30-55 years old and 8 of them were above 55 years old.
- The participants were healthy and had no chronic illness.
- The mean of HbA_{1C} was 3.43% with standard deviation of 1.13.
- The maximum value was 5.3% and the minimum was 1.4% (table 1).
- The exercise for most of participants was not structured exercise but most of them were physically active, 65% of them answered that they are practicing exercise as a part of their housekeeping and work. Figure 3
- No correlation was found between FBG, Hb level, daily calorie intake, BMI, and the level of HbA_{1c}. Table 2 shows that the all P-values are greater than (5%).
- No correlation was found between age and the level of HbA_{1c}. Table 3

4. Discussion

According to our study, Sudanese females living in Khartoum statehave clearly lower normal values of HbA_{1C} when compared to international reference values. Subsequently, using these international thresholds in our local population might result in under diagnosing diabetes and slowing the recognition of cases of poor control subjecting them to complications of the disease before aggressive measurement can be instituted

The normal mean of HbA_{1C} in Sudanese adult female was 3.4% and the range was (1.4%-5.3%), this is lower than

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international range (4.7-6.3) [12]. Most likely this large differences is due to racial, ethnic and genetic factors.

Our results mirrors result of similar studies conducted in some population of similar different racial backgrounds [23][24]

American medical association added HbA_{1C} as a criterion in the diagnosis of diabetes mellitus and considered 6.5 as the cut off point for diagnosis [13]. Based in our findings, the maximum HbA_{1C} for Sudanese females is 5.3%, a figure with a significant gap from 6.5 and still lagging away from the international threshold for the diagnosis of any evolving diabetes. So one can expect that relying on international reference values, will diagnose diabetes in this group only when HbA_{1C} increased significantly indicating poor control.

The age was above 30years old, there was weak correlation between age and HbA_{1C}. There was no correlation between HbA_{1C} and daily calorie intake (r=.-0.13)(p-value:0.5) same as the finding byA.E.Buyken [30].

Minimum BMI was 16.4 and the maximum was 31.9 the mean was 25.09 in relation to A_{1C} r=0.2 , p-value :0.3 ,Boeing et al mentioned that increasing in BMI results in increasing in HbA_{1C} [31].

In this study most of the participants were physically active according to their jobs but we did not compare between the physical activity and HbA_{1c} statistically because the exercise was not structured .Most of studies mentioned the importance of exercise in lowering HbA_{1C}, but negative association was reported by Modan [26].

5. Conclusion and Recommendations

The normal range value for HbA_{1c} in Sudanese female between (1.4 % – 5.3%). HbA_{1c} is not affected by daily calorie intake, BMI, Hb level, or FBG may be due to the small sample size .

In conclusion, The international normal references of HbA_{1C} cannot be applied in Sudanese females because of the large difference between values.

Sudanese reference for normal range of HbA_{1C} must be available in all laboratories for clinical use. We recommend more studies with large sample size to be conduct in different states of Sudan.

6. Acknowledgment

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8. Conflicts of interest

There are no conflicts of interest.

9. Ethical approval

The study was approved by the Institutional Ethics Committee

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Table 1: Statically analysis of HbA_{1c} and other variables

Variables	Minimum	Maximum	Mean	Std.deviation
BMI	16.46	31.90	25.09	4.21
Hb	9.50	13.50	11.87	1.35
FBG	48.00	110.00	83.25	19.36
HbA_{1c}	**1.40**	**5.30**	**3.43**	**1.13**

Table 2: The simple correlation factor

variables	Pearson correlation	HbA_{1c}	Statistical significance
Age	Correlation	0.078	Insignificant
	P-value	0.742	
Weight	Correlation	0.160	Insignificant
	P-value	0.501	
Height	Correlation	0.119	Insignificant
	P-value	0.618	
BMI	Correlation	0.229	Insignificant
	P-value	0.332	
Hb	Correlation	0.032	Insignificant
	P-value	0.892	
FBG	Correlation	0.056	Insignificant
	P-value	0.814	

HbA_{1c}& Age groups:

Table 3: HbA_{1c} & age groups

Age groups	HbA_{1c}
26 – 35	3.5 ±1.1045
36 – 45	3.2 ± 1.07
46 – 55	3.5 ± 1.53
Above 55	3.64 ± 1.33

Figure1compares the normal mean of HbA_{1C}in the study population with international values.
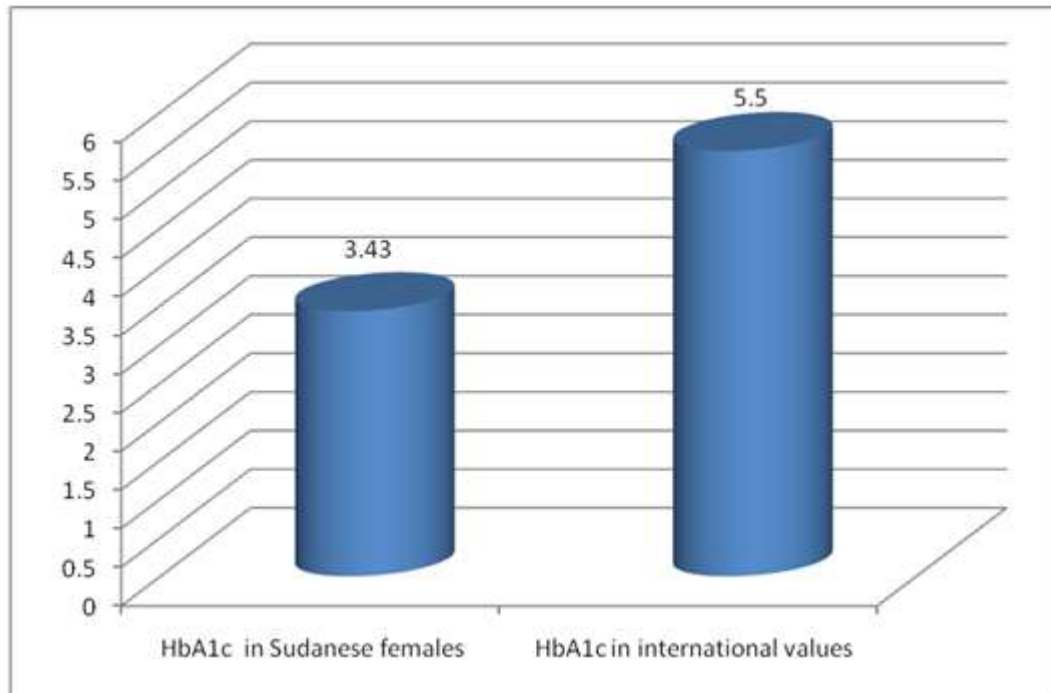
Figure 2: Show the difference between the normal range and mean of HbA_{1C} in Sudanese females and the international values.
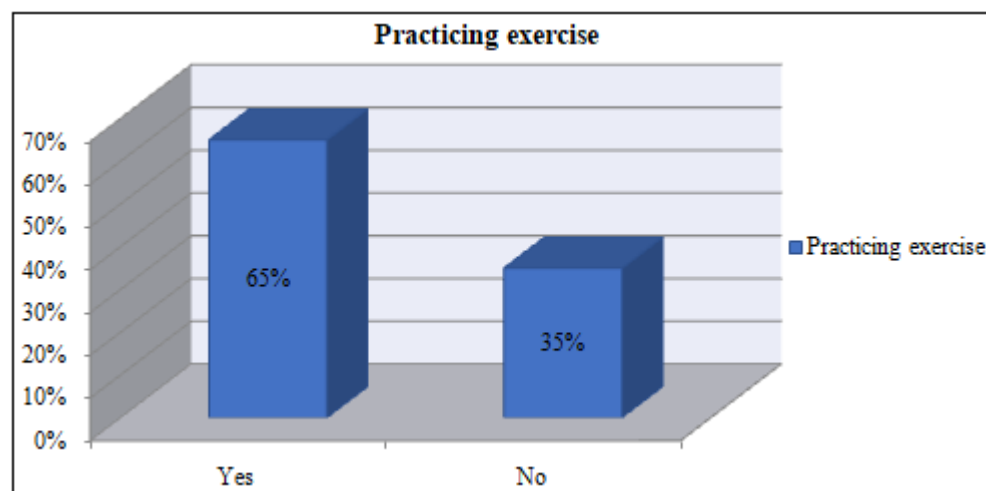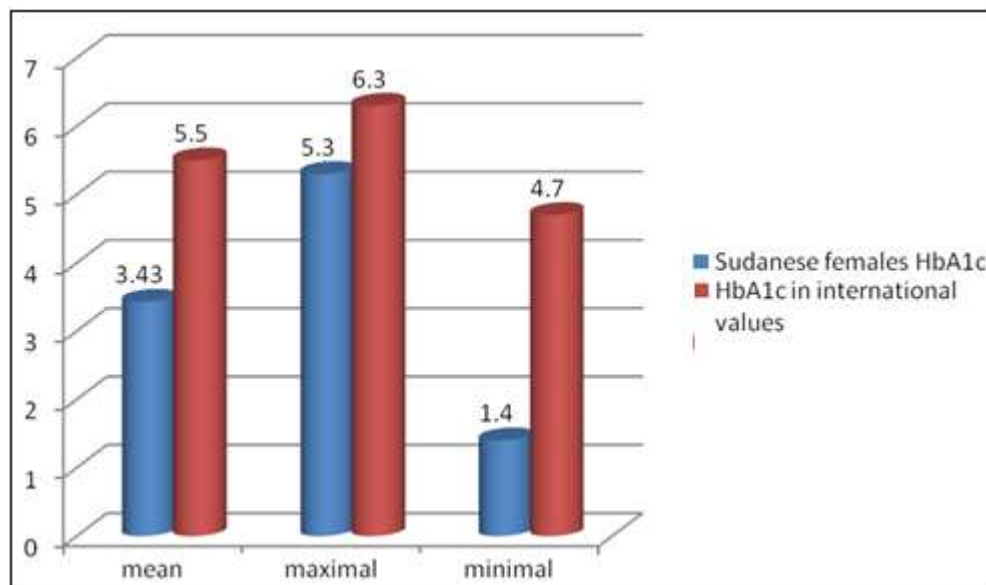

Figure 3: exercise percentage, source prepared by the researcher (Excel tool).