


Glycated hemoglobin (HbA1c) as diagnostic criteria for diabetes: the optimal cut-off points values for the Pakistani population; a study from second National Diabetes Survey of Pakistan (NDSP) 2016–2017

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ABSTRACT

Aim Glycated hemoglobin (HbA1c) cut-off values as diagnostic tool in diabetes and prediabetes with its concordance to oral glucose tolerance test (OGTT) in Pakistani population.

Methodology Data for this substudy was obtained from second National Diabetes Survey of Pakistan (NDSP) 2016–2017. With this survey, 10834 individuals were recruited and after excluding known subjects with diabetes, 6836 participants fulfilled inclusion criteria for this study. Demographic, anthropometric and biochemical parameters were obtained. OGTT was used as standard diagnostic tool to screen population and HbA1c for optimal cut-off values. Participants were categorized into normal glucose tolerance (NGT), newly diagnosed diabetes (NDD) and prediabetes.

Results Out of 6836 participants, 4690 (68.6%) had NGT, 1333 (19.5%) had prediabetes and 813 (11.9%) had NDD by OGTT criteria with median (IQR) age of 40 (31–50) years. Optimal HbA1c cut-off point for identification of diabetes and prediabetes was observed as 5.7% ((AUC (95% CI)=0.776 (0.757 to 0.795), $p<0.0001$)) and 5.1% ((AUC (95% CI)=0.607 (0.590 to 0.624), $p<0.0001$)), respectively. However, out of 68.6% NGT subjects identified through OGTT, 24.1% and 9.3% participants were found to have prediabetes and NDD, respectively by using HbA1c criteria. By using both OGTT and HbA1c criteria, only 7.9% and 7.3% were observed as prediabetes and diabetes, respectively.

Conclusion Findings from second NDSP demonstrated disagreement between findings of OGTT and HbA1c as diagnostic tool for Pakistani population. As compared with international guidelines, HbA1c threshold for prediabetes and NDD were lower in this part of world. HbA1c as diagnostic tool might require ethnic or regional-based modification in cut-off points, validated by relevant community-based epidemiological surveys.

INTRODUCTION

Globally, type 2 diabetes is a burgeoning public health challenge and a significant

Significance of this study

What is already known about this subject?

- ▶ HbA1c offers some advantages over fasting glucose and oral glucose tolerance test (OGTT). Racial and ethnic variations are observed as diagnostic tool for diabetes.

What are the new findings?

- ▶ The findings from second NDSP demonstrated the disagreement between the findings of OGTT and HbA1c as diagnostic tool for the Pakistani population. As compared to International guidelines, HbA1c threshold for prediabetes and NDD were found to be lower in this part of the world.
- ▶ HbA1c as a diagnostic tool might require ethnic or regional-based modification in cut-off points, validated by relevant community based epidemiological surveys.

How might these results change the focus of research or clinical practice?

- ▶ HbA1c as a diagnostic tool has its limitations. Local and regional guidelines should place HbA1c as diagnostic and prognostic tool in accordance with the available publish data.

contributor to non-communicable disease-related morbidity and mortality, particularly in resource-constrained countries.^{1 2} In Pakistan, recent second National Diabetes Survey of Pakistan (2nd NDSP) 2016–2017 has estimated the overall prevalence of diabetes to be 26.3%.³

Oral glucose tolerance test (OGTT) has been considered as the standardized diagnostic criteria for the classification of diabetes and prediabetes for decades.^{4 5} The uncertainty of fasting phase, poor concordance of



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fasting plasma glucose (FPG), 2-hour glucose tolerance (2hGT) values, day-to-day high glucose fluctuations and an alternative to glucose measurements for the diagnosis of diabetes has long been sought.^{6 7} Performance of HbA1c with OGTT for diagnosing diabetes and prediabetes has been compared in many studies.⁸ Racial and ethnic variations in HbA1c values were also observed for the same degree of hyperglycemia affecting its utilization as a modality for diagnosing diabetes.^{1 9} HbA1c offers some advantages over fasting glucose and 2hGT (OGTT) tests, as it can be performed at any time of the day with no requirement for overnight fasting and glucose challenge. The results do not get affected by delayed analysis after collection if properly stored as per the standard guidelines.^{1 10}

Studies have symbolized the evidence that convenience of sampling, unaffected outcome with dietary intake or compliance with fasting and stabilized analytical concentration in blood make HbA1c as a useful screening tool for diabetes in population and its complications, however, it is still not a validated tool for new diagnosis of diabetes in low-income and middle-income world.^{11 12} The common underlying causes for underestimation of HbA1c due to either hemoglobinopathies, hemolysis, hypertriglyceridemia, drugs and vitamin supplementations further raises the concern for diagnosing diabetes at 6.5% (48 mmol/mol).^{13–16}

It, therefore, seems imperative to determine the regional optimal cut-off values for HbA1c in order to have a useful, convenient and reliable tool for identifying the subjects with prediabetes and diabetes. Hence, this study aims to evaluate HbA1c cut-offs as a diagnostic tool in diabetes and prediabetes with its concordance to the OGTT in Pakistani population.

METHODOLOGY

Data for this substudy was obtained from 2nd NDSP (2016–2017).³ Duration of the survey was 19 months from February 2016 to August 2017. Informed consent was obtained from each participant.³

The main objective of the survey was to assess the prevalence of type 2 diabetes and its associated risk factors in urban and rural areas of all four provinces of Pakistan that includes Punjab, Sindh, Khyber Pakhtunkhwa (KPK) and Baluchistan (as defined in the latest available census). The estimated sample size was 10 800. Known and unknown diabetics, hypertensives and with other cardiometabolic risk factors were included as per designed methodology. Sample was taken from each province (stratum) with respect to its population size. In each province, tehsils or towns as subclusters were selected from identified clusters using probability proportional to size technique as defined in 2nd NDSP (2016–2017). Identified clusters were selected using the 'rule of thumb': number of clusters (k)=(sample size of stratum/2)^{0.5}. Total of 21 (tehsils/towns) from urban and 23 (tehsils/towns) from rural areas were selected for the survey. Households were

identified and selected members of every 10th household were advised to come to the camp after an overnight fast. Probability sampling and multistage stratified sampling technique were used to calculate the sample size.³

A predesigned questionnaire comprising detailed demographic, medical, social and obstetric history was filled by trained medical and research officers via a detailed interactive session. Self-questionnaire filling was not allowed. Unanswered or indecisive responses were considered as negative. Blood samples were drawn for the assessment of biochemical parameters. The clinical and anthropometric measurements included height, weight, blood pressure, waist circumference and waist-to-hip ratio, while biochemical parameters were OGTT, HbA1c and fasting lipid. Specifications of equipment was uniform throughout the study for standardization and to ensure quality control and sample were run as per standardized protocol.^{3 17} In the survey, OGTT was used as a standard diagnostic test to screen the population. In this substudy, HbA1c using the American Diabetes Association (ADA) criteria was also evaluated in the diagnosis of prediabetes and diabetes with its optimal cut-off values. To determine and differentiate between the optimal HbA1c cut-offs for Pakistani population and its concordance with OGTT, all those subjects who had known type 2 diabetes or were on antidiabetes treatment, and subjects missing their HbA1c levels were excluded from this substudy.

In the 2nd NDSP (2016–2017), 10 834 individuals were recruited for diabetes, out of which 6836 participants fulfilling inclusion criteria were included (participants with known diabetes and who have missing HbA1c were excluded from this substudy).³ Participants were categorized as normal glucose tolerance (NGT), newly diagnosed diabetes (NDD) and prediabetes. Known diabetes was considered if the subject had been diagnosed as having diabetes by a physician or definition by WHO.^{5 18} Diabetes and prediabetes (intermediate hyperglycemia) were diagnosed according to WHO categorization as done in the 2nd NDSP (2016–2017).³ As per WHO definition, results of plasma glucose testing were categorized as follows: isolated impaired fasting glucose (IFG)—FPG level between 110 and 125 mg/dL with 2-hour postglucose level (PGL) \leq 140 mg/dL. Isolated impaired glucose tolerance—fasting glucose level $<$ 110 mg/dL and 2-hour PGL between 141 and 199 mg/dL. Newly diagnosed diabetes was defined as FPG level \geq 126 mg/dL or 2-hour PGL \geq 200 mg/dL or both. For the diagnosis of diabetes using HbA1c as a diagnostic tool, the ADA standards of care were used. HbA1c \geq 6.5% (48 mmol/mol) was diagnosed as diabetes, while HbA1c between 5.7% and 6.4% (39 and 46 mmol/mol) was considered as prediabetes with results of available OGTT.^{3 5 19}

Height, body weight, waist circumference and blood pressure were measured using standardized techniques. Body mass index (BMI) was calculated as weight in kilograms divided by height in meter squared (kg/m²). Blood pressure was measured with a mercury

sphygmomanometer. Plasma glucose was measured using the glucose oxidase peroxidase method, total cholesterol by cholesterol oxidase phenol 4-amino antipyrine peroxidase (CHOD-PAP) method, triglycerides by glycerol phosphate oxidase-p-amino phenazone method, high-density lipoprotein cholesterol by homogeneous enzymatic calorimetric method, low-density lipoprotein cholesterol by CHOD-PAP method and HbA1c by high-performance liquid chromatography method as reported earlier in 2nd NDSP (2016–2017).³

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences V.20. Variables were expressed as median (IQR) or proportions. Kruskal-Wallis H non-parametric test was used to compare continuous variables and χ^2 test for categorical variables. With OGTT test as the gold standard, receiver operating characteristic curve (ROC) was used for the multiclass variable to define area under the curve (AUC) of HbA1c for diagnosing diabetes and prediabetes. ROC curve was used with OGTT (categorical variable) as state variable and HbA1c (continuous variable) as test variable. The cut-off for HbA1c was noted where sensitivity and specificity were optimal. Data were divided with respect to age and gender for both diabetics and prediabetics. The optimal HbA1c cut-off value was selected where sum of sensitivity and specificity was maximum. Statistical significance was determined as a two-sided $p < 0.05$.

RESULTS

OGTT was performed for 10834 individuals, however, only 6836 participants fulfilled the inclusion criteria for this substudy. Out of 6836 participants, 4690 (68.6%) had NGT, 1333 (19.5%) had prediabetes and 813 (11.9%) had NDD by OGTT criteria.

Table 1 shows the characteristics of subjects by glucose status according to OGTT in all three groups. Female participants were dominant 3952 (57.8%) as compared with males 2884 (42.2%). The median (IQR) age of participants was 40 (31–50) years. Positive family history of diabetes mellitus was 827 (22.2%) in NGT group, 353 (30.0%) in prediabetes and 183 (28.8%) in NDD group. Significantly higher results were observed for baseline and biochemical parameters in prediabetes except for primary education or more and HDL as compared with participants with NGT, while in NDD, this trend was observed for all variables except for LDL as compared with NGT group.

Table 2 is showing the optimal HbA1c cut-off point for identification of diabetes as 5.7% (39 mmol/mol) ((AUC (95% CI)=0.776 (0.757 to 0.795), $p < 0.0001$)) with a sensitivity and specificity of 59.5% and 85.9%, respectively. The sensitivity and specificity of HbA1c value of 6.5% (48 mmol/mol) (recommended by ADA) to classify diabetes were 43.40% and 92.60%, respectively.⁵ The optimal HbA1c cut-off point for identification of prediabetes was 5.1% (32 mmol/mol) ((AUC (95% CI)=0.607

Table 1 Characteristics of subjects by glucose status according to OGTT

Variable	Overall	Normal	Prediabetes	NDD
N	6836	4690 (68.6%)	1333 (19.5%)	813 (11.9%)
Age (years)	40 (31–50)	39 (29–49)	45 (35–55)***	49 (40–58)***
Gender				
Male	2884 (42.2%)	1963 (41.9%)	562 (42.2%)	359 (44.2%)
Female	3952 (57.8%)	2727 (58.1%)	771 (57.8%)	454 (55.8%)
Positive family history of DM	1363 (24.6%)	827 (22.2%)	353 (30.0%)***	183 (28.8%)***
Primary education or more	3045 (53.3%)	2132 (54.3%)	604 (52.2%)	309 (49.1%)*
Tobacco	814 (14.1%)	508 (12.8%)	199 (16.9%)***	107 (16.7%)**
Body mass index (kg/m ²)	26.3 (22.8–30.3)	26.2 (22.5–30.1)	26.5 (23.3–30.4)*	27.1 (23.7–30.7)**
Waist circumference (cm)	91 (81–99)	89 (81–98)	92 (86–102)***	94 (86–103)***
Systolic blood pressure (mm Hg)	120 (110–130)	120 (110–130)	120 (115–140)***	130 (120–140)***
Diastolic blood pressure (mm Hg)	80 (71–90)	80 (70–90)	80 (80–90)***	80 (80–90)***
Total cholesterol (mg/dL)	185 (151–222)	183 (149–218)	188 (157–227)**	192 (154–230)**
Triglyceride (mg/dL)	146 (102–218)	138 (97–206)	155 (108–226)***	183 (123–269)***
High-density lipoprotein (mg/dL)	31 (21.9–40)	31.6 (22.4–40.1)	30.4 (22.0–39.7)	29.0 (20.1–37.1)***
Low-density lipoprotein (mg/dL)	118.9 (95.1–145.6)	117.3 (95–143.4)	122 (97–150.4)***	121.5 (94.2–149.8)
HbA1c (%)	5.1 (4.8–5.5)	5.0 (4.8–5.3)	5.2 (4.9–5.6)***	6.2 (5.3–8.4)***
HbA1c (mmol/mol)	32 (29–37)	31 (29–34)	33 (30–38)***	44 (34–68)***

Data presented as median (IQR) or n (%)

* $P < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

DM, diabetes mellitus; HbA1c, glycated hemoglobin; NDD, newly diagnosed diabetes; OGTT, oral glucose tolerance test.

Table 2 Sensitivity and specificity of different HbA1c cut-off value for diagnosing diabetes and prediabetes

Status	HbA1c % (mmol/mol)	Sensitivity (%)	Specificity (%)
Diabetes	5.1 (32)	79.0	57.7
	5.2 (33)	75.4	64.8
	5.3 (34)	72.3	71.3
	5.4 (36)	67.7	76.1
	5.5 (37)	63.8	79.9
	5.6 (38)	61.0	84.1
	5.7 (39)	59.5	85.9
	5.8 (40)	57.1	87.7
	5.9 (41)	54.2	89.1
	6.0 (42)	52.8	90.1
	6.1 (43)	51.0	90.7
	6.2 (44)	47.6	91.6
	6.3 (45)	46.7	91.9
	6.4 (46)	45.0	92.3
6.5 (47)	43.4	92.6	
Prediabetes	4.7 (28)	96.1	23.4
	4.8 (29)	80.3	30.6
	4.9 (30)	73.9	38.7
	5 (31)	64.6	51.2
	5.1 (32)	56.9	59.7
	5.2 (33)	50.8	65.22
	5.3 (34)	40.7	74.7
	5.4 (36)	34.7	79.1
	5.5 (37)	30.1	82.7
	5.6 (38)	24.1	86.4
	5.7 (39)	21.6	88.0

HbA1c presented % (mmol/mol).

Bold values indicate optimal cut-point chosen by Youden index (J) method.

HbA1c, glycated hemoglobin.

(0.590 to 0.624), $p < 0.0001$) with a corresponding sensitivity and specificity of 56.9% and 59.7%, respectively. The sensitivity and specificity of HbA1c value of 5.7% (39 mmol/mol) (recommended by ADA) to classify prediabetes were 21.3% and 88.2%, respectively.⁵ Sensitivity and specificity of other HbA1c cut-off values are displayed in table 2.

Table 3 shows the stratified analysis of HbA1c for diagnosing diabetes and prediabetes. The optimal HbA1c cut-off values for detecting NDD and prediabetes were 5.62% (38 mmol/mol) (AUC (95% CI)=0.776 (0.748 to 0.804)) and 5.20% (33 mmol/mol) (AUC (95% CI)=0.612 (0.585 to 0.638)) in males and 5.80% (40 mmol/mol) (AUC (95% CI)=0.777 (0.752 to 0.802)) and 5.11% (32 mmol/mol) (AUC (95% CI)=0.604 (0.581 to 0.626)) in females, respectively. Age-stratified analysis of HbA1c shows the optimal HbA1c cut-off point for diabetes as 5.11% (32

mmol/mol) and prediabetes as 4.79% (29 mmol/mol) for youth (20–29 years). This trend was 5.94% (41 mmol/mol) and 5.16% (33 mmol/mol) in 30–39 years, 5.80% (40 mmol/mol) and 5.30% (34 mmol/mol) in 40–49 years, 5.85% (40 mmol/mol) and 5.25% (34 mmol/mol) in 50–59 years and 5.67% (38 mmol/mol) and 5.11% (32 mmol/mol) in ≥ 60 years for diabetes and prediabetes, respectively.

Table 4 presents the frequency of NDD and prediabetes by HbA1c and OGTT standard. Among 4690 (100%) subjects with NGT by OGTT, 1647 (35.1%) and 636 (13.6%) were identified as prediabetes and NDD, respectively, by HbA1c criteria and only 2407 (51.3%) were observed with NGT by both criteria. However, out of 1333 (100%) participants with prediabetes diagnosed by OGTT, 474 (35.6%) and 319 (23.9%) were identified with NGT and diabetes by HbA1c criteria, respectively, and 540 (40.5%) were identified prediabetes by both criteria. On the other hand, a total of 813 (100%) subjects were diagnosed with diabetes by OGTT, of whom 135 (16.6%) and 182 (22.4%) were identified with NGT and prediabetes by HbA1c criteria and only 496 (61.0%) with NDD by both criteria.

In table 5, the group by OGTT not HbA1c was compared with group by HbA1c not OGTT. By ADA criteria for HbA1c cut-off values, 955 (13.9%) and 1829 (26.7%) participants were detected as NDD and prediabetes, respectively. While 317 (4.6%) and 793 (11.6%) were diagnosed as NDD and prediabetes by OGTT cut-off values, respectively. Only 496 (7.3%) were diagnosed as NDD and 540 (7.9%) as prediabetes by both (HbA1c and OGTT) criteria. Further analysis demonstrates that in NDD, significant results were observed for age, waist circumference, systolic and diastolic blood pressure, HbA1c, fasting blood sugar and random blood sugar in group by HbA1c not OGTT as compared with group by OGTT not HbA1c. This trend was significant in prediabetes for all variables except for age, gender, primary education or more, systolic blood pressure and LDL in group diagnosed by HbA1c not OGTT as compared with group by OGTT not HbA1c.

DISCUSSION

In this substudy, all participants compared by both criteria were suggesting that HbA1c as a screening tool for diabetes and prediabetes in comparison to OGTT has non-similar performance. However, the optimal HbA1c threshold for diagnosing diabetes and prediabetes was found to be lower than ADA criteria for our population. Our results are consistent in general to previous studies, that FPG (OGTT or fasting only) measurement performs better than HbA1c for screening NDD and prediabetes.^{7 12 13} But these results as opposed to other study suggesting that the FPG and HbA1c have similar performance as screening modalities for diabetes in a multi-racial Asian population.²⁰

Table 3 Stratified analysis of HbA1c for diagnosing diabetes and prediabetes

	Optimal HbA1c % (mmol/mol) cut-off point	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P value
Diabetes					
Gender					
Male	5.62 (38)	61.6	83.8	0.776 (0.748 to 0.804)	0.96
Female	5.80 (40)	57.7	88.2	0.777 (0.752 to 0.802)	
Age (years)					
20–29	5.11 (32)	50	72.2	0.645 (0.567 to 0.723)	0.025
30–39	5.94 (41)	49.5	91.3	0.747 (0.694 to 0.801)	0.888
40–49	5.80 (40)	61	84.4	0.757 (0.717 to 0.797)	0.87
50–59	5.85 (40)	64.6	81.9	0.779 (0.739 to 0.818)	0.388
60+	5.67 (39)	65.7	75.1	0.752 (0.710 to 0.794)	
Prediabetes					
Gender					
Male	5.20 (33)	48.6	69.4	0.612 (0.585 to 0.638)	0.662
Female	5.11 (32)	58.6	57.5	0.604 (0.581 to 0.626)	
Age (years)					
20–29	4.79 (29)	77.7	34	0.562 (0.516 to 0.608)	0.676
30–39	5.16 (33)	51.5	62.6	0.588 (0.552 to 0.624)	0.682
40–49	5.3 (34)	46.4	68.7	0.568 (0.532 to 0.604)	0.781
50–59	5.25 (34)	58.2	84.9	0.581 (0.539 to 0.624)	0.873
60+	5.11 (32)	69.9	87.1	0.576 (0.532 to 0.620)	0.771

HbA1c presented % (mmol/mol).

AUC, area under the curve; HbA1c, glycated hemoglobin.

In this study, only 7.9% and 7.3% participants were identified as prediabetes and NDD, respectively, by using both OGTT and HbA1c criteria. This trend was higher by using only HbA1c cut-offs of ADA criteria as compared with using only OGTT. HbA1c used in diagnosing or screening diabetes has been debated for many years. The HbA1c >6.1% (43 mmol/mol) was the recommended optimum cut-off point for diabetes in most reviewed studies; however, there is also an argument for population-specific cut-off points as optimum cut-offs vary by ethnic group and population prevalence of diabetes.²¹ In previous study, HbA1c cut-off point of 5.6% (38 mmol/mol) was identified for undiagnosed type 2 diabetes, and used by the Japanese Diabetes Society as a supplementary diagnostic criterion.²²

Similarly, in our population, the optimal HbA1c cut-off point 5.7% (39 mmol/mol) was able to identify people

with undiagnosed diabetes and 5.1% (32 mmol/mol) had an increased risk for progression to type 2 diabetes with high predictive sensitivity than recommended HbA1c values by ADA. Hence, these cut-offs similar to other reported studies are too high to use for screening diabetes in our populations.^{5 23 24} The discrepancy between our study and others may be caused by differences in study design, study participants demography and area location. Racial differences may affect optimal HbA1c threshold for diagnosing diabetes, therefore, more and more data are still needed from all over the world.^{25 26}

Age-stratified analysis of HbA1c for diagnosing diabetes and prediabetes in our study shows the specific trends that also suggest the age-specific diagnostic threshold should be considered, if HbA1c being recommended as diagnostic standard similar to previous studies.^{6 23} Studies in Dutch and US population also present the HbA1c

Table 4 Population distribution classified by HbA1c and OGTT standard

OGTT	HbA1c cut-off point			Total
	<5.1% (32 mmol/mol)	5.2%–5.6% (33–38 mmol/mol)	≥5.7% (≥39 mmol/mol)	
NGT	2407 (51.3%)	1647 (35.1%)	636 (13.6%)	4690 (100%)
Prediabetes	474 (35.6%)	540 (40.5%)	319 (23.9%)	1333 (100%)
NDD	135 (16.6%)	182 (22.4%)	496 (61.0%)	813 (100%)

HbA1c, glycated hemoglobin; NDD, newly diagnosed diabetes; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

Table 5 Characteristics of participants identified as diabetes and prediabetes based on OGTT and HbA1c

Variable	Newly diagnosed diabetes			Prediabetes		
	By OGTT not HbA1c	By HbA1c not OGTT	By both	By OGTT not HbA1c	By HbA1c not OGTT	By both
n	317	955	496	793	1829	540
Age (years)	45 (35–56)	45 (37.75–55)**	50 (42–60)	44 (35–55)	40 (31–50)	45 (35–55)
Gender						
Male	141 (44.5%)	399 (41.8%)	218 (44%)	344 (43.4%)	725 (39.6%)	218 (40.4%)
Female	176 (55.5%)	556 (58.2%)	278 (56%)	449 (56.6%)	1104 (60.4%)	322 (59.6%)
Positive family history of DM	71 (29.5%)	183 (23.7%)	112 (22.6%)	212 (30.8%)	336 (21.5%)***	141 (29%)
Primary education or more	127 (53.4%)	410 (48.6%)	182 (36.7%)	362 (53.2%)	801 (50.7%)	242 (44.8%)
Tobacco	39 (15.8%)	122 (14.3%)	68 (17.3%)	129 (18.6%)	212 (13%)*	70 (14.5%)
Alcohol	1 (0.4%)	14 (1.7%)	14 (3.8%)	13 (2.0%)	29 (1.9%)*	7 (1.5%)
Body mass index (kg/m ²)	26.58 (22.72–30.15)	27.64 (24.38–31.25)	27.53 (24.16–30.98)	26.57 (23.54–30.73)	26.71 (23.18–30.86)*	26.48 (23.19–29.74)
Waist circumference (cm)	93 (85.25–104)	92 (84–102)*	95 (86–102)	91.4 (86–101)	90 (81–99)	93 (86–102)
Systolic blood pressure (mm Hg)	120 (110–140)	120 (120–135.5)**	127 (120–140)	120 (110–135)	120 (110–130)	120 (110–134)
Diastolic blood pressure (mm Hg)	80 (80–90)	87 (80–90)**	80.5 (80–90)	85 (80–90)	80 (80–90)*	80 (78–90)
Total cholesterol (mg/dL)	182.5 (147.5–218)	193 (158.25–229)	199 (159–236)	184 (153–221.75)	188 (153–226)**	192 (161–231)
Triglyceride (mg/dL)	150 (104–220.5)	170.5 (117.25–249.5)	204 (145.25–312)	151.5 (108–222.75)	144 (103–223)**	161 (108–230)
High-density lipoprotein (mg/dL)	28.9 (20.11–38)	31 (22.4–40)	29 (20.28–36.77)	30.25 (22–39.08)	32 (22.08–41)*	30.5 (21.8–40)
Low-density lipoprotein (mg/dL)	118 (92.67–143.41)	121 (100.34–147.59)	124.08 (95.1–152.8)	119.92 (97.22–148.08)	119.08 (96.45–145.28)	124.5 (98.1–152.5)
HbA1c (%)	5.1 (4.9–5.4)	6.4 (5.9–8.04)**	7.8 (6.4–9.2)	5 (4.79–6)	5.3 (5.13–5.4)**	5.3 (5.2–5.4)
HbA1c (mmol/mol)	32 (30–36 mmol/mol)	46 (41–64 mmol/mol)**	62 (46–77 mmol/mol)**	31 (29–42 mmol/mol)	34 (33–36 mmol/mol)**	34 (33–36 mmol/mol)
FBS (mg/dL)	133 (118.5–156.75)	88(76–100)**	175 (138–240)	99 (88–110)	85 (76–95)**	100 (88–110)
RBS (mg/dL)	204 (166–234)	123 (102–144)**	249 (204–326)	153 (143–165)	111 (96–126)**	150 (142–162)

Data presented as median (IQR) or n (%).

Group by OGTT not HbA1c was compared with group by HbA1c not OGTT.

* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

DM, diabetes mellitus; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance test; RBS, random blood sugar.

cut-off of 5.8% (40 mmol/mol) and 5.7% (39 mmol/mol), respectively.^{27 28} Accordingly, it seems difficult to develop a common HbA1c cut-off worldwide. Gender differences have also been reported in HbA1c levels being higher in men. This study showed no statistically significant difference in genders using both criteria. In a recent study, high specificity and sensitivity of HbA1c versus OGTT was noted after excluding 16% participants with anemia, but relatively very low number of these participants diagnosed with OGTT were compared with HbA1c, non-representative results.¹⁹ In this substudy, anemia was not determined as a confounding factor beyond the scope of 2nd NDSP (2016–2017) reported as our limitations.³ However, HbA1c is considered as an effective, more convenient and good alternative to FPG to diagnose complications in subjects with known diabetes, better indicator of chronic glycemic levels, has less pre-analytic instability and does not need a fasting or timed sample. Therapeutic decisions are also based on HbA1c value, so to improve the ability for screening prediabetes and NDD, additional FPG or OGTT tests are still necessary.²⁹

Based on topical results, considering HbA1c as a diagnostic tool we also have to diagnose anemia first. It is also a debate for new researchers regarding different HbA1c cut-offs for males and females by WHO and ADA similar to hemoglobin. Therefore, it is necessary to carefully choose HbA1c or FPG (OGTT) as diagnostic criteria.

Strengths and limitations

An important high point of this substudy is that it is the first study to compare the effect of Pakistani ethnic group on the HbA1c cut-off values for the diagnosis of dysglycemia. HbA1c cut-off values were determined in concordance to OGTT is another strength. We lack data regarding anemia, kidney functions, medication history as confounding factors affecting HbA1c. Apart from few limitations, as subpaper of 2nd NDSP (2016–2017) having community representative data, the findings of this study will contribute to open new horizons of research to use the HbA1c as a diagnostic tool.

CONCLUSION

The findings from 2nd NDSP demonstrated the disagreement between the findings of OGTT and HbA1c as diagnostic tool for the Pakistani population. As compared with international guidelines, HbA1c threshold for prediabetes and NDD were found to be lower in this part of the world. HbA1c as a diagnostic tool might require ethnic or regional-based modification in cut-off points, validated by relevant community-based epidemiological surveys.

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REFERENCES

- 1 Radhakrishna P, Vinod KV, Sujiv A, *et al*. Comparison of Hemoglobin A_{1c} with Fasting and 2-h Plasma Glucose Tests for Diagnosis of Diabetes and Prediabetes among High-risk South Indians. *Indian J Endocrinol Metab* 2018;22:50–6.
- 2 Yoon K-H, Lee J-H, Kim J-W, *et al*. Epidemic obesity and type 2 diabetes in Asia. *Lancet* 2006;368:1681–8.
- 3 Basit A, Fawwad A, Qureshi H, *et al*. Prevalence of diabetes, prediabetes and associated risk factors: second national diabetes survey of Pakistan (NDSP), 2016–2017. *BMJ Open* 2018;8:e020961.
- 4 American Diabetes Association. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes—2019*. *Diabetes Care* 2019;42:S13–28.

- 5 World Health Organization. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation*, 2006.
- 6 Zhou X, Pang Z, Gao W, *et al.* Performance of an A1c and fasting capillary blood glucose test for screening newly diagnosed diabetes and pre-diabetes defined by an oral glucose tolerance test in Qingdao, China. *Diabetes Care* 2010;33:545–50.
- 7 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014;37:S81–90.
- 8 Nair M, Prabhakaran D, Narayan KMV, *et al.* HbA(1c) values for defining diabetes and impaired fasting glucose in Asian Indians. *Prim Care Diabetes* 2011;5:95–102.
- 9 Herman WH, Cohen RM. Racial and ethnic differences in the relationship between HbA1c and blood glucose: implications for the diagnosis of diabetes. *J Clin Endocrinol Metab* 2012;97:1067–72.
- 10 Nazir A, Papita R, Anbalagan VP, *et al.* Prevalence of diabetes in Asian Indians based on glycated hemoglobin and fasting and 2-h post-load (75-g) plasma glucose (CURES-120). *Diabetes Technol Ther* 2012;14:665–8.
- 11 Selvin E, Crainiceanu CM, Brancati FL, *et al.* Short-Term variability in measures of glycemia and implications for the classification of diabetes. *Arch Intern Med* 2007;167:1545–51.
- 12 Lu ZX, Walker KZ, O'Dea K, *et al.* A1C for screening and diagnosis of type 2 diabetes in routine clinical practice. *Diabetes Care* 2010;33:817–9.
- 13 Kumar PR, Bhansali A, Ravikiran M, *et al.* Utility of glycated hemoglobin in diagnosing type 2 diabetes mellitus: a community-based study. *J Clin Endocrinol Metab* 2010;95:2832–5.
- 14 Bhowmik B, Diep LM, Munir SB, *et al.* HbA(1c) as a diagnostic tool for diabetes and pre-diabetes: the Bangladesh experience. *Diabet Med* 2013;30:e70–7.
- 15 Mohan V, Vijayachandrika V, Gokulakrishnan K, *et al.* A1C cut points to define various glucose intolerance groups in Asian Indians. *Diabetes Care* 2010;33:515–9.
- 16 Ho-Pham LT, Nguyen UDT, Tran TX, *et al.* Discordance in the diagnosis of diabetes: comparison between HbA1c and fasting plasma glucose. *PLoS One* 2017;12:e0182192.
- 17 World Health Organization. *Good laboratory practice training manual for the trainer: a tool for training and promoting good laboratory practice (GLP) concepts in disease endemic countries*. 2 edn, 2008. <http://www.who.int/tdr/publications/documents/glp-trainer.pdf>
- 18 American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 2016;39:S13–22.
- 19 Aamir AH, UI-Haq Z, Mahar SA, *et al.* Diabetes prevalence survey of Pakistan (DPS-PAK): prevalence of type 2 diabetes mellitus and prediabetes using HbA1c: a population-based survey from Pakistan. *BMJ Open* 2019;9:e025300.
- 20 Lim W-Y, Ma S, Heng D, *et al.* Screening for diabetes with HbA1c: test performance of HbA1c compared to fasting plasma glucose among Chinese, Malay and Indian community residents in Singapore. *Sci Rep* 2018;8:12419.
- 21 Cavagnoli G, Comerlato J, Comerlato C, *et al.* HbA(1c) measurement for the diagnosis of diabetes: is it enough? *Diabet Med* 2011;28:31–5.
- 22 Nakagami T, Tominaga M, Nishimura R, *et al.* Is the measurement of glycated hemoglobin A1c alone an efficient screening test for undiagnosed diabetes? Japan national diabetes survey. *Diabetes Res Clin Pract* 2007;76:251–6.
- 23 Choi SH, Kim TH, Lim S, *et al.* Hemoglobin A1c as a diagnostic tool for diabetes screening and new-onset diabetes prediction: a 6-year community-based prospective study. *Diabetes Care* 2011;34:944–9.
- 24 Tanaka Y, Atsumi Y, Matsuoka K, *et al.* Usefulness of stable HbA(1c) for supportive marker to diagnose diabetes mellitus in Japanese subjects. *Diabetes Res Clin Pract* 2001;53:41–5.
- 25 Bennett CM, Guo M, Dharmage SC. HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review. *Diabet Med* 2007;24:333–43.
- 26 Nida S, Khan DA, Ijaz A, *et al.* Determination of mean glycated haemoglobin in healthy adults of a local population. *J Coll Physicians Surg Pak* 2017;27:399–403.
- 27 van 't Riet E, Alsema M, Rijkkelijkhuizen JM, *et al.* Relationship between A1c and glucose levels in the general Dutch population: the new Hoorn study. *Diabetes Care* 2010;33:61–6.
- 28 Rohlfing CL, Little RR, Wiedmeyer HM, *et al.* Use of GHB (HbA1c) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 2000;23:187–91.
- 29 Sherwani SI, Khan HA, Ekhzaimy A, *et al.* Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016;11:BMIS38440.