COMPARISON OF DIAGNOSTIC ACCURACY OF GLYCATED HEMOGLOBIN (HBA1C) AND 2-HOUR POST GLUCOSE FOR DIABETES USING AMERICAN DIABETIC ASSOCIATION (ADA) CUT OFFS IN PAKISTANI POPULATION

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ABSTRACT

Objective: To determine median HbA1c level in patients screened for DM and compare the diagnostic accuracy of HbA1c with the currently recommended ADA cut off, using 2 hours (h) plasma glucose (2-h PG) post 75 grams oral glucose tolerance test (OGTT) as gold standard.

Study Design: Cross sectional study.

Place and Duration of Study: Aga Khan University Hospital Karachi, from Jul 2014 to Nov 2015.

Patients and Methods: Consecutive subjects screened by OGTT for diabetes mellitus (DM) were included. Blood sample for plasma glucose and HbA1c were analyzed on ADVIA 1800.

Results: Total 146 subjects were included with median age 45 (IQR 54.2-35) years; 53% (n=77) being females. Median HbA1c of the study subjects was 6% (IQR 6.6-5.6). Positive correlation was observed between age and HbA1c [5.7% (IQR 6.2-5.3) <40 y, 6.2% (IQR 6.8-5.8) in >40 y subjects; r 0.34, *p*=0.000). Males had higher HbA1c than females [6.1% (IQR 6.8-5.7) years. 5.9% (IQR 6.4-5.4); r 0.17, *p*=0.036). HbA1c levels were significantly different amongst the different ethnic groups residing in Pakistan (*p*-value<0.03). HbA1c was positively correlated with FPG (r=0.59, *p*-value<0.001) and 2-h PG (r=0.56, *p*-value<0.001). Sensitivity, specificity, PPV, NPV for HbA1c at cutoff of 6.5% in diagnosing diabetes mellitus (DM) were 70%, 89%, 72% and 80% respectively.

Conclusion: High median HbA1c levels is noted in our subjects. At cut off level of HbA1c ≥6.5%, 70% of subjects with DM were diagnosed using ADA criteria of 2-h PG for diabetes as gold standard. Ethnic differences were also observed in levels of HbA1c. There is a need to define cut off for our population.

Keywords: Diabetes Mellitus, Ethnic Groups, Glycosylated Hemoglobin (HbA1c).

INTRODUCTION

Incidence of Diabetes Mellitus (DM) is on rise worldwide. World Health Organization (WHO) has predicted a 170% rise in its incidence in developing countries, which accounts for 228 million patients with DM; consisting 75% of the total world diabetic population[1]. In Pakistan prevalence of DM in three provinces, i.e., Sindh, Baluchistan and Khyber Pakhtunkhwa is 13.9%, 8.6% and 11.7% respectively[2,3]. In Pakistan, prevalence of DM is high ranging from 7.6 to 11%[4]. Diagnosis of DM is a challenge, as patient remains asymptomatic for several years and would not be tested[5,6]. Commonly used diagnostic tests are fasting plasma glucose (FPG) and 75-gm oral glucose tolerance test (OGTT). However, these tests require not only overnight fasting, but results are also affected by calorie restriction, dietary in take of last 72 hours and exercise prior to testing. Also glucose level shows diurnal variation with higher level in morning and lower in afternoon; missing cases of undiagnosed DM if glucose estimation is performed in afternoon[7]. In addition, temperature affects glucose measurement by decreasing level by 3-8mg/dl per hour of glucose, when sample is kept at room temperature[8,9]. Most factors that affect FPG have minimal or no effect on HbA1c level. Short term lifestyle changes, recent food in take or little exercise do not affect HbA1c level. Samples are also stable at room temperature for up to 8 hours and analytical variability is <2%. Added advantage of HbA1c is that it predicts a subject's glycemic control over a period of 2-3 months[9].

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Previously only FPG and OGTT were included in diagnostic criteria for DM. In 2009 an International Expert Committee comprised of representatives of the American Diabetes Association (ADA), International Diabetes Federation (IDF) and European Association for Study of Diabetes (EASD) recommended that HbA1c can be utilized for labeling DM at a cutoff of ≥6.5% and using assay techniques which are certified by National Glycohemoglobin Standardization Program (NGSP) and traceable to Diabetes Control and Complications Trial (DCCT) reference method. In 2011 WHO also endorsed the recommendations of the International Expert Committee. HbA1c is now commonly used for diagnosing DM world-wide, but very few reports have shown its utility in diagnosing DM in our population. As the cutoffs defined for HbA1c are developed in Caucasian population. So, we wanted to evaluate the diagnostic accuracy of the ADA defined HbA1c cutoff in our population. Literature has reported that ethnic differences also affect HbA1c level, and no such study observing differences in HbA1c level due to ethnicities has been done in our population. This study was designed to calculate the median HbA1c level in patients suspected of DM, and compare the diagnostic accuracy of HbA1c with the currently recom-mended ADA cut off, using OGTT as gold standard and to evaluate HbA1c level in different ethnicities of Pakistan.

PATIENTS AND METHODS

A cross sectional study was conducted from July 2014 to November 2015 at the Section of Chemical Pathology, Department of Pathology & Laboratory Medicine and Department of Medicine, Aga Khan University Karachi Pakistan. Study was commenced after taking approval from institution's ethical review committee (ERC no: 2306-Med-ERC-12). All consecutive subjects aged 18 to 65 years, referred for screening of DM by OGTT, at the Clinical Laboratory of AKUH were approached and included in the study after informed consent. Subjects on medications affecting glucose or HbA1c level e.g. corticosteroids, pregnancy, history of blood transfusion in last 3 months, chronic kidney disease, known hemoglobinopathies, anemia (hemoglobin of <11.0 g/dl in females, and <13.7g/dl in males), and those taking iron or vitamin B12 supplementation were excluded from the study.

After informed consent demographic details including weight, height, waist circumference, ethnicity (based on ancestral language) along with smoking history and family history of DM were recorded on a preformed questionnaire at the time of OGTT. Blood samples were collected in vacutainer containing EDTA for HbA1c and sodium fluoride for glucose. Samples for FPG were collected after a minimum of 8 hours overnight fasting and samples for 2-h PG were taken after oral administration of a standard dose of 75 g anhydrous glucose dissolved in water. Samples for FPG and 2-h PG were analyzed immediately, while whole blood samples for HbA1c were stored at -80°C until analysis. Whole blood HbA1c was analyzed by immunoassay method on ADVIA 1800 chemistry analyzer (Siemens Diagnostics, NY, US) using manufacturer provided recommendations. HbA1c assay is NGSP certified and traceable to the DCCT reference method. For assessing system performance, three levels of quality control materials (low, medium and high) were run before analysis of each batch. Cutoffs used for HbA1c were normal ≤5.7%, prediabetes 5.7-6.4%, and diabetic ≥6.5%[10,11]. Plasma glucose was measured by hexokinase method on ADVIA 1800 chemistry analyzer (Siemens Diagnostics, NY, US). System performance was assessed using three levels of quality control i.e. low, medium and high. Clinical laboratory of AKUH is participating in external proficiency testing program of College of American Pathologists (CAP) during this study period and performance was acceptable. Cutoffs for FPG are, for normal <100mg/dl, prediabetes 100 to 125mg/dl and diabetic ≥126mg/dl and cutoffs for 2-h PG are normal <140mg/dl, prediabetes 140-199mg/dl and diabetic ≥200mg/d.[10,11]. Data was analyzed using the Statistical Package for the Social Sciences (SPSS version 19.0) for testing normality Kolmogorov-Smirnov test was

applied. Median value and IQR were computed for skewed quantitative variable and mean ± SD for others; whereas frequency and percentage were calculated for categorical variables. Subjects were labeled normal weight, overweight and obese using criteria proposed for Asian populations by International Association for the Study of Obesity and International Obesity Task Force of World Health Organization 2001[12,13]. Median levels of HbA1c, FPG and 2-h PG were compared amongst ethnic groups by Kruskal-Wallis test taking $p \leq 0.05$ as significant. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) at 6.5% cutoff level of HbA1c for diabetes were calculated using 2×2 table.

RESULTS

Total of 146 subjects who underwent OGTT for diagnosis of DM, over a period of 16 months, were included in this study. Test of normality showed that data for BMI, waist circumference, FPG, 2-h PG and HbA1c was skewed. Median age of study subjects was 45 (IQR 54.2-35) years, with 38% (n=55) subjects being <40 years of age and 62% (n=91) >40 years. Majority were females 53% (n=77). Subjects with family history of diabetes were 65% (n=95) while 17.8% (n=26) were smokers. Majority of subjects were Sindhis, Punjabis and Urdu speaking or muhajirs (immigrants from India), while Pakhtuns and Balochis were categorized as others, as their number was small as shown in table-I. Median BMI of the subjects was 27kg/m^2 (IQR 30.5-24.3) with 17.8% (n=26) were normal with median BMI 21.9 (IQR 22.3-19.9), 32.2% (n=47) being over weight with BMI 25.1 (IQR 26.3-24.5) and 50% (n=73) subjects were obese having BMI 30.5 (IQR 35-28.7). Median waist circumference was 35.5 cm (IQR 40-33) with 22% (n=32) female [median waist circumference 40cm (IQR 40-38)], while 15% (n=22) males [median waist circumference 42cm (IQR 44-40)] had high waist circumference. Median HbA1c of the study subjects was in prediabetic range; 6% (IQR 6.6-5.6). Positive correlation was observed between age and HbA1c [5.7% (IQR 6.2-5.3) <40 y and 6.2% (IQR 6.8-5.8) in >40y subjects; r 0.34, p=0.000). While amongst genders; males had higher HbA1c level [6.1% (IQR 6.8-5.7) as compared to female with level of 5.9% (IQR 6.4-5.4); r 0.17, p=0.036). No significant difference was observed for HbA1c, FPG, 2-h PG levels, age, BMI and waist circumference amongst ethnicities; as shown in table-I. While table-II shows the frequency of subjects diagnosed as normal, pre-diabetic and diabetic according to the current ADA criteria of FPG and 2-h PG levels in OGTT and corresponding median HbA1c levels in each group according to ADA cut-off. On basis of HbA1c 46% [n=67; median HbA1c levels 6% (IQR 6.2-5.8)] were labeled as pre-diabetic and 28% [n=41; median HbA1c 7% (IQR 7.55-6.75)] were labeled diabetic. Out of 22 subjects identified as diabetic on FPG, mean HbA1c was impaired in 2 subjects and

Table-I: Demographic and biochemical parameters including ethnicity in study subjects.

Parameters	Overall	Urdu speaking	Punjabi	Sindhi	Others	*p*-value
n (%)	146	85 (58)	28 (19)	19 (13)	14 (10)	-
Age (years) Median with IQR	45 (54.2-35)	45 (53-35)	43.5 (53.7-35.5)	40 (56-35)	27.9 (31.1-25)	0.524
BMI (kg/m^2) Median with IQR	27 (30.5-24.3)	26.59 (30.2-23.6)	27.8 (32-24.7)	27.4 (30.3-23.2)	27.9 (31.1-25)	0.669
Waist cicumference Median with IQR	35.5 (40-33)	35 (40-32)	38 (40-34)	36 (40-34)	36.5 (40.2-31.5)	0.579
HbA1c (%) Median with IQR	6 (6.6-5.6)	6 (6.6-5.5)	5.9 (6.2-5.45)	6 (7.4-5.6)	6.2 (7-5.85)	0.216
FPG (mg/dl) Median with IQR	94.5 (110.5-86.7)	94 (107-84)	94 (105.7-87)	102 (125-91)	104.5 (129.5-87.2)	0.18
2-h PG (mg/dl) Median with IQR	135.5 (206-110.7)	134 (196-105)	128 (172-114)	156 (269-117)	176 (240.7-123.5)	0.157

normal in 1 subject (table-II). One subject out of 22 diabetic on FPG was identified as normal on 2-hr PG value. Among 29% (or 42/146) of the subjects identified as diabetic by 2-h PG; only 71% (or 30/42) patients had HbA1c ≥6.5% while 19% (or 8/42) had impaired and 9.5% (or 4/42) had normal HbA1c. Using 2-h PG as gold standard, sensitivity, specificity, PPV, NPV for HbA1c at cutoff of 6.5% were 70%, 89%, 72% and 80% respectively. While sensitivity, specificity, PPV, NPV of FPGat cutoff of 126mg/dl for diagnosing diabetes mellitus was 67%, 84%, 79% and 73% respectively.

Table-II: Distribution of fasting plasma glucose (FPG) and 2-hour PG levels in study subjects according to ADA criteria and comparison with HbA1c levels (n=146).

		Plasma glucose (mg/dl)		HbA1c (%)						
OGTT	Distributions based on cutoff	n (%)	Median (IQR)	Overall Median (IQR)	HbA1c <5.7		HbA1c 5.7-6.4		HbA1c ≥6.5	
					n (%)	Median (IQR)	n (%)	Median (IQR)	n (%)	Median (IQR)
FPG (mg/dl)	Normal <100	87 (60)	88 (94-81)	5.7 (6-5.37)	35 (24)	5.3 (5.4-5)	2 (1.4)	5.4	1 (0.7)	5.1
	Pre-diabetes ≥100-125	37 (25)	109 (115-104)	6.3 (6.8-6)	44 (30)	5.9 (6.1-5.72)	21 (14.4)	6.2 (6.3-5.95)	2 (1.4)	6.1
	Diabetic ≥126	22 (15)	143 (184-133.5)	7.3 (8.3-6.6)	7 (5)	6.8 (6.8-6.6)	15 (10.3)	6.8 (7.1-6.7)	19 (13)	7.4 (8.6-7)
2-hPG (mg/dl)	Normal <140	76 (52)	114 (125.7-102)	5.7 (6-5.3)	35	5.3 (5.4-5)	44	5.9 (6.1-5.7)	7	6.8 (6.8-6.6)
	Pre-diabetes ≥ 140-199	28 (19)	163 (177.7-150.7)	6.1 (6.3-5.9)	2	5.4	21	6.2 (6.3-5.95)	15	6.8 (7.1-6.7)
	Diabetic ≥200	42 (29)	246 (269-222)	6.8 (7.5-6.35)	1	5.1	2	6.1	19	7.4 (8.6-7)

Values are expressed in frequency (%) and Median (IQR). 2-h PG is 2-h Plasma glucose post oral glucose load of 75 gram and FPG is Fasting plasma glucose.

DISCUSSION

Prevalence of DM keeps increasing in this part of the world and screening our population

remains a challenge. Additionally, glycemic control is fundamental to the management of diabetes, as optimal control prevents the risk of complications related to uncontrolled DM. Both plasma glucose and HbA1c have been used as a tool for monitoring and predicting risk of developing complications. 2010 ADA guidelines, updated in 2015 recommends that HbA1c can be used for of DM[10,11]. Since then HbA1c is being increasingly used for screening DM. Overall mean HbA1c is high in subjects screened by OGTT included in this study. Subjects with normal 2-h PG had mean HbA1c of 5.6 ± 0.5%. Compared to these, subjects with impaired glucose tolerance and diabetic had higher HbA1c levels; 6.2% ± 0.6 and 7.1% ± 1 respectively. Higher mean HbA1c levels are also observed in other studies in newly diagnosed diabetic patients as compared to normal subjects[14,15]. Mean HbA1c also shows positive correlation with age, suggesting that elderly subjects are at higher risk of developing diabetes[16,17]. Similar findings are reported by different studies from our part of the world[18,19]. After stratification based on ethnicities, our results show no significant difference in HbA1c levels. Although literature have reported that HbA1c levels varies in different ethnicities, the exact cause for which is not known. However, no such variation can be observed in this study. Variation in HbA1c in Indian population has been observed, where different HbA1c cut off values for southern and northern Indian had been reported; ≥6.0% vs. ≥6.4% respectively[19,20]. These findings suggest that larger prospective outcome based studies are required to establish separate HbA1c cut-off for diagnosing diabetes in our population. There is a considerable debate regarding role of HbA1c as diagnostic marker for screening of DM[19,20] Present study also demonstrates HbA1c misdiagnosed 13 cases as compared to 2-h PG. On assessing the HbA1c cutoff level of 6.5% (for labeling DM) using 2-h PG as the reference, showed sensitivity of 70% and specificity of 89%, which is almost similar to FPG. Findings are similar to a study conducted in India which showed sensitivity and specificity of 65% and 88% at cut off level of 6.5% of HbA1c[15]. Good screening tests should have sensitivity of at least 80% or above so that the suspected cases are not missed on screening. This advocates that using HbA1c cut off >6.5 for our population can miss few cases of diabetes compared to 2-h PG and newer cut offs for HbA1c should be derived for our population. Similar findings wereobserved by Likhari *et al*[21] who showed that South Asians had higher mean HbA1c level of 6.5% ± 0.7 than Caucasian 6.1 ± 0.6%. In another study by Razak *et al*[22] reported higher mean HbA1c in non-diabetic population among South Asians (5.9%) as compared to Chinese (5.7%) and Europeans (5.4%). These findings advocate that probably different cut offs should be used for HbA1c for our population and it should be used in combination with clinical history and other biochemical tests when diagnosing diabetes. Limitation of this study includes small sample size of different ethnic groups. A larger prospective study is required to confirm these findings. Also in this study HbA1c was performed only once and ideally it should be repeated in subjects with abnormal results to confirm findings.

CONCLUSIONS

Higher median HbA1c levels are seen in patients suspected of DM. Sensitivity of HBA1c is 70% using ADA cut-off of 6.5% using 200mg/dl 2-h PG levels in our pilot study.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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