Determining the Reference Range Values of Glycosylated Hemoglobin (HbA1c) by Immunoturbid Assay in Iraqi Population

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Abstract

Hemoglobin A1c is the most parameters for the monitoring of metabolic control of patients with diabetes mellitus. The aim of this study is to determine the reference range of glycosylated hemoglobin (HbA1c%) in an Iraqi population (males and females) by using immune method with agglutinations.

Blood samples were collected from 100 healthy subjects (50 females and 50 males), age ranged between (20-75) years old. The reference value of HbA1c % was (5.34 ± 0.67) % in females and (5.67 ± 0.73) % in males. The present study found a very high positive significant difference between HbA1c % and BMI in both gender. A positive significant was observed between HbA1c % with systolic and diastolic blood pressure in males, whereas there is no significant differences between HbAIc % and blood pressure in females.

الخلاصة

يعد الهيموغلوبين من نوع A1c من اكثر المتغيرات المستخدمة للسيطرة في مراقبة العملية الايضية في مرضى السكر. ان الهدف من هذه الدراسة هو تعيين مدى القيم الطبيعية للهيموغلوبين A1c (HbA1c%) لدى السكان العراقيين من الذكور والاناث وياستخدام طريقة تلازن الاختبار المناعي. جمعت نماذج دم من 100 شخص سوي (50 اناث و 50 ذكور) تراوحت اعمارهم بين 20- 75 سنة. كان مدى القيم الطبيعية لهيموغلوبين A1c في الاناث (5.34 ± 0.67) % وفي الذكور (5.67 ± 0.73) %. وجد في هذه الدراسة بان هناك علاقة معنوية موجبة بين HbA1c % ودليل كتلة الجسم في كلا الجنسين. لوحظ بان هناك علاقة معنوية موجبة بين HbA1c % وضغط الدم الانقباضي والانبساطي في الذكور ، في حين لم يلحظ اي اختلاف معنوي بين HbA1c % وضغط الدم في الاناث.

Introduction

Glycated or glycosylated hemoglobin A1c, HbA_1c, A1C, or Hb_1c, HbA1c, formed through the non enzymatic binding of circulating glucose to hemoglobin. Higher levels of glucose in blood contribute to more binding and consequents higher levels of glycosylated hemoglobin [1, 2]. Glycation occurs over the entire 90-120 day life span of the red blood cell [3]. HbA1c can consequently be interpreted as an average of the blood glucose present over the past 3-4 months [3]. Measurement of HbA1c is accepted as a useful index of mean blood glucose in the treatment of patients with diabetes [4, 5]. HbAIc < 6% is considered normal [4], its level as low as 6.2% have been cited as a risk threshold values for the development of cardiovascular complications [6, 7]. There are a number of techniques used to measure HbA1c, such as a high-performance liquid chromatography (HPLC) [2], immunoassay, affinity chromatography. Conversion between the units is by the following equation [8]:

IFCC- HbA1c (mmoL/moL) = [DCCT-HbA1c (%)− 2.15] x 10.929

IFCC: International Federation of Clinical Chemistry Units

DCCT: The Diabetes Control and Complications Trial

SUBJECTS AND METHODS

SUBJECTS

Specimen collection

Venous blood samples from 100 healthy subjects donors (random Iraqi people from urban and rural, which include 50 females and 50 males) were collected in EDTA coated tubes and were stored at 4 C° until analysis which occurred with the maximum of 7 days.

Methods

Determination of HbA1c concentration

HbAIc was measured by immunoturbidimetric assay with automatic analyzer (ACCENT-200 for HbA1c directed kit, PZ Comary, Poland).

The present method utilizes the interaction of antigen antibody to directly determine the HbA1c concentration in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse anti human HbA1c monoclonal antibody is added, latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat anti- mouse IgG polyclonal anti body interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on the surface of latex particles. The amount of agglutination is measured as absorbance on λmax 630 nm. The HbA1c value was obtained from a calibration curve, as shown in Figure (1).

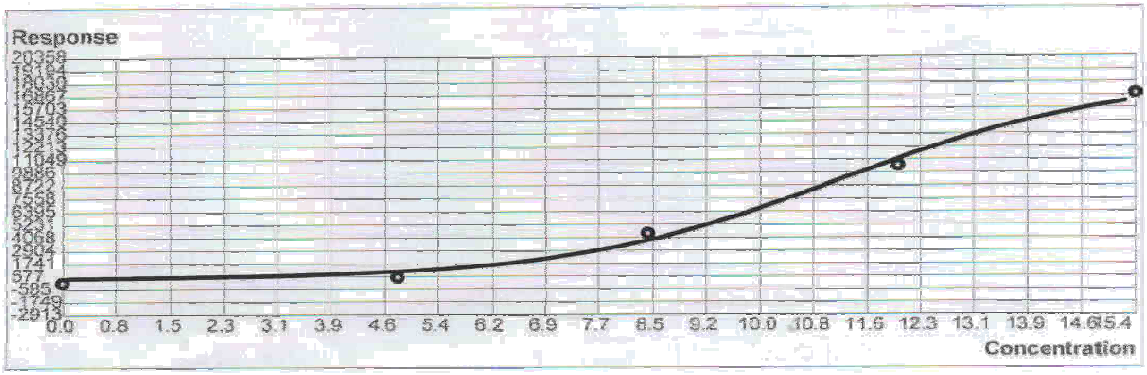

Figure (1): Standard curve for determination of HbA1c %.

Calculation:

The HbA1c % was calculate according to the following equation:

$$R = R_0 + K \frac{1}{1+\exp[-(a + b \ln C + cC)}$$

Where:

Rule = Logistic – Log 5P R= Response R_0= Response to calibrator zero

K= constant which equal to 17997.08952 a= -6.02419

b= 0.48275 c= absorption C= concentration

Measurment of Blood pressure Level

The levels of blood pressure were done after 5 minutes of resting using mercury sphygmomanometer then take the mean of two reading for both systolic and diastolic blood pressure [9]. The mean of 2 readings was used for classification of blood pressure into optimal (systolic < 120 mm Hg and diastolic < 80 mm Hg), normal (systolic 120 to 129 mm Hg or diastolic 80 to 84 mm Hg).

Measurment of Weight and Height

The weight and height were measured using DETECTO- MEDIC by BROOKLYN. N. Y. USA. Body Mass Index (BMI) was calculated as the weight in kilogram (Kg) per height in meter squared (m^2) [10].

BMI (Kg / m^2) = Weight (Kg)/ Height (m^2)

Statistical Methods

The data was analyzed on the computer statistical programmed SPSS version 13. The mean ± SD was also computed for the comparison of results. The comparison between two groups was tested by Students' t- test; whereas one – way analysis of variance ANOVA was used for comparison data in three groups. Results were considered statistically significant in p value is less than 0.05.

Results and Discussion

Glycated hemoglobin is recommended for both (a) checking blood sugar control in people who might be per diabetic and (b) monitoring blood sugar control in patients with more elevated levels termed diabetes mellitus [11]. This study provides a reference values for HbA1c distribution among subjects aged 20-75 years old in Iraq, who were evaluated by age , gender, schooling region (urban and rural), job, BMI, and blood pressure. Table (1) shows a measurements of different parameters used in this study as mean ± SD.

Table (1): Data (mean ± SD) of Age, BMI, HbA1c, Diastolic and Systolic Blood Pressure.

Parameters	Gender	Number	mean	SD	SE	P
Age (Years)	F*	50	36.88	13.81	1.99	0.428
	M**	50	39.14	14.25	2.04	
BMI (Kg/m^2)	F*	50	28.25	5.04	0.71	0.530
	M**	50	28.89	5.06	0.72	
HbA1c %	F*	50	5.14	0.67	0.16	0.305
	M**	50	5.33	0.73	0.17	
Diastolic BP (mmHg)	F*	50	77.35	8.11	1.16	0.0001
	M**	50	82.86	6.46	0.92	
Systolic BP (mmHg)	F*	50	122.86	9.79	1.40	0.0001
	M**	50	129.59	12.24	1.75	

F* : Female M*: Male

Table (1) shows there is no significant differences in HbA1c, age, and BMI when compared females to males (P<0.305), (P<0.428), (P<0.530) respectively, while, there was a highly significant differences in both diastolic and systolic blood pressure (P<0.0001). Table (1) refers to the mean levels of HbA1c was 5.14% in females and 5.33% for males, this individual values showed a normal distribution about this mean in Iraqi population because there is no consensus on either a reference method or glycosylated hemoglobin standard, the HbA1c levels in both males and females in Iraqi population have been determined. The data shows there is no significant difference between males and females. The method was found to be both easy to used and very reproducible. The reference range values obtained for the Iraqi male population differ from values of male Kuwaiti population (4.8% ±0.5%) this is may be due to life style of Iraqi population and nutrition system [(2)]. In general the reference range (that found in healthy persons) is about 4% - 4.6% . The 2010 American Diabetes Association Standard for Medical Care in Diabetes added the HbA1c ≥ 6.5% as other criteria for the diagnosis of diabetes [(12)]. Our data revealed there is no effect of gender and region (urban and rural) upon HbA1c %. Table (2) and (3) represent the variable parameters in healthy Iraqi subjects distributing according to age (less and more than 40 years old).

Table (2): HbA1c%, BMI, Diastolic and Systolic Blood Pressure in females blood donors in Iraq.

Parameters	Females	N	mean	SD	SE	P<
Age (Years)	Less than 40	34	29.68	5.98	1.026	0.001*
	More than 40	16	54.36	11.59	3.096	
BMI (Kg/m^2)	Less than 40	34	26.76	4.59	0.788	0.002*
	More than 40	16	31.41	4.57	1.143	
HbA1c %	Less than 40	34	5.18	1.29	0.214	0.020*
	More than 40	16	5.96	0.63	0.155	
Diastolic BP (mmHg)	Less than 40	34	76.06	6.59	1.145	0.112
	More than 40	16	80.00	10.33	2.582	
Systolic BP (mmHg)	Less than 40	34	118.49	6.18	1.077	0.0001*
	More than 40	16	131.88	9.81	2.453	

***P<0.05.**

Table (3): HbA1c%, BMI, Diastolic and Systolic Blood Pressure in blood males donors in Iraq.

Parameters	Females	N	mean	SD	SE	P<
Age (Years)	Less than 40	33	31.06	5.44	0.99	0.001*
	More than 40	17	55.81	12.08	3.02	
BMI (Kg/m^2)	Less than 40	33	27.85	5.01	0.87	0.038*
	More than 40	17	31.02	4.58	1.14	
HbA1c %	Less than 40	33	5.43	1.01	0.17	0.041*
	More than 40	17	6.17	1.41	0.35	
Diastolic BP (mmHg)	Less than 40	33	81.51	5.65	0.98	0.035*
	More than 40	17	85.62	7.27	1.81	
Systolic BP (mmHg)	Less than 40	33	125.45	6.65	1.15	0.0001*
	More than 40	17	138.12	16.41	4.10	

***P<0.05.**

Table (2) shows a significant difference (P<0.02) in HbA1c (5.18% ± 1.24%) in females less than 40 years old and (5.96% ±0.62%) %) in females more than 40 years old. Also there is a significant difference in age, BMI, and systolic blood pressure, while there is no significant difference in diastolic blood pressure in both cases. Our data revealed an age –depended increase in HbA1c%, this result were in good agreements with Hashimoto *et al* [13].

Table (3) shows the mean ± SD of HbA1c% and other parameters which used in this study according to divided the males into less and more than 40 years old. The data obtained revealed to significant differences in all parameters which used in this study in males with ages less than 40 years old compared with males more than 40 years old. The HbA1c% was found to be (5.43% ± 1.01%) in males less than 40 years old, while the percentage of HbA1c found to be (6.17% ± 1.41%) in males more than 40 years old. The results also showed that HbA1c% in males was higher than in females; these results were agreed with Saaddline *et al* [14]. According to WHO (The Guidliness of WHO- ISH, 1999) [15], the optimal blood pressure is (120/80 mmHg) and the normal blood pressure is (130/85 mmHg). In our data the raised in systolic and diastolic blood pressure in both males and females which aged was more than 40 years old, may be due to obesity [16]. Also we found that blood pressure was higher in males than females, our data was agreements with He and Whetton *et al* [17].

Body mass index (BMI) is a measurements that is associated with the body fat, it is also predicts the development of health problems which related to excess weight. For these reasons, BMI is widely used by health care providers [18,19]. In this study the parameters was distributed according to BMI were defined normal (BMI < 25 Kg/m^2), over weight (BMI 25.0 – 29.9 Kg/m^2), and obesity (BMI ≥ 30).

Our data shows no significant differences changes in all parameters which measured in the three groups of healthy Iraqi females which distributed according to BMI. These results may be explained that the mass of the body did not effect on HbA1c % in healthy females population as shown in Table (4).

Table (4): Age, HbA1c%, Diastolic and Systolic Blood Pressure in blood females donors in Iraq which distributed according to BMI.

Parameters	BMI	N	mean	SD	SE	P<
Age (Years)	< 25	17	31.41	11.46	2.78	0.091
	25.0 – 29.9	16	36.46	15.68	4.04	
	≥ 30	17	41.76	13.12	3.18	
HbA1c %	< 25	17	4.92	0.59	0.14	0.073
	25.0 – 29.9	16	5.51	1.19	0.29	
	≥ 30	17	5.78	1.35	0.31	
Systolic BP (mmHg)	< 25	17	120.58	9.66	2.34	0.409
	25.0 – 29.9	16	122.0	10.14	2.61	
	≥ 30	17	125.0	9.85	2.32	
Diastolic BP (mmHg)	< 25	17	75.88	7.12	1.72	0.499
	25.0 – 29.9	16	76.0	9.85	2.54	
	≥ 30	17	78.88	8.32	1.96	

The results in Table (5) showed significant differences between three groups in both HbA1c % and systolic blood pressure. The three groups did not significant differ in age nor in diastolic blood pressure. Our data showed there are only 13 (26 %) males had a normal BMI, about 42% overweight and 32% obese. We observed that the males with normal BMI have a lower reference range of HbA1c% (4.95 %) and approximately optimal blood pressure, whereas in obese males they have a higher reference range in HbA1c% (6.39 %), systolic blood pressure (134.37 ± 17.11), and diastolic blood pressure (85.62 ± 6.29).

The higher prevalence may be risk factors for infection with diabetes mellitus and cardiovascular disease [20].

Table (5): Age, HbA1c%, Diastolic and Systolic Blood Pressure in blood males donors in Iraq which distributed according to BMI.

Parameters	BMI	N	mean	SD	SE	P<
Age (Years)	< 25	13	31.90	11.90	3.85	0.112
	25.0 – 29.9	21	42.76	15.93	3.47	
	≥ 30	16	40.50	11.65	2.91	
HbA1c %	< 25	13	4.95	0.59	0.17	0.004*
	25.0 – 29.9	21	5.56	1.02	0.22	
	≥ 30	16	6.39	1.36	0.34	
Systolic BP (mmHg)	< 25	13	122.72	4.67	1.40	0.042*
	25.0 – 29.9	21	130.47	8.04	1.75	
	≥ 30	16	134.37	17.11	4.27	
diastolic BP (mmHg)	< 25	13	81.81	4.04	1.21	0.127
	25.0 – 29.9	21	82.38	5.38	1.17	
	≥ 30	16	85.62	6.29	1.57	

***P<0.05.**

All major institution like international Expert Committee Report, drawn from the international Diabetes Federation (IDF), the European Association for the study of diabetes (EASD), and the American Diabetes Association (ADA), suggest the hemoglobin A1c level of 6.5 % as a diagnostic level [21].

Conclusion

Our study provided a reference levels for HbA1c distribution among Iraqi population (males and females) aged 20-75 years old and show significant differences in HbA1c levels that are explained by age, gender, BMI, systolic and diastolic blood pressure and demographic variables.

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