Iran Red Crescent Med J. 2016 August; 18(8):e31254. doi: 10.5812/ircmj.31254.

Published online 2016 June 21. **Research Article**

Optimal Glycemic and Hemoglobin A1c Thresholds for Diagnosing Diabetes Based on Prevalence of Retinopathy in an Iranian Population

Naser Samadi Aidenloo,[1] Alireza Mehdizadeh,[2,*] Neda Valizadeh,[2] Mohammad Abbaszadeh,[1] Siavash Qarequran,[2] and Hamidreza Khalkhali[3]

[1]Department of Ophthalmology, Urmia University of Medical Sciences, Urmia, IR Iran
[2]Department of Endocrinology, Urmia University of Medical Sciences, Urmia, IR Iran
[3]Department of Biostatistics and Epidemiology, Urmia University of Medical Sciences, Urmia, IR Iran

[*]*Corresponding author*: Alireza Mehdizadeh, Department of Endocrinology, Urmia University of Medical Sciences, Urmia, IR Iran. Tel: +98-9143407326, Fax: +98-4433469935, E-mail: armehdizadeh2014@gmail.com

Received 2015 July 03; **Revised** 2015 September 15; **Accepted** 2015 October 07.

Abstract

Background: The use of glycemic thresholds for diabetes diagnosis is controversial. However, no information is available regarding glycemic and glycated hemoglobin (HbA1c) thresholds for detecting diabetic retinopathy (DR) in the Iranian population.
Objectives: The main purpose of the current investigation was to examine the association of fasting plasma glucose (FPG) and HbA1c levels with diabetic retinopathy (DR), and to determine the relevant cut-off levels in an Iranian population.
Patients and Methods: This cross-sectional, population-based study was performed during 2012-2013 in Urmia, the capital of West Azerbaijan province, Iran. The subjects were 3,010 Iranians aged 40-81 years. The FPG levels were determined using the glucose oxidase method whereas, the HbA1c values were measured using a standardized assay by high performance liquid chromatography. DR was evaluated by an examination of the fundus photograph of each eye. The photographs were graded according to the international clinical diabetic retinopathy disease severity scale by photograph graders who were masked to the clinical information.
Results: Of the subjects, 59 had DR. The prevalence of DR increased steeply between the ninth and the tenth deciles for both variables. The ROC curve analysis showed overall glycemic thresholds for DR of 6.5 mmol/L (117 mg/dL) for FPG and 6.2% (44 mmol/mol) for HbA1c. The sensitivities and specificities were 78.0% and 87.1% for FPG and 89.8% and 89.5% for HbA1c, respectively. The areas under the ROC curves indicated that HbA1c was a stronger discriminator of retinopathy: the area under curve was 0.880 for FPG and 0.946 for HbA1c $P < 0.001$). However, the thresholds for detecting DR for the two measures showed no significant differences after excluding individuals receiving anti-hyperglycemic medication.
Conclusions: These findings suggest that the HbA1c and FPG thresholds for detecting diabetes in the Iranian population are lower than the current diagnostic criteria.

Keywords: Diabetic Retinopathy (DR), Hemoglobin A1c (HbA1c), Fasting Plasma Glucose (FPG), Diagnostic Criteria

1. Background

The world health organization (WHO) has published several guidelines since 1965 for the diagnosis of diabetes mellitus (DM) (1). One of the preferred diagnostic criteria is the determination of the glycemic thresholds (2) by calibrating glycemic tests against diabetic retinopathy (DR) observations (3). DR is an early and specific clinical complication of diabetes and is used as the basis for determining diagnostic cut-off points for DM (4, 5). Several population-based studies have investigated the relationship between the prevalence of retinopathy and some glycemic measures such as fasting plasma glucose (FPG), 2-hour post oral glucose load plasma glucose (2hPG), and glycated hemoglobin (HbA1c) levels (2, 5-10). These investigations and many other published clinical studies have recommended diagnostic cut-off points for diabetes as an FPG level of $\geq$ 7.0 mmol/L (126 mg/dL) and an HbA1c level of $\geq$ 6.5% ($\geq$ 48 mmol/mol) (11-13).

Other studies in the literature have suggested somewhat different optimal cut-off values, based on the presence of DR. The differences in these recommendations could partly arise due to the differences in the various studies, such as the subject population, ethnicity, or age range of the included subjects (2, 14-16). The different statistical methods utilized in these studies could also have affected the final decisions on cut-off points (17, 18). In addition, the glycemic cut-off points associated with DR may also be affected by disease control within the sample population (2). On the other hand, almost all of these investigations adopted a very broad definition of retinopathy that included many cases of mild retinopathy, which is claimed to have causes other than hyperglycemia (19). Hence, a better result might be obtained by limiting the inclusion crite-

ria to diabetes-specific retinopathy (moderate or more severe levels of retinopathy) that can be clearly attributed to hyperglycemia.

The incidence of diabetes has steadily and dramatically increased throughout the world, including in Iran. The prevalence of DM is estimated as 24% among Iranian individuals older than 40 years of age and it increases by 0.4% with each year after 20 years of age (20). The dramatic increase in the diabetic population is inevitably accompanied by increased diabetic complications and enormous health costs. Therefore, early detection and screening of high-risk individuals for diabetes is of great importance and interest to investigators and health care providers.

2. Objectives

The current investigation was undertaken to examine the relationships between DR prevalence and FPG and HbA1c levels and to determine the optimal FPG and HbA1c cut-off points for diagnosing diabetic retinopathy in a population from Northwest Iran. These glycemic measures were also compared for their efficacy in detecting DR as a truly diabetic state.

3. Patients and Methods

3.1. Study Population

This cross-sectional, population-based study was conducted between September 2012 and August 2013 in Urmia, the capital of West Azerbaijan province, Iran. Participants were recruited by voluntary participation through advertisement among citizens who were $\geq$ 40 years of age. Individuals were selected using a systematic random sampling method that selected every *n*th subject. The value of *n* depended on the number of patients expected on each day of testing, in order to include 15 participants per day. The eligibility criteria included an age of $\geq$ 40 years, fully completed questionnaires, no missing values for HbA1c or FPG, and sufficient mental and physical ability to participate. We also limited the inclusion criteria to diabetes-specific retinopathy (moderate or more severe levels of retinopathy). The exclusion criteria included a previous history of endocrinopathies; hypothyroidism; liver, kidney, or heart failure; neoplasia; hemoglobinopathies; anemia; pregnancy; evidence of any other ocular diseases, except for refraction errors; hypertension; a history of major surgery; blood transfusion within the previous 6 months; severe illness; and weight gain or loss > 10% during the past 3 months. After clinical screening (medical history, physical examination, and laboratory tests), a total of 3,010 non-hypertensive subjects were randomized into the study, as described above. All included subjects received baseline examinations, including FPG and HbA1c measurements, an ophthalmic examination, and blood pressure measurement, in addition to completing a general health and lifestyle questionnaire. Participant educational status, ethnicity, anti-hypertensive and anti-hyperglycemic medications, smoking status, and prevalent medical conditions were obtained by this questionnaire. Individuals with systolic/diastolic blood pressures higher than 140/90 mmHg or subjects who were using anti-hypertensive medications were considered as hypertensives and were excluded from the study. The study protocol was approved by the ethics committee of the Urmia medical university and written informed consent was obtained from all the participants.

3.2. Measurements

Blood samples were collected after a minimum fasting time of 8 hours for the determination of plasma glucose and HbA1c levels. Plasma glucose was determined by the glucose oxidase method, whereas HbA1c was measured by high performance liquid chromatography (Tosoh G7, Tokyo, Japan).

3.3. Ophthalmic Examination and Definition of Diabetic Retinopathy

All participants received eye examinations by an ophthalmologist and had a bilateral retinal photograph taken of the fundus through dilated pupils. After pupil dilatation with 1.0% tropicamide and 10% phenylephrine, fundus photographs (45° color digital images of the retina) were taken from both eyes of each participant by a technologist using a Topcon TRC-NW7SF fundus camera (Topcon corporation, Tokyo, Japan). The first image was centered on the macula, whereas the second one was centered on the optic nerve. The photographs were assessed according to the international clinical DR severity scale (21) by photographic graders who were blinded to the clinical information of participants. This scale rates DR at five different levels: (i) no retinopathic changes (equivalent to the early treatment of diabetic retinopathy study [ETDRS] scale level 10); (ii) mild non-proliferative retinopathy (NPDR) (equivalent to ETDRS level 20); (iii) moderate NPDR (equivalent to ETDRS levels 35, 43, and 47); (iv) severe NPDR (equivalent to ETDRS levels 53A-53E); and (v) proliferative retinopathy (PDR) (equivalent to ETDRS levels 61 or higher). The final DR grading for each participant was determined according to the diagnosis in the more severely affected eye. As mentioned previously, the presence of DR was defined as the presence of moderate (level iii) or severe non-proliferative (level iv) DR, or proliferative (level v) DR in either eye.

3.4. Statistical Analysis

All statistical analyses were performed using the SPSS (statistical package for the social sciences ver. 17). All data were presented as a frequency percentage for categorical variables and as the mean ± standard deviation (SD) for continuous variables. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off value of HbA1c and FPG for identifying DR. The sensitivity was defined as the measured level of HbA1c or FPG that correctly identified subjects with DR, while the specificity cut-off point was defined as the level that correctly identified subjects who did not have DR. The Youden index (sensitivity + specificity - 1) was utilized to evaluate the discriminatory power of FPG and HbA1c for predicting DR (22). The discriminatory power of HbA1c and FPG levels for retinopathy was further assessed as the area under the receiver operating characteristic curve (AUC), where an AUC of 1 would indicate perfect predictive value, with no false positives or false negatives, and an AUC of 0.5 would indicate a discrimination no better than chance. The difference in the AUC was estimated by the method of DeLong et al. (23). The effect of HbA1c and FPG concentrations on the risk of DR was evaluated by multiple logistic regression analysis, using HbA1c and FPG categories as independent variables and DR as a response variable. Diabetes was diagnosed based on an HbA1c reading of ≥ 6.5% (48 mmol/mol), FPG of ≥7.0 mmol/L (126 mg/dL), self-reported diagnosed diabetes, or current use of anti-hyperglycemic medications. Two-sided P values less than 0.05 were considered statistically significant for all analyses.

4. Results

After exclusion of the non-eligible subjects, 3010 individuals (1413 men and 1597 women) participated in this investigation. The mean concentration of HbA1c was 48.5 ± 0.2 mmol/mol (5.6 ± 0.1 %) and the mean level of FPG was 5.9 ± 1.9 mmol/L (106.2 ± 34.2 mg/dL) in our population. Table 1 shows the demographic and clinical characteristics of the studied population. The mean age of the participants was 54.7 ± 8.4 years, and men comprised 46.9% of the group. The individuals with DR had higher FPG, HbA1c, and BMI levels when compared with subjects without DR. The patient group also had a higher prevalence of diabetes. Among the cases with diabetes, those with DR had a longer duration of diabetes.

The overall prevalence of diabetic retinopathy in the investigated population was about 2 %. A total of 35 participants had moderate NPDR, 13 had severe NPDR, and 11 had PDR. Among those with diabetes, the prevalence of diabetic retinopathy was 32.6% (95% CI: 23.1-54.7%), including a prevalence of 33.7% (95% CI: 20.4-67.7%) in those with HbA1c ≥ 6.5% (48 mmol/mol), 40.1% (95% CI: 27.2-71.7%) in those with known diabetes, and 2.2 % (95% CI: 0.0-5.8) in individuals with normal HbA1c and normal FPG.

Figure 1 shows the prevalence of DR by deciles of the distribution of the FPG and HbA1C levels. These plots suggest a curvilinear relationship between FPG or HbA1C and retinopathy. The prevalence of DR was very low in the first through ninth deciles for both glycemic measures (6.3-7.0 mmol/L for FPG and 6.4-6.8% for HbA1c), but started to increase markedly between the ninth and the tenth deciles for both variables. Indeed, the prevalence of DR in the tenth deciles was 9.0% and 10.3% for FPG and HbA1c respectively, whereas the prevalence in the ninth deciles was 1.2% for FPG and 1.3% for HbA1c.

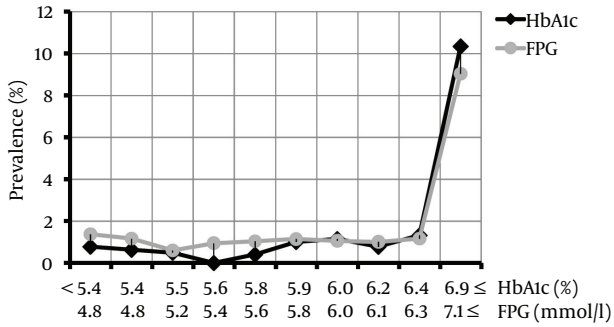

Figure 1. The Prevalence of Diabetic Retinopathy According to Glycated Hemoglobin (HbA1c) and Fasting Plasma Glucose (FPG) Deciles

Figure 2 displays the ROC curves for FPG and HbA1c in detecting DR. The overall optimal cut-off points of HbA1c and FPG values for diagnosis of DR, determined by maximizing the sensitivity and specificity, were 6.5 mmol/L (117 mg/dL) for FPG and 6.2% (44 mmol/mol) for HbA1c. The sensitivity and specificity were 78.0% and 87.1% for FPG and 89.8% and 89.5% for HbA1c, respectively (Figure 2). We also evaluated the ability of the HbA1c and FPG measures to identify the presence of DR by comparing the AUCs for these two glycemic measures (Figure 2). The AUC for HbA1c was 0.946 (95% CI: 0.877-0.978) and was significantly larger than that for FPG [0.880 (95% CI: 0.791-0.930); p for difference < 0.001] (Figure 2). After excluding individuals receiving anti-hyperglycemic medication, the thresholds for detecting DR were 6.6 mmol/L (118.8 mg/dL) for FPG and 5.9% (41 mmol/mol) for HbA1c. In this case, the AUC for FPG was 0.845 (95% CI: 0.748-0.943), whereas the area under the ROC curve for HbA1c was 0.876 (95% CI: 0.699-0.941). These measures showed no significant differences in terms of detecting DR (P = 0.116).

Table 2 presents the diagnostic properties for diabetic

Table 1. Clinical and Biochemical Characteristics of the Study Population

	Total	Retinopathy	No retinopathy	P Value
Frequency, N	3010	59	2951	
Age, y, mean ± SD	54.7 ± 8.4	52.8 ± 7.5	54.9 ± 8.0	0.562
Males/Females, No. (%)	1413/1597	22/37	1391/1560	0.133
BMI, Kg/m^2, mean ± SD	23.3 ± 0.5	25.8 ± 0.6	23.1 ± 0.4	0.025
Smokers, No. (%)	356 (11.8)	6 (10.2)	350 (11.9)	0.893
Diabetes, No. (%)	327 (10.9)	14 (23.7)	313 (10.6)	0.001
Diabetic duration, y, mean ± SD	7.3 ± 0.6	11.11 ± 0.6	7.1 ± 05	0.031
FPG, mmol/L, mean ± SD	5.9 ± 1.9	8.9 ± 2.0	5.9 ± 0.3	< 0.001
HbA1c, mmol/mol, mean ± SD	48.5 ± 0.2	72.1 ± 1.4	47.9 ± 0.1	< 0.001
HbA1c (%), mean ± SD	5.6 ± 0.1	8.5± 0.3	5.5 ± 0.1	< 0.001

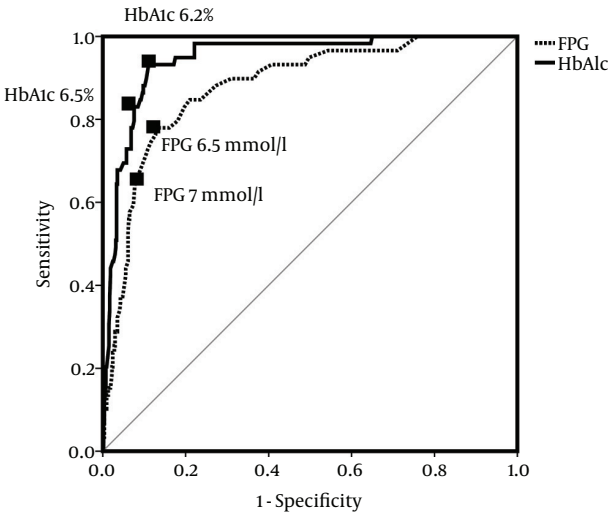

Figure 2. Thresholds of Fasting Plasma Glucose (FPG) and Glycated Hemoglobin (HbA1c) for Diabetic Retinopathy from Receiver-Operating Characteristic (ROC) Curve Analyses

retinopathy (i.e., sensitivity, specificity, positive predictive value, negative predictive value, and Youden index) at various cut-off points of FPG and HbA1c. These parameters were evaluated by calculating the sensitivity and specificity of each threshold using the ROC curve, as shown elsewhere (10, 24). Below our determined threshold values, the prevalence of DR was 0.9% (95% CI: 0.10-2.31) for the threshold of HbA1c [6.2% (44 mmol/mol)], 1.32% (95% CI: 0.18-3.56) for the threshold of FPG (6.5 mmol/L, 117 mg/dL), and 0.09% (95% CI: 0.03-0.17) for the threshold of both HbA1c and FPG. The positive and negative predictive values (PPV and NPV) of FPG cut-off point (6.5 mmol/L, 117 mg/dL) were 10.8% and 96.7%, respectively, while the PPV and NPV of the HbA1c cut-off point [6.2% (44 mmol/mol)] were 14.6% and 98.1%, respectively. The pre-existing thresholds of 6.5% (48 mmol/mol) for HbA1c and 7.0 mmol/L (126 mg/dL) for FPG for diagnosing diabetes showed 83.1% and 64.4% sensitivity, respectively. The specificity of these pre-existing thresholds for diagnosing diabetes was 91.5% for HbA1c and 92.3% for FPG. As shown in Table 2, the use of these pre-existing thresholds improved the specificity and the positive predictive value. On the contrary, the negative predictive value and the sensitivity of the pre-existing thresholds were reduced compared with the use of the optimal glycemic thresholds suggested from this study.

Table 3 presents the association of FPG and HbA1c with DR in two models: an unadjusted model and a model adjusted for gender, age, and smoking. The categories used in Table 3 are as follows: i) concentrations less than our cut-offs (< 6.2% for HbA1c and < 6.5 mmol/L for FPG); ii) concentrations between our cut-offs and those proposed by the American diabetes association (ADA) (6.2-6.4% for HbA1c and 6.5-6.9 mmol/L for FPG); and iii) concentrations greater than the ADA proposed cut-offs (6.5% $\leq$ for HbA1c and 7.0mmol/L $\leq$ for FPG). The odds ratio (OR) for DR increased with increasing categories of FPG and HbA1c (Reference = lowest category), both before and after adjustment.

5. Discussion

The glycemic thresholds for diabetes diagnosis have been determined separately in several countries, including the USA, Japan, Egypt, South Korea, China, Australia, France, and Singapore (2, 6-10, 15, 17, 18, 25-27). However, no information has yet been provided for the Iranian population. This community-based cross-sectional study revealed a significant increase in the prevalence of diabetic

Table 2. Percentage of Cases, Sensitivity, Specificity, and Positive and Negative Predictive Values at Different Fasting Plasma Glucose (FPG) and Glycated Hemoglobin (HbA1c) Cutoffs

Cutoff	Number of Retinopathy Cases/Total Number of Participants		Sensitivity (%)	Specificity (%)	Youden Index	PPV (%)	NPV (%)
	Below the Cutoff (%)	Above the Cutoff (%)					
HbA1c							
> 5.5 %	1/1049 (0.1)	58/1961 (3.0)	98.3	35.5	0.34	3.0	99.9
> 6.0 %	3/2302 (0.1)	56/708 (7.9)	94.9	77.9	0.73	7.9	99.5
> 6.1 %	4/2450 (0.2)	55/560 (9.8)	93.2	82.9	0.76	9.8	99.2
> 6.2 %	6/2647 (0.2)	53/363 (14.6)	89.8	89.5	0.79	14.6	98.1
> 6.3 %	7/2669 (0.3)	52/341 (15.2)	88.1	90.2	0.78	15.2	97.6
> 6.4 %	9/2689 (0.3)	50/321 (15.6)	84.7	90.8	0.76	15.6	96.8
> 6.5 %	10/2710 (0.4)	49/300 (16.3)	83.1	91.5	0.75	16.3	96.2
> 6.7 %	13/2752 (0.5)	46/258 (17.8)	78.0	92.8	0.71	17.8	94.2
> 6.9 %	16/2799 (0.6)	43/211 (20.4)	72.9	94.3	0.67	20.4	91.3
> 7.0 %	18/2822 (0.6)	41/189 (21.7)	69.5	95.0	0.64	21.7	89.2
> 7.5 %	21/2875 (0.7)	38/135 (28.1)	64.4	96.7	0.61	28.1	82.2
FPG							
> 5.5 mmol/L	2/1286 (0.2)	57/1724 (3.3)	96.6	43.5	0.40	3.3	99.9
> 6.0 mmol/L	4/1515 (0.3)	55/1495 (3.7)	93.2	51.2	0.44	3.7	99.7
> 6.1 mmol/L	5/1846 (0.3)	54/1164 (4.6)	91.5	62.4	0.54	4.6	99.6
> 6.2 mmol/l	6/1989 (0.3)	53/1021 (5.2)	89.8	67.2	0.57	5.2	99.4
> 6.3 mmol/L	7/2152 (0.3)	52/858 (6.1)	88.1	72.7	0.61	6.1	99.1
>6.4 mmol/L	9/2340 (0.4)	50/670 (7.4)	84.7	79.0	0.64	7.5	98.6
> 6.5 mmol/L	13/2583 (0.5)	46/427 (10.8)	78.0	87.1	0.65	10.8	96.7
> 6.7 mmol/L	16/2645 (0.6)	43/365 (11.8)	72.9	89.1	0.62	11.8	95.3
> 6.9 mmol/L	19/2704 (0.7)	40/306 (13.1)	67.8	91.0	0.59	13.1	93.3
> 7.0 mmol/L	21/2745 (0.8)	38/265 (14.3)	64.4	92.3	0.57	14.3	91.6
> 7.5 mmol/L	26/2788 (0.9)	33/222 14.9)	55.9	93.6	0.50	14.9	87.9

Table 3. Association Between the Categories of Glycated Hemoglobin (HbA1c) and Fasting Plasma Glucose (FPG) and the Risk of Diabetic Retinopathy (DR)

Categories (%)	HbA1c		Categories (mmol/L)	FPG	
	Crude OR (95% CI)	Adjusted OR (95% CI)		Crude OR (95% CI)	Adjusted OR (95% CI)
i) < 6.2	1	1	**i) < 6.5**	1	1
ii) 6.2-6.4	7.13 (1.89-25.16)	8.64 (2.11-26.33)	**ii) 6.5-6.9**	13.43 (3.87-29.73)	19.23 (5.44-49.36)
iii) 6.5 ≤	35.85 (10.09-78.32)	44.14 (14.55-100.26)	**iii) 7.0 ≤**	37.35 (7.83-116.54)	43.42 (9.64-124.74)

retinopathy significantly between the 9th and 10th deciles for both FPG and HbA1c. In addition, the optimal thresholds for detecting prevalent DR in our population, based on ROC analyses, were 6.2% (44 mmol/mol) for HbA1c and 6.5 mmol/L (117 mg/dL) for FPG, which were lower than those currently used as diagnostic criteria for diabetes.

The diagnostic cut-offs for HbA1c or FPG in diabetes have been modified according to the optimal sensitivity and specificity for DR (28). In 1997, the ADA recommended lowering the threshold for FPG from 7.8 mmol/L (140.4 mg/dL) to 7.0 mmol/L (126 mg/dL), because multiple investigations had shown a linear increase in the prevalence of retinopathy above this level and the latter cut-off correlated better with a 2h plasma glucose ≥ 11.1 mmol/L (3, 7, 17). In 2010, based on a data pooling analysis of five studies, the ADA proposed HbA1c ≥ 48 mmol/mol (6.5 %) as the diagnostic cut-off point for diabetes (1, 6, 29).

As already mentioned, the optimal cut-off thresholds for FPG and HbA1c for defining DR can vary between populations. Previous ROC curve analyses indicated an optimal FPG threshold level of 7.1 mmol/L (127.8 mg/dL) in Australia (the AusDiab study) (18), 6.7 mmol/L (120.6 mg/dL) in the US population (NHANES III) (7), and 6.8 mmol/L (122.4 mg/dL) in the Pima Indian population (8). The DETECT-2 collaboration writing group, which investigated approximately 45,000 participants, showed glycemic thresholds

for diabetes-specific retinopathy (defined as moderate or more severe DR) of 6.4% (46 mmol/mol) for HbA1c and 6.5 mmol/L (117 mg/dL) for FPG (6). Our optimal HbA1c threshold for DR was similar to those of other studies, such as the fifth Korea national health and nutrition examination survey (6.2%; 44 mmol/ mol) (10), a Japanese population study (6.2%; 44 mmol/ mol) (30), and an Egyptian study (6.3%; 45 mmol/ mol) (17), but our values were higher than those other investigations, such as the AusDiab study (6.1%; 43 mmol/mol), NHANES 2005-2006 (5.5%; 37 mmol/mol), and a Japanese study (5.3-5.7%; 34-39 mmol/mol) (14, 15, 18). In the case of FPG, our cut-off value was consistent with the results obtained by the Hisayama Studies (6.4 and 6.5 mmol/L; 115.2 and 117 mg/dL) (9, 15), a Chinese population study (6.4 mmol/L, 115.2 mg/dL) (2), and the DETECT-2 investigation (6.6 mmol/L, 118.8 mg/dL) (6).

The use of HbA1c measurement to diagnose diabetes remains somewhat controversial (31). HbA1c has several diagnostic benefits over FPG, such as the independence from fasting, lower day-to-day variation compared with FPG, lower pre-analytical instability, and lower biologic variability (10, 32). These advantages have implications for the early identification and treatment of undiagnosed diabetes. Our analysis demonstrated a significantly larger area under the ROC curve for HbA1c than for FPG when considering the whole population. However, this difference lost statistical significance when individuals receiving anti-hyperglycemic medication were excluded. This finding indicates that the discriminative ability of HbA1c for detecting individuals with diabetes or with a high risk for diabetes was comparable to that of FPG. This was consistent with the findings of the Korean diabetes association (KDA) (12).

The strengths of our study include its population-based design with a homogeneous ethnic background, the precise interpretation of retinal photography by an ophthalmologist, and stringent inclusion criteria. In fact, the studied subjects were limited to those who were non-hypertensive and had diabetes-specific retinopathy. These criteria reduced the chance of observing the spurious results. However, some limitations should also be acknowledged. First, our analyses included people with previous or current hypoglycemic treatment. Hypoglycemic medications could have affected the levels of glycemia and might have influenced the distribution of HbA1c or FPG levels. Second, this study was based on cross-sectional data, which might have affected the threshold values of the glycemic measures. Ideally, the diagnostic thresholds would be derived from prospective studies, which would allow a more accurate evaluation of the relationship between glycemic measures and incident microvascular complications. Third, other glycemic measures, such as 2-hour PG, glycated albumin (GA), and 1,5-anhydroglucitol (1,5-AG) values, were not included in this analysis.

In conclusion, our study showed that the HbA1c and FPG thresholds for diagnosing diabetes in an Iranian population were lower than the current diagnostic criteria. According to the present investigation, the prevalence of retinopathy increased when the HbA1c and FPG cut-off values of 6.2% (44 mmol/mol) and 6.5 mmol/L (117 mg/dL), respectively, were utilized. In addition, HbA1c showed a better discriminatory power than FPG for detecting the presence of retinopathy. A follow-up study with a larger sample size is needed to confirm the relationship of FPG and HbA1c to the incidence of DR.

Acknowledgments

The authors acknowledge all the participants of this study, as well as the nursing staff of the Imam Khomeini hospital for helping with data collection.

Footnotes

Authors' Contribution: Naser Samadi Aidenloo: designing the study, writing the manuscript and patients' examination; Alireza Mehdizadeh: designing the study, drafting the manuscript, obtaining finding and acquisition of data; Neda Valizadeh: designing the study, acquisition of data, and critical revision of intellectual contents; Mohammad Abbaszadeh: writing the manuscript, patients' examination; Siavash Qarequran: acquisition of data and analysis and interpretation of data; Hamidreza Khalkhali: analysis and interpretation of data; All authors have read and approved the content of the manuscript.

Funding/Support: This study was supported by Urmia University of Medical Sciences

References

1. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2010;**33**:62–9. doi: 10.2337/dc10-S062. [PubMed: 20042775].
2. Xin Z, Yuan MX, Li HX, Hua L, Feng JP, Shi J, et al. Evaluation for fasting and 2-hour glucose and HbA1c for diagnosing diabetes based on prevalence of retinopathy in a Chinese population. *PLoS One.* 2012;**7**(7):40610. doi: 10.1371/journal.pone.0040610. [PubMed: 22808204].
3. McCance DR, Hanson RL, Pettitt DJ, Bennett PH, Hadden DR, Knowler WC. Diagnosing diabetes mellitus–do we need new criteria?. *Diabetologia.* 1997;**40**(3):247–55. doi: 10.1007/s001250050671. [PubMed: 9084961].
4. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet.* 2010;**376**(9735):124–36. doi: 10.1016/S0140-6736(09)62124-3. [PubMed: 20580421].

5. Wong TY, Liew G, Tapp RJ, Schmidt MI, Wang JJ, Mitchell P, et al. Relation between fasting glucose and retinopathy for diagnosis of diabetes: three population-based cross-sectional studies. *Lancet.* 2008;**371**(9614):736–43. doi: 10.1016/S0140-6736(08)60343-8. [PubMed: 18313502].
6. Colagiuri S, Lee CM, Wong TY, Balkau B, Shaw JE, Borch-Johnsen K, et al. Glycemic thresholds for diabetes-specific retinopathy: implications for diagnostic criteria for diabetes. *Diabetes Care.* 2011;**34**(1):145–50. doi: 10.2337/dc10-1206. [PubMed: 20978099].
7. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care.* 1997;**20**(7):1183–97. [PubMed: 9203460].
8. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ.* 1994;**308**(6940):1323–8. [PubMed: 8019217].
9. Mukai N, Yasuda M, Ninomiya T, Hata J, Hirakawa Y, Ikeda F, et al. Thresholds of various glycemic measures for diagnosing diabetes based on prevalence of retinopathy in community-dwelling Japanese subjects: the Hisayama Study. *Cardiovasc Diabetol.* 2014;**13**:45. doi: 10.1186/1475-2840-13-45. [PubMed: 24533962].
10. Park YM, Ko SH, Lee JM, Kim DJ, Kim DJ, Han K, et al. Glycaemic and haemoglobin A1c thresholds for detecting diabetic retinopathy: the fifth Korea National Health and Nutrition Examination Survey (2011). *Diabetes Res Clin Pract.* 2014;**104**(3):435–42. doi: 10.1016/j.diabres.2014.04.003. [PubMed: 24785739].
11. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care.* 2012;**35**(6):1364–79. doi: 10.2337/dc12-0413. [PubMed: 22517736].
12. Ko SH, Kim SR, Kim DJ, Oh SJ, Lee HJ, Shim KH, et al. 2011 clinical practice guidelines for type 2 diabetes in Korea. *Diabetes Metab J.* 2011;**35**(5):431–6. doi: 10.4093/dmj.2011.35.5.431. [PubMed: 22111032].
13. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes M, Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig.* 2010;**1**(5):212–28. doi: 10.1111/j.2040-1124.2010.00074.x. [PubMed: 24843435].
14. Cheng YJ, Gregg EW, Geiss LS, Imperatore G, Williams DE, Zhang X, et al. Association of A1C and fasting plasma glucose levels with diabetic retinopathy prevalence in the U.S. population: Implications for diabetes diagnostic thresholds. *Diabetes Care.* 2009;**32**(11):2027–32. doi: 10.2337/dc09-0440. [PubMed: 19875604].
15. Miyazaki M, Kubo M, Kiyohara Y, Okubo K, Nakamura H, Fujisawa K, et al. Comparison of diagnostic methods for diabetes mellitus based on prevalence of retinopathy in a Japanese population: the Hisayama Study. *Diabetologia.* 2004;**47**(8):1411–5. doi: 10.1007/s00125-004-1466-8. [PubMed: 15309291].
16. Sivaprasad S, Gupta B, Gulliford MC, Dodhia H, Mohamed M, Nagi D, et al. Ethnic variations in the prevalence of diabetic retinopathy in people with diabetes attending screening in the United Kingdom (DRIVE UK). *PLoS One.* 2012;**7**(3):32182. doi: 10.1371/journal.pone.0032182. [PubMed: 22412857].
17. Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, et al. Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. *Diabetes Care.* 1997;**20**(5):785–91. [PubMed: 9135943].
18. Tapp RJ, Zimmet PZ, Harper CA, de Courten MP, McCarty DJ, Balkau B, et al. Diagnostic thresholds for diabetes: the association of retinopathy and albuminuria with glycaemia. *Diabetes Res Clin Pract.* 2006;**73**(3):315–21. doi: 10.1016/j.diabres.2006.02.008. [PubMed: 16644057].
19. van Leiden HA, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, et al. Risk factors for incident retinopathy in a diabetic and nondiabetic population: the Hoorn study. *Arch Ophthalmol.* 2003;**121**(2):245–51. [PubMed: 12583792].
20. Haghdoost AA, Rezazadeh-Kermani M, Sadghirad B, Baradaran HR. Prevalence of type 2 diabetes in the Islamic Republic of Iran: systematic review and meta-analysis. *East Mediterr Health J.* 2009;**15**(3):591–9. [PubMed: 19731775].
21. Wilkinson CP, Ferris F3, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology.* 2003;**110**(9):1677–82. doi: 10.1016/S0161-6420(03)00475-5. [PubMed: 13129861].
22. Schisterman EF, Faraggi D, Reiser B, Hu J. Youden Index and the optimal threshold for markers with mass at zero. *Stat Med.* 2008;**27**(2):297–315. doi: 10.1002/sim.2993. [PubMed: 17624866].
23. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* 1988;**44**(3):837–45. [PubMed: 3203132].
24. Cho NH, Kim TH, Woo SJ, Park KH, Lim S, Cho YM, et al. Optimal HbA1c cutoff for detecting diabetic retinopathy. *Acta Diabetol.* 2013;**50**(6):837–42. doi: 10.1007/s00592-013-0452-3. [PubMed: 23354926].
25. Mohan V, Sandeep S, Deepa M, Gokulakrishnan K, Datta M, Deepa R. A diabetes risk score helps identify metabolic syndrome and cardiovascular risk in Indians - the Chennai Urban Rural Epidemiology Study (CURES-38). *Diabetes Obes Metab.* 2007;**9**(3):337–43. doi: 10.1111/j.1463-1326.2006.00612.x. [PubMed: 17391160].
26. Wong TY, Cheung N, Tay WT, Wang JJ, Aung T, Saw SM, et al. Prevalence and risk factors for diabetic retinopathy: the Singapore Malay Eye Study. *Ophthalmology.* 2008;**115**(11):1869–75. doi: 10.1016/j.ophtha.2008.05.014. [PubMed: 18584872].
27. Massin P, Lange C, Tichet J, Vol S, Erginay A, Cailleau M, et al. Hemoglobin A1c and fasting plasma glucose levels as predictors of retinopathy at 10 years: the French DESIR study. *Arch Ophthalmol.* 2011;**129**(2):188–95. doi: 10.1001/archophthalmol.2010.353. [PubMed: 21320965].
28. Malkani S, Mordes JP. Implications of using hemoglobin A1C for diagnosing diabetes mellitus. *Am J Med.* 2011;**124**(5):395–401. doi: 10.1016/j.amjmed.2010.11.025. [PubMed: 21531226].
29. International Expert C. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care.* 2009;**32**(7):1327–34. doi: 10.2337/dc09-9033. [PubMed: 19502545].
30. Ito C. Evidence for diabetes mellitus criteria in 2010 using HbA1c. *Diabetology International.* 2012;**4**(1):9–15. doi: 10.1007/s13340-012-0086-7.
31. Waugh NR, Shyangdan D, Taylor-Phillips S, Suri G, Hall B. Screening for type 2 diabetes: a short report for the National Screening Committee. *Health Technol Assess.* 2013;**17**(35):1–90. doi: 10.3310/hta17350. [PubMed: 23972041].
32. Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C. *Diabetes Care.* 2011;**34**:184–90. doi: 10.2337/dc11-s216. [PubMed: 21525453].