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DIABETES PREVALENCE SURVEY OF PAKISTAN (DPS- PAK): Prevalence of type 2 Diabetes Mellitus and pre-diabetes using HbA1c: A population-based survey from Pakistan

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Abstract

Objectives

We conducted a Pakistan-wide community-based survey on the prevalence of type-2 diabetes using HbA1c. The only previous national survey was conducted in 1999 using Oral glucose tolerance test (OGTT).

Design, settings and participants

Multi-staged stratified cluster sampling was used for the representative selection of person aged≥20 years, residing in 378 sampled clusters of 16 randomly selected districts. Eligible participants were called to the nearby field clinic for HbA1c analyses. OGTT was conducted on a subsample of the participants. Overall and stratified prevalence of type-2 diabetes and its association with risk factors were estimated using logistic regression models through STATA-14.

Main outcome measures

Prevalence of pre-diabetes and type 2 diabetes

Results

Of 18,856 eligible participants the prevalence of pre-diabetes was 10.91% (95% CI 10.46, 11.36, n=2,057) and type-2 diabetes was 16.98% (95%CI 16.44, 17.51, n=3,201). Overall, the mean HbA1c level was 5.62% (SD 1.96), and among newly diagnosed was 8.56% (SD 2.08). The prevalence was highest in age 51-60 years (26.03%, p<0.001), no formal education (17.66%, p<0.001), class-3 obese (35.09%, p<0.001), family history (31.29%, p<0.001) and female (17.80%, p=0.009). On multivariate analysis, there was a significant dose-response relationship of type-2 diabetes with age, BMI, central obesity, family history, and hypertension and the inverse relationship with education. On a subsample (n=1,027), summary statistics for diagnosis of diabetes on HbA1c showed a sensitivity of 84.7%, specificity of 87.2%, and ROC area 0.86, compared to OGTT.

Conclusions

The prevalence of type-2 diabetes and pre-diabetes is higher than what was reported before in Pakistan and demands public health emergency against this deadly disease.

Key words (mesh terms):

Type 2 Diabetes Mellitus, diabetes, Prevalence, HbA1c, OGTT, obesity, pre-diabetes, Pakistan

Strength and limitations of this study

- This is the 2nd national prevalence study of type-2 diabetes mellitus from Pakistan conducted after 20 years and the first community based national study to use HbA1c as the diagnostic tool with a large sample of 18,856 subjects.
- This study presents estimates that are more valid and scientifically sound than the available evidence on type-2 diabetes mellitus prevalence from a country (Pakistan) that boasts a population of 220 million.
- Our findings have the potential to influence policy in developing countries and induce a shift towards the prevention and control of Non-communicable diseases.
- Limitation includes the lesser number (n=1,027) of 2 hours Oral Glucose Tolerance Test for comparisons with HbA1c (n=18,856).

Background

Type 2 Diabetes Mellitus is one of the most common public health issues and its incidence is on the rise particularly in the middle and low-income countries[1]. When associated with complications, type-2 diabetes can have a profound impact on the person with consequences on the society as a whole. It was thought to be the disease of the affluent and mostly in the urban areas but due to urbanization and the sedentary lifestyle it has affected developing nations, including Pakistan[2].

Pakistan is a South Asian country with an area of 796,095 km^2 and a population of 207.7 million people[3]. In terms of population, Pakistan is the 6^{th} most populous country and is the 36^{th} largest country in the world. The only previous national diabetes survey in Pakistan back in 1999 (published in 2007), reported the prevalence of type-2 diabetes as 11%using Oral Glucose Tolerance Test (OGTT) [4 5]. Part of the same survey separately reported the prevalence of type-2 diabetes in different provinces of Pakistan [6-8]. The International Diabetes Federation (IDF) reported in its atlas 20 the prevalence for Pakistan to be 6.8%, aged 20-79[9], but the health care professionals always believed this to be an underestimate as it is in majority based on OGTT only, 2 decades old national survey and there are conflicting findings with prevalence ranges from 7.2%-19.21% done in different regions of the country [5].

The American Diabetes Association (ADA) criteria for the diagnosis of diabetes require either fasting plasma glucose (FPG) or 75gm OGTT which is time-consuming, requires fasting and may not be reproducible as affected by acute glucose changes and lifestyle interventions[10 11]. In 2009, the International Expert committee on diabetes proposed new diagnostic criteria based on Glycated Haemoglobin (HbA1c), which captures chronic glucose exposure[12]. The proposed diagnostic threshold of 6.5% (48mmol/mol) was based on retinopathy risk at different levels of HbA1c as was the case with FPG and OGTT. This report was followed by a recommendation from the ADA. AnHbA1clevel of 6.5% (48mmol/mol) was agreed to be a diagnostic cut-off for the diagnoses of diabetes[13].

In summary, there is only one previous national level type-2 diabetes survey conducted in 1999 with sample size if 5433 using OGTT. In this study we investigated the prevalence of type-2 diabetes (using the HbA1c test) and its distribution across gender, age, rural and urban, education, Body Mass Index (BMI) WHO and Asian cut-offs, family history, smoking, and blood pressure among a large sample across Pakistan, aged 20 years and above. A sub subsample was tested to explore the diagnostic summary statistics for diagnosis of type-2 diabetes on HbA1c, compared to 2 hours OGTT.

Methods

The Department of Diabetes, Endocrine and Metabolic Diseases, Hayatabad and Department of Health, Government of Khyber Pakhtunkhwa, Pakistan with technical support from Institute of Public Health, Khyber Medical University Peshawar Pakistan, University of Manchester UK and Pakistan Endocrine Society have conducted a nationwide cross-sectional study for the prevalence of type-2 diabetes in April 2017 and completed in November 2017. Three teams under the supervision of epidemiologists were formulated and trained to take basic demographic data on the sample to be screened. These national samples were selected based on a stratified two-stage cluster design, including all metropolitan cities of Pakistan and randomly selected districts (both rural and urban settings) within each province. The sample included districts from central and south of Punjab province (Lahore, Multan, Bahawalpur, Rahim Yar Khan),interior Sindh (Larkana,Dadoo, Sukkur), central Sindh (Karachi), northern Khyber Pakhtunkhwa (KP) (Haripur), central (Peshawar)and southern KP province(Karak), Baluchistan province(Quetta), capital territory(Rawalpindi-Islamabad), Azad Jammu Kashmir (Muzaffarabad), Frontier region Peshawar and the Khyber Agency in Federal Administered Tribal Area (FATA).

Sample size and sampling methodology:

The sample size was estimated for the provinces of the country based on recent census results. The sample size was estimated based on an expected prevalence of 12% with 20% precision and a design effect of 2[6]. For 95% confidence interval and an additional of 32% for non-responders, keeping in view an exclusion rate due to an expected high prevalence of anaemia, the sample size was 4407 approximated to 4500 in order to have 50 subjects from each cluster. The four provinces were included and Federal territory; AJK and FATA were considered as one province for the survey purpose because of their small size of the population. The number of eligible subjects was 4500*5 = 22500.

Three districts were randomly selected from each province and the sample size was equally divided on these districts. Fifteen hundred subjects (30 clusters, 50 subjects per cluster) were examined in each district. The sample was proportionately divided amongst urban and rural area. Probability proportionate to size (PPS) method was used to select clusters from villages in the rural settings of the district. In urban settings, clusters were selected from charges and circles (defined in the national census) using PPS method.

Maps were obtained from the census office of selected villages/charges/circles (V/C/C). Maps of each V/C/C was divided into equal segments such that each segment has approximately 50 persons 20 years and above. One segment was randomly selected and every house within the segment was included. All persons 20 and above living in that house were examined until 50 number is reached. Any person who was absent on the day of survey till evening time was terminally ill, or fitted into excluding criteria or who refused were marked as non-responders.

The ethical approval was granted from Khyber Medical University ethical board (DIR/KMU-EB/SP/000395). Conditions that may affect HBA1C including anaemic subjects with haemoglobin less than 12gm/dl in women and less than 14 gm/dl in men, self-reported renal and hepatic dysfunction, recent blood transfusion, and use of erythropoietin anyone aged below 20 years or refused to participate were excluded. Face to face interviews was conducted at participant's homes to collect information on demographics (including age, gender, residential area, formal education, family history of diabetes, and smoking status) in the paper questionnaire in local languages. Eligible participants were called to a central point established in hujra (local public gathering place) where their Haemoglobin was tested using mission plus Haemoglobin meter (reflectance photometer technique) Acon Laboratories, Inc., San Diego (coefficient of variance CV:3%). Blood pressure was measured using an automated digital blood pressure monitor Konfort Model AS-351in the lying position with the average of three readings was recorded. Weight in kilograms and height in meters was recorded and used to calculate Body Mass Index.

Diabetes status was assessed for HbA1c on blood samples using National Glycohemoglobin Standardization Program (NGSP) certified FIA 8000 immunoassay analyser (lateral flow chromatography colloidal gold) traceable to diabetes control and complication trial (DCCT) reference method(CV: 3-5%). To compare the results from HbA1c, 2 hours OGTT was conducted on a random sample of participants from all clusters (n=1,027) in the specified standard laboratory using Cobas C311 Roche Diagnostics. Participants were given vouchers for free OGTT test within 7 days in a nearby laboratory using Cobas C311 Roche Diagnostics.

Definitions

Age was categorised into six groups: 20-30, 31-40, 41-50, 51-60 and 61 and above years. The residential area was classified as urban and rural based on local government criteria. Formal education status was self-reported was categorized as no formal education, primary, secondary and graduation/post graduation. BMI was categorised on WHO criteria into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), class I obese (30-34.9 kg/m^2), class II obese (35-39.9 kg/m^2), and class III obese (>40 kg/m^2)(World Health Organization., 1995). Waist Circumference (WC) was categorized into normal-weight (0-93.99), overweight (94-102) and obese (102 & above).Waist to hip ratio (WHR) was categorized into normal-weight (0-0.89), overweight (0.90-0.99) and obese (1 & above)[14].The family history of diabetes was categorised to negative or positive on participant's self-reporting based on physician's diagnoses. Smoking status was categorized as never, ex or current smoker. Systolic and diastolic blood pressure was measured using standard procedure and was hypertension was based on blood pressure measurement of ≥140/90 mmHg or anti-hypertensive medication. Patients were considered as known type-2 diabetes on the basis of self-reporting and or being on dietary or exercise advice, oral anti-diabetic medications or insulin. This self-reported group of patients could either be on individual medications or on different drug combinations or diet and exercise therapy.

Type 2 diabetes was diagnosed based on HbA1c results keeping the WHO levels of non diabetic (<5.69 % DCCT aligned/38 mmol/mol IFFC units), pre-diabetes (5.7-6.49% DCCT aligned/ 39-47mmol/mol IFFC units), diabetes (≥6.5 % DCCT aligned /48 mmol/mol IFFC units). For univariate and multivariate logistic regression models, diabetes was dichotomized to No (0; HbA1C level <6.5) and Yes (1; HbA1C level ≥6.5).

Statistical Analyses

Differences in the characteristics of participants by diabetes category were analysed using the χ^2 test for categorical data and ANOVA for continuous data. We examined the association between diabetes and risk factors i.e., age, gender, residence area, education, BMI, family history of diabetes, smoking, systolic and diastolic blood pressure, using univariate and multivariate logistic regression models. Taking OGTT as the standard on a sub subsample, the diagnostic summary statistics (sensitivity, specificity, ROC area, positive and negative predictive value) for diagnosis of diabetes on HbA1c was measured. All statistical analyses were performed using Stata version 14 (StataCorp, College Station, Texas). Statistical significance was defined as $p<0.05$ and analysis were adjusted for the cluster design.

Patient and Public Involvement statement:

Patients were not involved in this study.

Results

Of the 22,500 participants, 3,644 (16%) were anaemic and therefore excluded from the study. Out of the remaining, 18,856participantsaged 20 and above, were actually examined from 378 clusters of which 216 were rural generating a response rate of 84%. The mean age was 45.23 years (standard deviation [SD] 13.97 years), 10,116 (53.55%) were men, 4,148 (21.96%) were hypertensive and those with higher blood pressure were advised to check their blood pressure by visiting their doctors, 13,834 (73.24%) had no formal education, and 1,209 (6.40) had graduation, and 6,010 (31.81%) had family history of type-2 diabetes. Overall on WHO cut-off 345 (1.83%) were underweight, 6,839 (36.20%) normal-weight, 8,038 (42.55%) overweight, 2,864 (15.16%) class I obese, 633 (3.35%) class II obese, and 172 (0.91%) class III obese. On WC cut-off (n=12865) 1,142 (13.32%) were normal-weight, 357 (15.40%) were overweight and 543 (27.51%) were obese. On central obesity cut-off (WHR, n=12865) 658 (15.41%) were normal weight, 1171 (15.68%) were overweight and 213 (18.90%) were obese. Mean systolic blood pressure was 126.30mmHg (SD 14.2) and diastolic blood pressure was 83.24mmHg (SD 10.2).

Overall, 3,201 subjects (16.98% (95%CI 16.44, 17.51) had type-2 diabetes based on HbA1c screening. Pre-diabetes was present in 2,057 subjects 10.91% (95% CI 10.46, 11.36).The mean HbA1c level of the entire cohort (n=18856) was 5.62% (SD 1.96), among known type-2 diabetes (n=2179) had 8.68% (SD 2.70) and newly diagnosed type-2 diabetes (n=1577) had 8.56% (SD2.08). The prevalence of diabetes differed significantly by age, education, BMI, WC, WHR, family history, and blood pressure (Table 1) (Figure 1). The prevalence of diabetes was highest in age 51-60 years (26.03%, p<0.001), no formal education (17.73%, p<0.001), class 3 obese (32.19%, p<0.001), and with a positive family history of diabetes (31.34%, p<0.001) (Figure 1). There were also statistically significant differences in diabetes prevalence by gender (female 17.85%, p=0.01), rural/urban (rural 19.09%, p<0.001) and that smoking status (p=0.008).

On univariate logistic regression analysis, there was a significant association between age, gender, education, BMI category, family history, blood pressure and diabetes (p<0.005)(Table 2). On multivariate logistic regression, there was significantly higher risk of diabetes with age (adjusted Odds Ratio [OR] 2.03, 95% CI 3.39, 4.87 and 4.93, p< 0.001, aged 31-40 years, 41-50 years, 51-60 years and 61 years and above respectively, compared to age 20-30 years), BMI (adjusted OR 1.54, 95% CI 2.13, 2.44,p-value <0.001 for class 1, class II and class III obese respectively, compared to normal weight) with evidence of dose-response relationship. Similarly, there was a significantly higher risk of diabetes with lower educational attainment (adjusted OR 1.83, 95% CI 1.39, 1.57, no formal education, primary and secondary education respectively, compared to graduates). There was a significantly higher risk of diabetes in people with a positive family history (adjusted OR 3.94, 95% CI 3.6, 4.3 p< 0.001), than with no family history of diabetes. There was no significant association with smoking and rural/urban area.

Among 1,029 participants who were tested for 2hrs OGTT, in addition to HBA1C, the mean 2 hours OGTT was 200.26 (SD 91.7), and the median was 178 (inter-quartile range 100). Taking the OGTT as the gold standard, HbA1c recommended cut-off for diabetes showed a sensitivity of 84.7% (95% CI 80.8, 88) and Specificity of 87.2% (95% CI 84.3, 89.8%), ROC area 0.86 (95% CI 0.84, 0.88), positive predictive value 81.9% (95% CI 77.9, 85.4), and negative predictive value 89.3 (86.5, 91.6) (Table 3).

Discussion:

This is the first community based national study done in the region based on HbA1c and with the eligible 18,856 subjects from the country as sample size makes this study largest to date from Pakistan. The prevalence of type-2 diabetes all across Pakistan was 16.98% (95%CI 16.44, 17.51) and pre-diabetes was 10.91% (95% CI 10.46, 11.36). This is higher than found in the only previous national survey conducted in1999 (n=5433) using OGTT. There was a significantly higher risk of type-2 diabetes with increasing systolic blood pressure, age, BMI, WC, WHR with evidence of a dose-response relationship. Similarly, there was a significant inverse relationship

of type-2 diabetes with the level of formal education. The risk of diabetes increases 2.68 times with a prior family history. The hba1c level had good sensitivity and specificity level for the diagnoses of type-2 diabetes compared to a 2-hour OGTT level and is feasible in community settings for screening purposes.

Previous national prevalence study used OGTT where almost 80% of the subjects were women as conducted in the morning time (4). In contrast, our study screening was done all through the day due to which working men had equal opportunity to be part of the sample and constituted 50% of the study population. OGTT was the gold standard for the diagnosis but because of the length of time which is required for the test and the fact that person had to be fasting; making it very difficult to do a standard test without errors. Also, high temperatures in South Asia make it difficult to carry out while keeping the sample steady for transportation to the laboratory. To minimize the effect of temperature and transportation errors on HbA1c all tests were conducted in the field.

Recently a study carried out in 15 states of India showed the prevalence of type-2 diabetes ranging between 4%-13.6% and showed variation due to age, male sex, obesity, family history as independent risk factors using capillary FBG for diagnosis [15]. Capillary blood sample for epidemiological studies is not an ideal test but the authors acknowledged the logistic hindrance in carrying out venous sample test in the field. High prevalence in another Indian study was reported ranging from 12.1-14% for diabetes using OGTT on a sample size of 11,216 subjects[16].

A study conducted in Bangladesh was based on capillary fasting level prevalence type-2 diabetes in the range of 4.3% in a rural setting[17]. Risk factors were positive family history for diabetes, age, high BMI and low socio-economic status, similar to our study. Although these are geographically distant areas, the risk factors showed commonality in both studies, which suggests these risk factors as an important tool for mass screening[18].

The age sex-standardized prevalence of type-2 diabetes for Sri Lankans was 10.3% based on OGTT[19]. The risk factors were almost the same as seen in our study. The investigators found dysglycaemia in almost 21.8% participants and predicted that this would lead to higher prevalence in the years to come. Another study from Sri Lanka indicated a prevalence of 14.2% basis of FBG[20]. FBG as well as, OGTT blood glucose levels, may not be reproducible in an epidemiological survey if the individual is changing lifestyle with diet and exercise. With HbA1c used in our study makes our study more scientific, addressing issues pertaining to sampling errors in the local environment.

The Asian population is known to have a significantly higher risk of developing diabetes and its related complications as predicted by IDF projections [1]. It makes it very important from a

public health point of view to identify high-risk individuals at an early stage. The HbA1c has been used successfully in community settings[21]. National health survey New Zealand in 2008-9 used HbA1c to identify high-risk individuals with diabetes and pre-diabetes[22]. A study in Japan revealed that a combination of tests including FBG and HbA1c yields more diabetes cases compared to any of these tests alone[23].

WHO experts have accepted HbA1c as a diagnostic tool provided quality assurance tests are in place and there are no conditions present which precludes its accurate measurement. New Zealand society for the study of diabetes and Australian diabetes Society has already endorsed HbA1c as a test for the diagnosis of diabetes[24 25]. Recently prevalence study done in Korea concluded that FBG level results in underestimation of diabetes and pre-diabetes[26]. This study suggested the use of standardized HbA1c as a diagnostic tool for diagnosis of type-2 diabetes.

Our study had the strength that we carried out both HbA1c and OGTT on a subsample. We found that HbA1c level had a good sensitivity and specificity level for diagnoses of diabetes compared to 2-hrs OGTT level. HbA1C has the advantage of being a simple test and less time to consume, making it an ideal test for community surveys in our populations. In Pakistan, as there is no effective and practical primary care (General Practice) concept and hence most of the population is not screened at primary level and person present to tertiary care with florid complications. This demands a test which can be done in the community setting without any preparations like HbA1c for screening purpose. There will always be the argument about the cost of the test and whether this to be used for screening purposes, however disadvantaged population need to be accessed to improve diagnosis and care of diabetes[27], needless to say, early diagnosis will also reduce diabetes-related complications.

The central Government of Pakistan has developed and agreed on Non- Communicable Diseases (NCD) National Action Plan including diabetes, however, it was never implemented. After the 18th Amendment in the constitution of Pakistan in 2010, provinces are responsible for making and implementing their own health policies and the role of central Government is limited to coordination among the different provinces[28]. There is a dire need that based on the agreed NCD National Action Plan each province should build their capacity for implementing it at both primary and secondary level. Pakistan is a signatory to the Sustainable Development Goal 2030 and under which actions, like increasing access to universal health coverage, increasing coverage of health insurance program and adopting family medicine approach, will not only be major steps towards prevention and control of diabetes but all NCDs.

Conclusions:

This second national diabetes prevalence study conducted after 20 years and the first one in the region using HbA1c identified a huge population of type-2 diabetes and pre-diabetes group. Those who are obese, with no formal education, older, family history of diabetes and

hypertensive merit close attention and timely intervention. HbA1c is an applicable test in community settings in developing countries and it has a good correlation with 2 hours OGTT.

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Contributors AHA, ZUH conceived the study. AHA, SM, FQ, AS, AJ, OI, AR, IA, HA collected the data. ZUH, AHA, SF, AH, conducted the analysis and wrote the manuscript. All authors reviewed the manuscript. AHA and ZUH contributed equally.

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Competing interest All authors have no competing interest to declare.

Patient Consent Written informed consent granted. The data is fully anonymised and neither the patient nor anyone else could identify the patient.

Ethical approval The ethical approval was granted from the Khyber Medical University ethical board (DIR/KMU-EB/SP/000395).

Data sharing statement No additional data are available

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Table 1 Characteristics of the participants by diabetes categories (n=18,856)

	Non-Diabetic N (%)	Pre-Diabetic N (%)	Diabetic N (%)	*P-value*
	13,598 (72.11)	2,057 (10.91)	3,201 (16.98)	
Age (years)				
20-30	2,772 (20.37)	218 (10.60)	176 (5.52)	<0.001
31-40	3,503 (25.74)	425 (20.66)	511 (16.01)	
41-50	3,802 (27.94)	654 (31.79)	1,033 (32.37)	
51-60	1,955 (14.37)	412 (20.03)	833 (26.10)	
61 & above	1,576 (11.58)	348 (16.92)	638 (19.99)	
Gender				
Male	7363 (54.15)	1099 (53.43)	1638 (51.17)	0.010
Female	6235 (45.85)	958 (46.57)	1563 (48.83)	
Education				
No formal education	9,853 (72.41)	1516 (73.70)	2,439 (76.43)	<0.001
Primary	1936 (14.23)	354 (17.21)	452 (14.16)	
Secondary	818 (6.01)	99 (4.81)	184 (5.77)	
Graduation	1001 (7.36)	88 (4.28)	116 (3.64)	
Area				
Urban	9,117 (67.00)	1,213 (58.97)	1,932 (60.55)	<0.001
Rural	4,491 (33.00)	844 (41.03)	1,259 (39.45)	
Family History				
Negative	10325 (75.93)	1210 (58.82)	1320 (41.24)	<0.001
Positive	3273 (24.07)	847 (41.18)	1881 (58.76)	
Smoking				
Never	12682 (93.26)	1893 (92.03)	2937 (91.75)	0.008
Ex-Smoker	275 (2.02)	49 (2.38)	91 (2.84)	
Current smoker	641 (4.71)	115 (5.59)	173 (5.40)	
Systolic BP (mean ±SD)	124.98	128.17	130.77	0.020
Diastolic BP (mean ±SD)	82.75	84.13	84.84	0.006
BMI				
Underweight (<18.5)	279 (2.05)	31 (1.51)	35 (1.09)	
Normal (18.5-<25)	5137 (37.78)	668 (32.47)	1019 (31.83)	<0.001
Overweight (25-<30)	5884 (43.27)	850 (41.32)	1288 (40.24)	
Obese1 (30-<35)	1844 (13.56)	396 (19.25)	621 (19.40)	
Obese 2 (35-<40)	372 (2.74)	83 (4.04)	178 (5.56)	
Obese 3 (≥40)	82 (0.60)	29 (1.41)	60 (1.87)	
Waist circumference *				
Normal Weight	6,787 (70.88)	645 (51.68)	1,142 (55.93)	
Over weight	1,676 (17.50)	285 (22.84)	357 (17.48)	<0.001
Obese	1,113 (11.62)	318 (25.48)	543 (26.59)	
Waist to Hip ratio *				
Normal Weight	3,219 (33.62)	394 (31.57)	658 (32.22)	
Over weight	5,528 (57.73)	768 (61.54)	1,171 (57.35)	
Obese	828 (8.65)	86 (6.89)	213 (10.43)	0.002

* n for Waist circumference & waist to hip ratio is 12865

Table 2 Logistic regression analysis of the participant characteristics associated with having diabetes (HbA1C ≥6.5 % DCCT aligned /48 mmol/mol IFFC units) (n=18,856)

	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age				
20-30	1		1	
31-40	2.21 (1.8, 2.6)	<0.001	2.03 (1.7, 2.4)	<0.001
41-50	3.93 (3.3,4.6)	<0.001	3.39 (2.9, 4.0)	<0.001
51-60	5.97 (5.0, 7.1)	<0.001	4.87 (4.1, 5.8)	<0.001
61 & above	5.63 (4.7, 6.7)	<0.001	4.93 (4.1, 6.0)	<0.001
Gender				
Male	1		1	
Female	1.12 (1.0,1.2)	0.003	1.04 (0.9, 1.1)	0.334
Education				
No formal education	2.02 (1.6,2.4)	<0.001	1.83 (1.5, 2.3)	<0.001
Primary	1.85 (1.49, 2.3)	<0.001	1.39 (1.1 , 1.8)	0.006
Secondary	1.89 (1.47,2.4)	<0.001	1.57 (1.2 , 2.0)	0.001
Graduation	1		1	
Area				
Urban	1		1	
Rural	1.26 (1.16, 1.34)	<0.001	1.08 (0.9, 1.2)	0.084
Family History of diabetes				
Negative	1		1	
Positive	3.98 (3.6,4.3)	<0.001	3.94 (3.6, 4.3)	<0.001
Smoking				
Never	1		1	
Ex- Smoker	1.39 (1.1, 1.8)	0.006	1.13 (0.9 , 1.5)	0.323
Current Smoker	1.13 (0.9,1.3)	0.14	1.06(0.9, 1.3)	0.571
Systolic BP	1.02 (1.021, 1.026)	<0.001	1.01 (1.01, 1.02)	<0.001
Diastolic BP	1.01 (1.01, 1.02)	<0.001	0.99 (0.9 , 1.0)	0.310
BMI				
Underweight (<18.5)	0.64 (0.4, 0.9)	0.001	0.71 (0.5, 1.0)	0.077
Normal (18.5-<25)	1		1	
Overweight (25-<30)	1.08 (0.9,1.2)	0.06	1.06 (0.9, 1.1)	0.182
Obese1 (30-<35)	1.57 (1.4,1.7)	<0.001	1.54 (1.3, 1.7)	<0.001
Obese 2 (35-<40)	2.22 (1.8,2.6)	<0.001	2.13 (1.7, 2.6)	<0.001
Obese 3 (≥40)	3.07 (2.2,4.2)	<0.001	2.44 (1.7, 3.5)	<0.001
Waist circumference *				
Normal Weight	1		1	
Over weight	1.18 (1.04, 1.34)	0.01	0.98 (0.8, 1.1)	0.774
Obese	2.46 (2.19, 2.77)	<0.001	1.86 (1.6, 2.2)	<0.001
Waist to Hip Ratio *				
Normal Weight	1		1	

Over weight	1.02 (0.92, 1.13)	0.40	0.8 (0.7, 0.9)	<0.001
Obese	1.27 (1.07, 1.51)	0.005	1.13 (0.9, 1.4)	0.205

* n for WC & WHR is 12865.

Table 3: Summary statistics for diabetes diagnosed by HbA1c compared to diabetes diagnosed by 2hrs OGTT (n=1,027)

			[95% Confidence Interval]	
Prevalence	Pr(A)	40.6%	37.6%	43.7%
Sensitivity	Pr(+|A)	84.7%	80.8%	88.0%
Specificity	Pr(-|N)	87.2%	84.3%	89.8%
ROC area	(Sens. + Spec.)/2	0.86	0.84	0.88
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	6.62	5.36	8.18
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	0.18	0.14	0.22
Odds ratio	LR(+)/LR(-)	37.62	26.34	53.73
Positive predictive value	Pr(A|+)	81.9%	77.9%	85.4%
Negative predictive value	Pr(N|-)	89.3%	86.5%	91.6%

Figure 1: Prevalence of diabetes (diagnosed by HbA1C ≥6.5 % DCCT aligned /48 mmol/mol IFFC units) by regions of Pakistan, age, gender, education, family history of diabetes, hypertension, waist circumference, waist to hip ratio and body mass index (n=18,856).

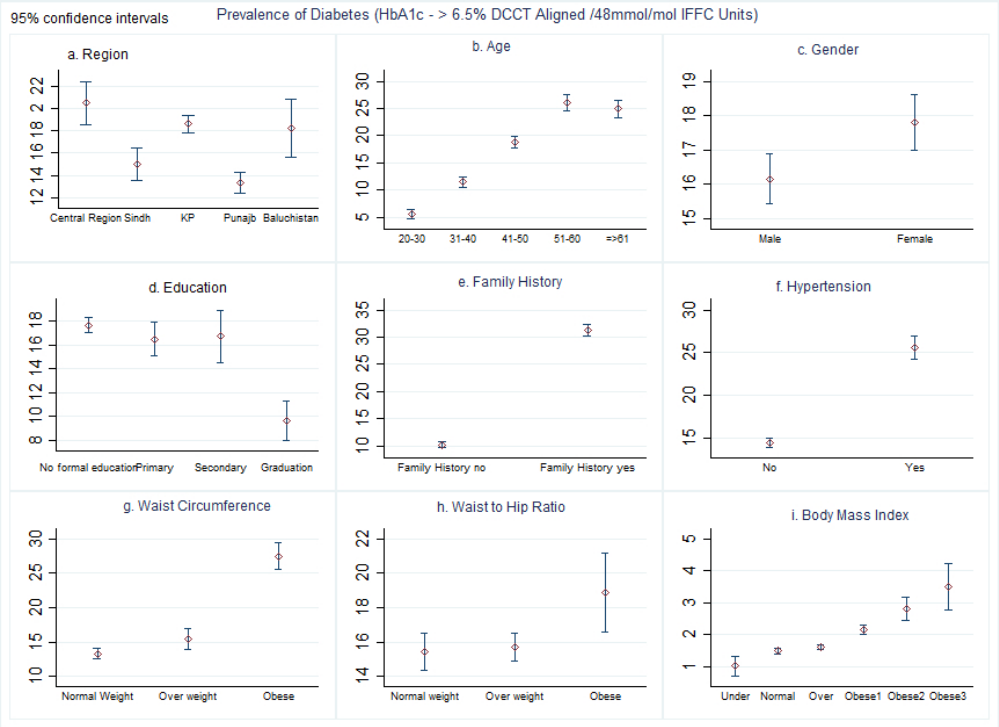

Figure 1: Prevalence of type 2 diabetes (diagnosed by HbA1C ≥6.5 % DCCT aligned /48 mmol/mol IFFC units) by regions of Pakistan, age, gender, education, family history, hypertension, WC, WHR and BMI (n=18,856)

304x221mm (72 x 72 DPI)

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	3
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	6-7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	7-8
Bias	#9	Describe any efforts to address potential sources of bias	8
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8
	#12b	Describe any methods used to examine subgroups and interactions	8
	#12c	Explain how missing data were addressed	8
	#12d	If applicable, describe analytical methods taking account of sampling strategy	8
	#12e	Describe any sensitivity analyses	8
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8-9
	#13b	Give reasons for non-participation at each stage	8
	#13c	Consider use of a flow diagram	6
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8-9

	#14b	Indicate number of participants with missing data for each variable of interest	8-9
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
	#16b	Report category boundaries when continuous variables were categorized	9
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	11
Generalisability	#21	Discuss the generalisability (external validity) of the study results	11
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

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DIABETES PREVALENCE SURVEY OF PAKISTAN (DPS- PAK): Prevalence of type 2 Diabetes Mellitus and pre-diabetes using HbA1c: A population-based survey from Pakistan

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Title Page:

DIABETES PREVALENCE SURVEY OF PAKISTAN (DPS- PAK): Prevalence of type 2 Diabetes Mellitus and pre-diabetes using HbA1c: A population-based survey from Pakistan

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Figures	01
Tables	03

Abstract

Objectives

We conducted a Pakistan-wide community-based survey on the prevalence of type-2 diabetes using HbA1c as the screening test. The aim was to cover all regions of Pakistan and the full spectrum of demographic situations.

Design, settings and participants

Multi-staged stratified cluster sampling was used for the representative selection of people aged≥20 years, residing in 378 sampled clusters of 16 randomly selected districts. Eligible participants were called to the nearby field clinic for HbA1c analyses. OGTT was conducted on a subsample of the participants. Overall and stratified prevalence of type-2 diabetes and its association with risk factors were estimated using logistic regression models through STATA-14.

Main outcome measures

Prevalence of pre-diabetes and type 2 diabetes

Results

Of 18,856 eligible participants the prevalence of pre-diabetes was 10.91% (95% CI 10.46, 11.36, n=2,057) and type-2 diabetes was 16.98% (95%CI 16.44, 17.51, n=3,201). Overall, the mean HbA1c level was 5.62% (SD 1.96), and among newly diagnosed was 8.56% (SD 2.08). The prevalence was highest in age 51-60 years (26.03%, p<0.001), no formal education (17.66%, p<0.001), class-3 obese (35.09%, p<0.001), family history (31.29%, p<0.001) and female (17.80%, p=0.009). On multivariate analysis, there was a significant dose-response relation of type-2 diabetes with age, BMI, central obesity, family history, and hypertension and an inverse relation with education. On a subsample (n=1,027), summary statistics for diagnosis of diabetes on HbA1c showed a sensitivity of 84.7%, specificity of 87.2%, and ROC area 0.86, compared to OGTT.

Conclusions

The prevalence of type-2 diabetes and pre-diabetes is much higher than previously thought in Pakistan. Comprehensive strategies need to be developed to incorporate screening, prevention and treatment of type 2 diabetes at a community level.

Key words (mesh terms):

Type 2 Diabetes Mellitus, diabetes, Prevalence, HbA1c, OGTT, obesity, pre-diabetes, Pakistan

Strength and limitations of this study

Strengths:

Our study has the strength that we carried out HbA1c on all participants and OGTT on a subsample.

Furthermore this is the largest ever national prevalence study of type-2 diabetes mellitus from Pakistan and the first community based national study to use HbA1c as the diagnostic tool.

Limitations:

The relatively low number (n=1,027) of 75g OGTT. Nevertheless the specificity and sensitivity of HbA1c versus OGTT was good.

We had to exclude 16% of recruited participants recruited because of anaemia.

Background

Type 2 Diabetes Mellitus is one of the most common public health issues worldwide and its incidence is on the rise particularly in middle and low-income countries[1]. When associated with complications, type-2 diabetes can have a profound impact on the person with consequences also for the society as a whole. It was previously thought to be a disease of the affluent and mostly prevalent in urban areas but due to urbanization and a more sedentary lifestyle for many people, it has affected developing nations, including Pakistan[2].

Pakistan is a South Asian country with an area of 796,095 km^2 and a population of 207.7 million people[3]. In terms of population, Pakistan is the 6^{th} most populous country and is the 36^{th} largest country by geographical area in the world. Before 2018, the only previous national diabetes survey in Pakistan in 1999 (published in 2007), reported the prevalence of type-2 diabetes as 11% using the Oral Glucose Tolerance Test (OGTT)[4 5]. Part of the same survey separately reported the prevalence of type-2 diabetes in different provinces of Pakistan [4 6-8]. The International Diabetes Federation (IDF) reported in its Atlas 20 the prevalence for Pakistan to be 6.8%, aged 20-79[9], but health care professionals with local insight always believed this to be an underestimate. Subsequently there were conflicting findings with prevalence ranges from7.2%-19.21% in different regions of the country[5].

The American Diabetes Association (ADA) criteria for the diagnosis of diabetes require either fasting plasma glucose (FPG) or 75gm OGTT which is time-consuming, requires fasting and may not always be reproducible[10 11]. In 2009, the International Expert committee on diabetes proposed new diagnostic criteria based on Glycated Haemoglobin (HbA1c), which captures chronic glucose exposure[12]. The proposed diagnostic threshold of 6.5% (48mmol/mol) was based on retinopathy risk at different levels of HbA1c as was the case with FPG and OGTT. This report was followed by a recommendation from the ADA. An HbA1c level of 6.5% (48mmol/mol) was agreed to be a diagnostic cut-off for the diagnoses of diabetes[13].

A previous national level type-2 diabetes survey was conducted in 1999 with a sample size of 5433 using OGTT. In the study described in this paper, we investigated the prevalence of type-2 diabetes(using the HbA1c test) and its distribution across gender, age, rural and urban, education, Body Mass Index (BMI) WHO and Asian cut-offs, family history, smoking, and blood pressure among a large sample across Pakistan, aged 20 years and above. A sub subsample was tested to explore the diagnostic summary statistics for diagnosis of type-2 diabetes on HbA1c, compared to 2 hours OGTT.

Methods

The Department of Diabetes, Endocrine and Metabolic Diseases, Hayatabad and Department of Health, Government of Khyber Pakhtunkhwa, Pakistan with technical support from Institute of Public Health, Khyber Medical University Peshawar Pakistan, University of Manchester UK and Pakistan Endocrine Society conducted a nationwide cross-sectional study for the prevalence of type-2 diabetes starting in April 2017 and completed in November 2017. Three teams under the supervision of epidemiologists were created and the field workers trained to take basic demographic data on the sample to be screened. These national samples were selected based on a stratified two-stage cluster design, including all metropolitan cities of Pakistan and randomly selected districts (both rural and urban settings) within each province. The sample included districts from central and south of Punjab province (Lahore, Multan, Bahawalpur, Rahim Yar Khan),interior Sindh (Larkana, Dadoo, Sukkur), central Sindh (Karachi), northern Khyber Pakhtunkhwa (KP) (Haripur), central (Peshawar) and southern KP province (Karak), Baluchistan province (Quetta), capital territory (Rawalpindi-Islamabad), Azad Jammu Kashmir (Muzaffarabad), Frontier Region Peshawar and the Khyber Agency in Federal Administered Tribal Area (FATA).

Sample size and sampling methodology:

The sample size was estimated for the provinces of the country based on recent census results. The sample size was estimated based on an expected prevalence of 12% with 20% relative precision and a design effect of 2[6]. For a 95% confidence interval and an additional adjustment of 32% for non-responders, keeping in view an exclusion rate due to an expected high prevalence of anaemia, the sample size was 4407 approximated to 4500 in order to have 50 subjects from each cluster. All provinces were included as was the Federal territory. AJK and FATA were considered as one province for the survey purpose because of their small size of the population. The number of eligible subjects was 4500*5 = 22500.

Three districts were randomly selected from each province and the sample size was equally divided on these districts. Fifteen hundred subjects (30 clusters, 50 subjects per cluster) were examined in each district. The sample was proportionately divided amongst urban and rural areas. Probability proportionate to size (PPS) method was used to select clusters from villages in the rural settings of the district. In urban settings, clusters were selected from charges and circles (defined in the national census) using the PPS method.

Maps were obtained from the census office of selected villages/charges/circles (V/C/C). Maps of each V/C/C was divided into equal segments such that each segment had approximately 50 persons 20 years and above. One segment was randomly selected and every house within the segment was included. All persons 20 and above living in that house were examined until the 50 number was reached. Any person who was absent on the day of survey until evening, was terminallyill, who fitted into exclusion criteria or who refused were marked as non-responders.

Ethical approval was granted from Khyber Medical University ethical board (DIR/KMU-EB/SP/000395).Conditions that may affect HbA1c including anaemic subjects with haemoglobin less than 12gm/dl in women and less than 14 gm/dl in men, self-reported renal and hepatic dysfunction, recent blood transfusion, and use of erythropoietin, age below 20 years or refusal to participate resulted in exclusion. Face to face interview was conducted at the participant's home to collect information on demographics (including age, gender, residential area, formal education, family history of diabetes, and smoking status) using a paper questionnaire in local languages. Eligible participants were called to a central point established in the hujra (local public gathering place) where their haemoglobin was tested using the Mission Plus Haemoglobin Meter (reflectance photometer technique) Acon Laboratories, Inc., San Diego (coefficient of variance CV: 3%). Blood pressure was measured using an automated digital blood pressure monitor Konfort Model AS-351in the lying position with the average of three readings was recorded. Weight in kilograms and height in meters was recorded and used to calculate Body Mass Index.

Diabetes status was assessed for HbA1c on blood samples using the National Glycohemoglobin Standardization Program (NGSP) certified FIA 8000 immunoassay analyser (lateral flow chromatography colloidal gold) traceable to diabetes control and complication trial (DCCT) reference method(CV: 3-5%). To compare the results from HbA1c, 2 hours OGTT was conducted on a random sample of participants from all clusters (n=1,027) in the specified standard laboratory using Cobas C311 Roche Diagnostics, Mannheim, Germany. Participants were given vouchers for free OGTT test within 7 days in a nearby laboratory using Cobas C311 Roche Diagnostics.

Definitions

Age was categorised into six groups: 20-30, 31-40, 41-50, 51-60 and 61 and above years. The residential area was classified as urban and rural based on local government criteria. Formal education status was self-reported was categorized as no formal education, primary, secondary and graduation/post graduation. BMI was categorised on WHO criteria into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), class I obese (30-34.9 kg/m^2), class II obese (35-39.9 kg/m^2), and class III obese (>40 kg/m^2)(World Health Organization., 1995). Waist Circumference (WC) was categorized into normal-weight (0-93.99), overweight (94-102) and obese (102 & above).Waist to hip ratio (WHR) was categorized into normal-weight (0-0.89), overweight (0.90-0.99) and obese (1 & above)[14].The family history of diabetes was categorised to negative or positive on the basis of the participant's self-reporting, based on physician's diagnoses. Smoking status was categorized as never, ex or current smoker. Systolic and diastolic blood pressure was measured using a standard procedure and was Hypertension was defined on a blood pressure measurement of ≥140/90 mmHg or anti-hypertensive medication. Patients were considered as known type-2 diabetes on the basis of self-reporting and or being on dietary or exercise advice, oral anti-diabetes medications or insulin.

This self-reported group of patients could either be on single medications or on different drug combinations or diet and exercise therapy.

Type 2 diabetes was diagnosed based on HbA1c results keeping the WHO levels of non diabetes (<5.69 % DCCT aligned/38 mmol/mol IFFC units), pre-diabetes (5.7-6.49% DCCT aligned/ 39-47mmol/mol IFFC units), diabetes (≥6.5 % DCCT aligned /48 mmol/mol IFFC units). For univariate and multivariate logistic regression models, diabetes was dichotomized to No (0; HbA1c level <6.5) and Yes (1; HbA1c level ≥6.5).

Statistical Analyses

Differences in the characteristics of participants by diabetes category were analysed using the χ^2 test for categorical data and ANOVA for continuous data. We examined the association between diabetes and risk factors i.e., age, gender, residence area, education, BMI, family history of diabetes, smoking, systolic and diastolic blood pressure, using univariate and multivariate logistic regression models. Multivariate regression analysis included all these variables.

Taking OGTT as the standard on a sub subsample, the diagnostic summary statistics (sensitivity, specificity, ROC area, positive and negative predictive value) for the diagnosis of diabetes using HbA1c were determined. All statistical analyses were performed using Stata version 14 (Stata Corp, College Station, Texas). Statistical significance was defined as $p<0.05$ and analysis were adjusted for the cluster design.

Patient and Public Involvement statement:

Patients were not involved in the study conception or design. There was consultation with interested representative public bodies but not with individual members of the public.

Results

Of the 22,500 participants, 3,644 (16%) were anaemic and therefore excluded from the study. Out of the remaining, 18,856participantsaged 20 and above, were actually examined from 378 clusters of which 216 were rural generating a response rate of 84%. The mean age was 45.23 years (standard deviation [SD] 13.97 years). 10,116 (53.55%) were men, 4,148 (21.96%) were hypertensive and those with higher blood pressure were advised to check their blood pressure by visiting their doctors. 13,834 (73.24%) had no formal education, and 1,209 (6.40%) had graduated. 6,010 (31.81%) had a family history of type-2 diabetes. Overall on WHO cut-off 345 (1.83%) were underweight, 6,839 (36.20%) normal-weight, 8,038 (42.55%) overweight, 2,864 (15.16%) class I obese, 633 (3.35%) class II obese, and 172 (0.91%) class III obese. On waist circumference (WC) cut-off (n=12865) 8,574 (66.64%) were normal-weight, 2,318 (18.02%) were overweight and 1,974 (15.34%) were obese. On central obesity cut-off (WHR, n=12865) 4,271 (33.20%) were normal weight, 7,467 (58.04%) were overweight and 1,127 (8.76%) were

obese. Mean systolic blood pressure was 126.30mmHg (SD 14.2) and diastolic blood pressure was 83.24mmHg (SD 10.2).

Overall, 3,201 subjects (16.98%, 95%CI 16.44, 17.51) had type-2 diabetes based on HbA1c screening. Pre-diabetes was present in 2,057 subjects 10.91% (95% CI 10.46, 11.36).The mean HbA1c level of the entire cohort (n=18856) was 5.62% (SD 1.96), among known type-2 diabetes (n=2179) had 8.68% (SD 2.70) and newly diagnosed type-2 diabetes (n=1577) had 8.56% (SD2.08). The prevalence of diabetes differed significantly by age, education, BMI, WC, WHR, family history, and blood pressure (Table 1) (Figure 1). The prevalence of diabetes was highest in age 51-60 years (26.03%, p<0.001), no formal education (17.73%, p<0.001), class 3 obese (32.19%, p<0.001), and with a positive family history of diabetes (31.34%, p<0.001) (Figure 1). There were also statistically significant differences in diabetes prevalence by gender (female 17.85%, p=0.01), rural/urban (rural 19.09%, p<0.001) and thatsmoking status (p=0.008).

On univariate logistic regression analysis, there was a significant association between age, gender, education, BMI category, family history, blood pressure and type 2 diabetes (p<0.005) (Table 2).

On multivariate logistic regression, there was significantly higher risk of diabetes with age (adjusted Odds Ratio [OR] 2.03, 3.39, 4.87 and 4.93, p< 0.001, aged 31-40 years, 41-50 years, 51-60 years and 61 years and above respectively, compared to age 20-30 years), BMI (adjusted OR 1.54, 2.13, 2.44,p-value <0.001 for class 1, class II and class III obese respectively, compared to normal weight) with evidence of a dose-response relationship. Similarly, there was a significantly higher risk of diabetes with lower educational attainment (adjusted OR 1.83, 1.39, 1.57, no formal education, primary and secondary education respectively, compared to graduates). There was a significantly higher risk of diabetes in people with a positive family history (adjusted OR 3.94, 95% CI 3.6, 4.3 p<0.001), than with no family history of diabetes. There was no significant association with smoking and rural/urban area.

Among 1,029 participants who were tested for 2hrs OGTT, in addition to HbA1c, the mean 2 hours OGTT was 200.26 (SD 91.7), and the median was 178 (inter-quartile range 100). Taking the OGTT as the gold standard, HbA1c recommended cut-off for diabetes showed a sensitivity of 84.7% (95% CI 80.8, 88) and Specificity of 87.2% (95% CI 84.3, 89.8%), ROC area 0.86 (95% CI 0.84, 0.88), positive predictive value 81.9% (95% CI 77.9, 85.4), and negative predictive value 89.3 (86.5, 91.6) (Table 3).

Discussion:

This is the first community based national study done in the region based on HbA1c and with the eligible 18,856 subjects from Pakistan this is the largest study to date from that country. The prevalence of type-2 diabetes all across Pakistan was 16.98% (95%CI 16.44, 17.51) and pre-

diabetes was 10.91% (95% CI 10.46, 11.36). This is higher than found in the only previous national survey conducted in1999 (n=5433) using OGTT. There was a significantly higher risk of type-2 diabetes with increasing systolic blood pressure, age, BMI, WC, WHR with evidence of a dose-response relationship. Similarly, there was a significant inverse relationship of type-2 diabetes with the level of formal education. The risk of diabetes increased 2.68 times with a prior family history. The HbA1c level had good sensitivity and specificity level for the diagnoses of type-2 diabetes compared to a 2-hour OGTT level and is therefore valid in community settings for screening purposes.

We have noted the findings of Basit et al [15]. The methodology for that study was based on 75g Oral Glucose Tolerance diagnostic test for glucose handling as opposed to HbA1c used in our study. While the prevalence of diabetes + pre-diabetes is different in the two studies, the point that both studies make is that both diabetes and pre-diabetes are much more prevalent than previously thought.

The previous national prevalence study conducted in 1999 used OGTT where almost 80% of the subjects were women as the test was conducted in the morning time [4] . In contrast, our study screening was done all through the day so that working men had an equal opportunity to be part of the study. They constituted 50% of the study population. OGTT is the gold standard for the type 2 diabetes screening but because of the length of time which is required for the test and the fact that person has to be fasting, it is very difficult to perform in many community settings in Pakistan. Also, the high temperatures in South Asia make it difficult to keep the sample stable for transportation to the laboratory. To minimize the effect of temperature and transportation errors on HbA1c, all tests were conducted in the field.

Recently a study carried out in 15 states of India showed that the prevalence of type-2 diabetes ranged between 4%-13.6% and showed variation due to age, male sex, obesity and family history using capillary FBG for diagnosis [16]. A capillary blood sample for epidemiological studies is not an ideal test but the authors acknowledged the logistic hindrance in carrying out venous sample test in the field. A high prevalence in another Indian study was reported ranging from 12.1-14% for diabetes using OGTT on a sample size of 11,216 subjects [17].

A study conducted in Bangladesh was based on capillary fasting level found a prevalence of type-2 diabetes of 4.3% in a rural setting[18]. Risk factors were positive family history for diabetes, age, high BMI and low socio-economic status, similar to our study. Although these are geographically distant areas, the risk factors showed commonality in both studies, which suggests these risk factors as an important tool for mass screening[19].

The age sex-standardized prevalence of type-2 diabetesfor Sri Lankans was 10.3% based on OGTT[20]. The risk factors were almost the same as seen in our study. The investigators found dysglycaemia in almost 21.8%participants and predicted that this would lead to a higher

prevalence of T2DM in the years to come. Another study from Sri Lanka indicated a prevalence of 14.2% basis of FBG[21]. FBG as well as, OGTT blood glucose levels, may not be reproducible in an epidemiological survey if the individual are changing lifestyle in terms of diet and exercise. The use of HbA1c in our study makes our study more scientific, addressing issues pertaining to sampling errors in the local environment.

The Asian population is known to have a significantly higher risk of developing diabetes and its related complications as predicted by IDF projections[1]. It makes it very important from a public health point of view to identify high-risk individuals at an early stage. The HbA1ctest has been used successfully in community settings[22]. A national health survey New Zealand in 2008-9 used HbA1c to identify high-risk individuals with diabetes and pre-diabetes[23]. A study in Japan revealed that a combination of tests including FBG and HbA1c yields more diabetes cases compared to any of these tests alone[24].

WHO experts have accepted HbA1c as a diagnostic tool provided quality assurance tests are in place and there are no conditions present, which preclude its accurate measurement. The New Zealand Society for the study of Diabetes and the Australian Diabetes Society have already endorsed HbA1c as a test for the diagnosis of diabetes[25 26].Recently a prevalence study done in Korea concluded that FBG testing results in underestimation of diabetes and pre-diabetes[27]. This study suggested the use of standardized HbA1c as a diagnostic tool for diagnosis of type-2 diabetes.

We found that HbA1c level had a good sensitivity and specificity level for diagnoses of diabetes compared to 2-hrs OGTT level. HbA1c has the advantage of being a simple test and less time consuming, making it an ideal test for community surveys in our populations. In Pakistan, as there is no effective primary care (general/ family practice) structure most of the population does not undergo primary screening for diabetes. Sometimes as the diagnosis is note made, people may present to tertiary care with florid complications.

Thus there is a strong case for applying HbA1c for screening purposes in the community setting. There will always be an argument about the cost of the test and whether this to be used for screening purposes. However particularly those at social disadvantage need to be undergo screening to improve the diagnosis timely treatment of diabetes[28]. Needless to say, early diagnosis will also reduce diabetes-related complications.

Strengths and limitations

Our study has the strength that we carried out HbA1c on all participants and OGTT on a subsample. Furthermore this is the largest ever national prevalence study of type-2 diabetes mellitus from Pakistan and the first community based national study to use HbA1c as the diagnostic tool.

Limitations are the relatively low number (n=1,027) of 75gOGTT. Nevertheless the specificity and sensitivity of HbA1c vs OGTT was good. We had to exclude 16% of recruited participants recruited because of anaemia.

The central Government of Pakistan has developed and agreed on Non- Communicable Diseases (NCD) National Action Plan including diabetes, however, it was never implemented. After the 18th Amendment in the constitution of Pakistan in 2010, provinces are responsible for making and implementing their own health policies and the role of central Government is limited to coordination among the different provinces[29]. There is a dire need that based on the agreed NCD National Action Plan each province should build their capacity for implementing it at both primary and secondary level. Pakistan is a signatory to the Sustainable Development Goals 2030document which outlines among its goals, increasing access to universal health coverage, increasing coverage of health insurance program and adopting a family medicine approach. When implemented, these will not only be major steps towards prevention and control of diabetes but all non-communicable diseases.

Conclusions:

This national diabetes prevalence study is the first one in the region using HbA1c identified a huge population of type-2 diabetes and pre-diabetes group. The prevalence of type-2 diabetes and pre-diabetes is much higher than previously thought in Pakistan. Comprehensive strategies need to be developed to incorporate screening, prevention and treatment of type 2 diabetes at community level. Those who are obese, with no formal education, older, family history of diabetes and hypertensive merit close attention and timely intervention.

HbA1c is an applicable test in community settings in developing countries and it has a good correlation with 2 hours OGTT. Our findings have the potential to influence policy in developing countries and induce a shift towards the prevention and control of non-communicable diseases.

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Contributors AHA, ZUH conceived the study. AHA, SM, FQ, AS, AJ, OI, AR, IA, HA collected the data. ZJ, NS study design and data monitoring. ZUH, AHA, SF, AH, conducted the analysis and wrote the manuscript. All authors reviewed the manuscript. AHA and ZUH contributed equally.

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Competing interest All authors have no competing interest to declare.

Patient Consent Written informed consent granted. The data is fully anonymised and neither the patient nor anyone else could identify the patient.

Ethical approval The ethical approval was granted from the Khyber Medical University ethical board (DIR/KMU-EB/SP/000395).

Data sharing statement No additional data are available

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Table 1 Characteristics of the participants by diabetes categories (n=18,856)

	Non-Diabetic N (%)	Pre-Diabetic N (%)	Diabetic N (%)	*P-value*
	13,598 (72.11)	2,057 (10.91)	3,201 (16.98)	
Age (years)				
20-30	2,772 (20.37)	218 (10.60)	176 (5.52)	<0.001
31-40	3,503 (25.74)	425 (20.66)	511 (16.01)	
41-50	3,802 (27.94)	654 (31.79)	1,033 (32.37)	
51-60	1,955 (14.37)	412 (20.03)	833 (26.10)	
61 & above	1,576 (11.58)	348 (16.92)	638 (19.99)	
Gender				
Male	7363 (54.15)	1099 (53.43)	1638 (51.17)	0.010
Female	6235 (45.85)	958 (46.57)	1563 (48.83)	
Education				
No formal education	9,853 (72.41)	1516 (73.70)	2,439 (76.43)	<0.001
Primary	1936 (14.23)	354 (17.21)	452 (14.16)	
Secondary	818 (6.01)	99 (4.81)	184 (5.77)	
Graduation	1001 (7.36)	88 (4.28)	116 (3.64)	
Area				
Urban	9,117 (67.00)	1,213 (58.97)	1,932 (60.55)	<0.001
Rural	4,491 (33.00)	844 (41.03)	1,259 (39.45)	
Family History				
Negative	10325 (75.93)	1210 (58.82)	1320 (41.24)	<0.001
Positive	3273 (24.07)	847 (41.18)	1881 (58.76)	
Smoking				
Never	12682 (93.26)	1893 (92.03)	2937 (91.75)	0.008
Ex-Smoker	275 (2.02)	49 (2.38)	91 (2.84)	
Current smoker	641 (4.71)	115 (5.59)	173 (5.40)	
Systolic BP (mean ±SD)	124.98	128.17	130.77	0.020
Diastolic BP (mean ±SD)	82.75	84.13	84.84	0.006
BMI				
Underweight (<18.5)	279 (2.05)	31 (1.51)	35 (1.09)	
Normal (18.5-<25)	5137 (37.78)	668 (32.47)	1019 (31.83)	<0.001
Overweight (25-<30)	5884 (43.27)	850 (41.32)	1288 (40.24)	
Obese1 (30-<35)	1844 (13.56)	396 (19.25)	621 (19.40)	
Obese 2 (35-<40)	372 (2.74)	83 (4.04)	178 (5.56)	
Obese 3 (≥40)	82 (0.60)	29 (1.41)	60 (1.87)	
Waist circumference *				
Normal Weight	6,787 (70.88)	645 (51.68)	1,142 (55.93)	
Over weight	1,676 (17.50)	285 (22.84)	357 (17.48)	<0.001
Obese	1,113 (11.62)	318 (25.48)	543 (26.59)	
Waist to Hip ratio *				
Normal Weight	3,219 (33.62)	394 (31.57)	658 (32.22)	
Over weight	5,528 (57.73)	768 (61.54)	1,171 (57.35)	
Obese	828 (8.65)	86 (6.89)	213 (10.43)	0.002

* n for Waist circumference& waist to hip ratio is 12865

Table 2 Logistic regression analysis of the participant characteristics associated with having diabetes (HbA1c≥6.5 % DCCT aligned /48 mmol/mol IFFC units) (n=18,856)

	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age				
20-30	1		1	
31-40	2.21 (1.8, 2.6)	<0.001	2.03 (1.7, 2.4)	<0.001
41-50	3.93 (3.3,4.6)	<0.001	3.39 (2.9, 4.0)	<0.001
51-60	5.97 (5.0, 7.1)	<0.001	4.87 (4.1, 5.8)	<0.001
61 & above	5.63 (4.7, 6.7)	<0.001	4.93 (4.1, 6.0)	<0.001
Gender				
Male	1		1	
Female	1.12 (1.0,1.2)	0.003	1.04 (0.9, 1.1)	0.334
Education				
No formal education	2.02 (1.6,2.4)	<0.001	1.83 (1.5, 2.3)	<0.001
Primary	1.85 (1.49, 2.3)	<0.001	1.39 (1.1 , 1.8)	0.006
Secondary	1.89 (1.47,2.4)	<0.001	1.57 (1.2 , 2.0)	0.001
Graduation	1		1	
Area				
Urban	1		1	
Rural	1.26 (1.16, 1.34)	<0.001	1.08 (0.9, 1.2)	0.084
Family History of diabetes				
Negative	1		1	
Positive	3.98 (3.6,4.3)	<0.001	3.94 (3.6, 4.3)	<0.001
Smoking				
Never	1		1	
Ex- Smoker	1.39 (1.1, 1.8)	0.006	1.13 (0.9 , 1.5)	0.323
Current Smoker	1.13 (0.9,1.3)	0.14	1.06(0.9, 1.3)	0.571
Systolic BP	1.02 (1.021, 1.026)	<0.001	1.01 (1.01, 1.02)	<0.001
Diastolic BP	1.01 (1.01, 1.02)	<0.001	0.99 (0.9 , 1.0)	0.310
BMI				
Underweight (<18.5)	0.64 (0.4, 0.9)	0.001	0.71 (0.5, 1.0)	0.077
Normal (18.5-<25)	1		1	
Overweight (25-<30)	1.08 (0.9,1.2)	0.06	1.06 (0.9, 1.1)	0.182
Obese1 (30-<35)	1.57 (1.4,1.7)	<0.001	1.54 (1.3, 1.7)	<0.001
Obese 2 (35-<40)	2.22 (1.8,2.6)	<0.001	2.13 (1.7, 2.6)	<0.001
Obese 3 (≥40)	3.07 (2.2,4.2)	<0.001	2.44 (1.7, 3.5)	<0.001
Waist circumference *				
Normal Weight	1		1	
Over weight	1.18 (1.04, 1.34)	0.01	0.98 (0.8, 1.1)	0.774
Obese	2.46 (2.19, 2.77)	<0.001	1.86 (1.6, 2.2)	<0.001
Waist to Hip Ratio *				
Normal Weight	1		1	

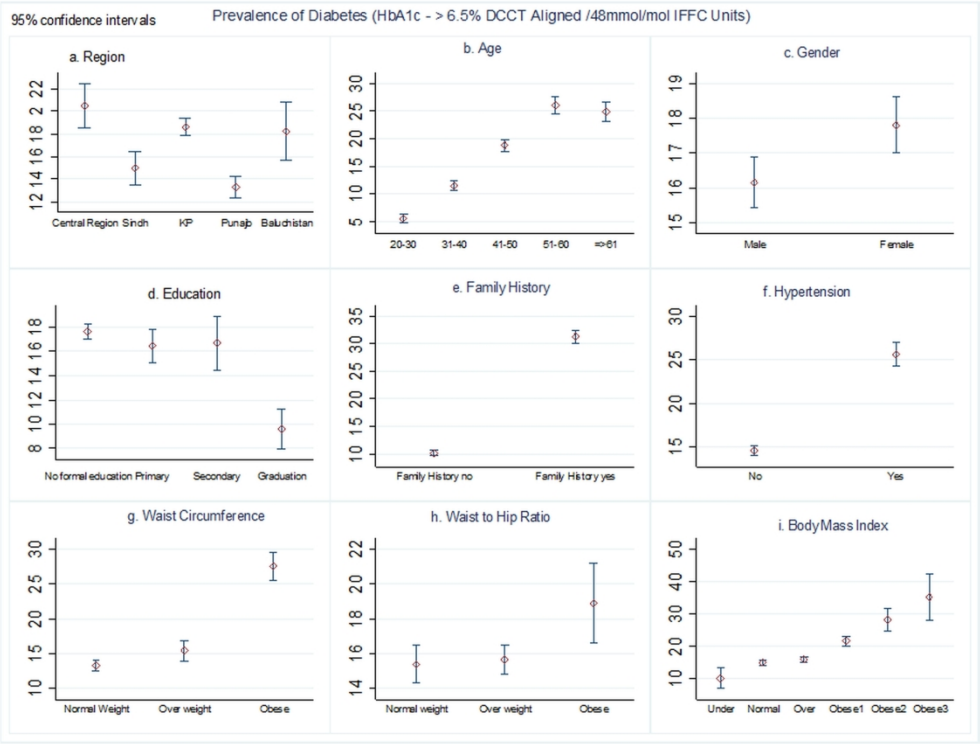
Over weight	1.02 (0.92, 1.13)	0.40	0.8 (0.7, 0.9)	<0.001
Obese	1.27 (1.07, 1.51)	0.005	1.13 (0.9, 1.4)	0.205

* n for WC &WHR is 12865. The following variables were included in the multivariate regression: Age, gender, education, residence area, family history of diabetes, smoking, systolic and diastolic blood pressure and BMI.

Table 3: Summary statistics for diabetes diagnosed by HbA1c compared to diabetes diagnosed by 2hrs OGTT (n=1,027)

			[95% Confidence Interval]	
Prevalence	Pr(A)	40.6%	37.6%	43.7%
Sensitivity	Pr(+|A)	84.7%	80.8%	88.0%
Specificity	Pr(-|N)	87.2%	84.3%	89.8%
ROC area	(Sens. + Spec.)/2	0.86	0.84	0.88
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	6.62	5.36	8.18
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	0.18	0.14	0.22
Odds ratio	LR(+)/LR(-)	37.62	26.34	53.73
Positive predictive value	Pr(A|+)	81.9%	77.9%	85.4%
Negative predictive value	Pr(N|-)	89.3%	86.5%	91.6%

Figure 1: Prevalence of diabetes (diagnosed by HbA1c≥6.5 % DCCT aligned /48 mmol/mol IFFC units) by regions of Pakistan, age, gender, education, family history of diabetes, hypertension, waist circumference, waist to hip ratio and body mass index (n=18,856).



Prevalence of diabetes (diagnosed by HbA1c≥6.5 % DCCT aligned /48 mmol/mol IFFC units)

246x187mm (300 x 300 DPI)

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	3
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	6-7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	7-8
Bias	#9	Describe any efforts to address potential sources of bias	8
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8
	#12b	Describe any methods used to examine subgroups and interactions	8
	#12c	Explain how missing data were addressed	8
	#12d	If applicable, describe analytical methods taking account of sampling strategy	8
	#12e	Describe any sensitivity analyses	8
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8-9
	#13b	Give reasons for non-participation at each stage	8
	#13c	Consider use of a flow diagram	6
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8-9

	#14b	Indicate number of participants with missing data for each variable of interest	8-9
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
	#16b	Report category boundaries when continuous variables were categorized	9
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	11
Generalisability	#21	Discuss the generalisability (external validity) of the study results	11
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

BMJ Open

DIABETES PREVALENCE SURVEY OF PAKISTAN (DPS- PAK): Prevalence of type 2 Diabetes Mellitus and pre-diabetes using HbA1c: A population-based survey from Pakistan

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Secondary Subject Heading:	Epidemiology
Keywords:	Type 2 Diabetes Mellitus, diabetes, HbA1c, Pakistan, pre-diabetes, Prevalence

Title Page:

DIABETES PREVALENCE SURVEY OF PAKISTAN (DPS- PAK): Prevalence of type 2 Diabetes Mellitus and pre-diabetes using HbA1c: A population-based survey from Pakistan

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Word count (main text)	3483
Figures	01
Tables	03

Abstract

Objectives

We conducted a Pakistan-wide community-based survey on the prevalence of type-2 diabetes using HbA1c as the screening test. The aim was to estimate diabetes prevalence across different demographic groups as well as all regions of Pakistan.

Design, settings and participants

Multi-staged stratified cluster sampling was used for the representative selection of people aged≥20 years, residing in 378 sampled clusters of 16 randomly selected districts, in this cross sectional study. Eligible participants had blood drawn for HbA1c analyses at field clinics near to their homes. The oral glucose tolerance test (OGTT) was conducted on a subsample of the participants. Overall and stratified prevalence of type-2 diabetes and its association with risk factors were estimated using logistic regression models.

Main outcome measures

Prevalence of pre-diabetes and type 2 diabetes

Results

Of 18,856 eligible participants the prevalence of pre-diabetes was 10.91% (95% CI 10.46, 11.36, n=2,057) and type-2 diabetes was 16.98% (95%CI 16.44, 17.51, n=3,201). Overall, the mean HbA1c level was 5.62% (SD 1.96), and among newly diagnosed was 8.56% (SD 2.08). The prevalence was highest in age 51-60 years (26.03%, p<0.001), no formal education (17.66%, p<0.001), class-3 obese (35.09%, p<0.001), family history (31.29%, p<0.001) and female (17.80%, p=0.009). On multivariate analysis, there was a significant association between type-2 diabetes and; older age, increase in BMI and central obesity, positive family history, and having hypertension and an inverse relation with education as a categorical variable. On a subsample (n=1,027), summary statistics for diagnosis of diabetes on HbA1c showed a sensitivity of 84.7%, specificity of 87.2%, and ROC area 0.86, compared to OGTT.

Conclusions

The prevalence of type-2 diabetes and pre-diabetes is much higher than previously thought in Pakistan. Comprehensive strategies need to be developed to incorporate screening, prevention and treatment of type-2 diabetes at a community level.

Key words (mesh terms):

Type 2 Diabetes Mellitus, diabetes, Prevalence, HbA1c, OGTT, obesity, pre-diabetes, Pakistan

Strength and limitations of this study

Strengths:

Our study has the strength that we carried out HbA1c on all participants and OGTT on a subsample.

Furthermore this is the largest ever national prevalence study of type-2 diabetes mellitus from Pakistan and the first community based national study to use HbA1c as the diagnostic tool.

Limitations:

The relatively low number (n=1,027) of 75g OGTT. Nevertheless the specificity and sensitivity of HbA1cversus OGTT was good.

We had to exclude 16% of recruited participants because of anaemia.

Background:

Type 2 Diabetes Mellitus is one of the most common public health issues worldwide and its incidence is on the rise particularly in middle and low-income countries[1]. When associated with complications, type-2 diabetes can have a profound impact on the person with consequences also for the society as a whole. Diabetes was previously thought to be a disease of the affluent and mostly prevalent in urban areas but due to urbanization, change in nutrition and a more sedentary lifestyle for many people, it has affected developing nations, including Pakistan[2].

Pakistan is a South Asian country with an area of 796,095 km^2 and a population of 207.7 million people[3]. In terms of population, Pakistan is the 6^{th} most populous country and is the 36^{th} largest country by geographical area in the world. Before 2018, the only previous national diabetes survey in Pakistan in 1999 (published in 2007), reported the prevalence of type-2 diabetes as 11%using the Oral Glucose Tolerance Test (OGTT)[4 5]. Part of the same survey separately reported the prevalence of type-2 diabetes in different provinces of Pakistan[4 6-8]. The International Diabetes Federation (IDF) reported in its Atlas 20 the prevalence for Pakistan to be 6.8%, aged 20-79[9], but health care professionals with local insight always believed this to be an underestimate. Subsequently there were conflicting findings with prevalence ranges from7.2%-19.21% in different regions of the country[5].

The American Diabetes Association (ADA) criteria for the diagnosis of diabetes require either fasting plasma glucose (FPG) or 75gm OGTT which is time-consuming, requires fasting and may not always be reproducible[10 11]. In 2009, the International Expert committee on diabetes proposed new diagnostic criteria based on Glycated Haemoglobin (HbA1c), which captures chronic glucose exposure[12]. The proposed diagnostic threshold of 6.5% (48mmol/mol) was based on retinopathy risk at different levels of HbA1c as was the case with FPG and OGTT. This report was followed by a recommendation from the ADA thatanHbA1clevel of 6.5% (48mmol/mol) be used as the diagnostic cut-off for the diagnoses of diabetes (this has not been validated in Pakistan)[13].

A previous national level type-2 diabetes survey was conducted in 1999 with a sample size of 5433 using OGTT. In the study described in this paper, we investigated the prevalence of type-2 diabetes (using the HbA1c test) and its distribution across gender, age, rural and urban, education, Body Mass Index (BMI) WHO and Asian cut-offs, family history, smoking and blood pressure among a large sample across Pakistan, aged 20 years and above. A subsample was tested to explore the diagnostic accuracy of HbA1c for diagnosis of type-2 diabetes, compared to the 2-hour OGTT.

Methods:

The Department of Diabetes, Endocrine and Metabolic Diseases, Hayatabad and Department of Health, Government of Khyber Pakhtunkhwa, Pakistan with technical support from Institute of Public Health, Khyber Medical University Peshawar Pakistan, University of Manchester UK and Pakistan Endocrine Society conducted a nationwide cross-sectional study for the prevalence of type-2 diabetes starting from April 2017 to November 2017. Three teams of trained field workers under the supervision of epidemiologists collected basic demographic data and blood samples from the selected sample. The study sample was selected based on a stratified two-stage cluster design, including all metropolitan cities of Pakistan and randomly selected districts (both rural and urban settings) within each province. The sample included districts from central and south of Punjab province (Lahore, Multan, Bahawalpur, Rahim Yar Khan),interior Sindh (Larkana, Dadoo, Sukkur), central Sindh (Karachi), northern Khyber Pakhtunkhwa (KP) (Haripur), central (Peshawar) and southern KP province (Karak), Baluchistan province (Quetta), capital territory (Rawalpindi-Islamabad), Azad Jammu Kashmir (Muzaffarabad), Frontier Region Peshawar and the Khyber Agency in Federal Administered Tribal Area (FATA).

Sample size and sampling methodology:

The sample size was estimated for the provinces of the country based on recent census results. The sample size was estimated based on an expected prevalence of 12% with 20% relative precision and a design effect of 2[6]. For a 95% confidence interval and an additional adjustment of 32% for non-responders, keeping in view an exclusion rate due to an expected high prevalence of anaemia, the sample size was 4407 approximated to 4500 in order to have 50 subjects from each cluster. All provinces were included as was the Federal territory. AJK and FATA were considered as one province for the survey purpose because of their small size of the population. The number of eligible subjects was 4500*5 = 22500.

Three districts were randomly selected from each province and the sample size was equally divided on these districts. Fifteen hundred subjects (30 clusters, 50 subjects per cluster) were examined in each district. The sample was proportionately divided amongst urban and rural areas. Probability proportionate to size (PPS) method was used to select clusters from villages in the rural settings of the district. In urban settings, clusters were selected from charges and circles (defined in the national census) using the PPS method.

Maps were obtained from the census office of selected villages/charges/circles (V/C/C). Maps of each V/C/C was divided into equal segments such that each segment had approximately 50 persons 20 years and above. One segment was randomly selected and every house within the segment was included. All persons 20 and above living in that house were examined until the 50 number was reached. Any person who was absent on the day of survey until evening, was terminally ill, who fitted into exclusion criteria or who refused were marked as non-responders.

Ethical approval was granted from Khyber Medical University ethical board (DIR/KMU-EB/SP/000395). Conditions that may affect HbA1c including anaemic subjects with haemoglobin less than 12gm/dl in women and less than 14 gm/dl in men, self-reported renal and hepatic dysfunction, recent blood transfusion, and use of erythropoietin, age below 20 years or refusal to participate resulted in exclusion. Face to face interview was conducted at the participant's home to collect information on demographics (including age, gender, residential area, formal education, family history of diabetes, and smoking status) using a paper questionnaire in local languages. Eligible participants were called to a central point established in the ***hujra*** (local public gathering place) where their haemoglobin was tested using the Mission Plus Haemoglobin Meter (reflectance photometer technique) Acon Laboratories, Inc., San Diego (coefficient of variance CV:3%). Blood pressure was measured using an automated digital blood pressure monitor Konfort Model AS-351in the lying position with the average of three readings was recorded. Weight in kilograms and height in meters was recorded and used to calculate Body Mass Index.

Diabetes status was assessed for HbA1c on blood samples using the National Glycohemoglobin Standardization Program (NGSP) certified FIA 8000 immunoassay analyser (lateral flow chromatography colloidal gold) traceable to diabetes control and complication trial (DCCT) reference method (CV: 3-5%). To compare the results from HbA1c, 2-hour OGTT was conducted on a random sample of participants from all clusters (n=1,027) in the specified standard laboratory using Cobas C311 Roche Diagnostics, Mannheim, Germany. Participants were given vouchers for free OGTT test within 7 days in a nearby laboratory using Cobas C311 Roche Diagnostics.

Definitions:

Age was categorised into six groups: 20-30, 31-40, 41-50,51-60 and 61 and above years. The residential area was classified as urban and rural based on local government criteria. Formal education status was self-reported was categorized as no formal education, primary, secondary and graduation/post-graduation. BMI was categorised on WHO criteria into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), class I obese (30-34.9 kg/m^2), class II obese (35-39.9 kg/m^2), and class III obese (>40 kg/m^2)(World Health Organization., 1995). Waist Circumference (WC) was categorized into normal-weight (0-93.99), overweight (94-102) and obese (102 & above).Waist to hip ratio (WHR) was categorized into normal-weight (0-0.89), overweight (0.90-0.99) and obese (1 & above)[14].The family history of diabetes was categorised to negative or positive on the basis of the participant's self-reporting, based on physician's diagnoses. Smoking status was categorized as never, ex or current smoker. Systolic and diastolic blood pressure was measured using a standard procedure and Hypertension was defined on a blood pressure measurement of ≥140/90 mmHg or anti-hypertensive medication. Patients were considered as known type-2 diabetes based on self-reporting and or being on dietary or exercise advice, oral anti-diabetes medications or insulin. This self-reported

group of patients could either be on single medications or on different drug combinations or diet and exercise therapy.

Type 2 diabetes was diagnosed based on HbA1c results in keeping with the WHO levels for non diabetes (<5.69 % DCCT aligned/38 mmol/mol IFFC units), pre-diabetes (5.7-6.49% DCCT aligned/ 39-47mmol/mol IFFC units), diabetes (≥6.5 % DCCT aligned /48 mmol/mol IFFC units). For univariate and multivariate logistic regression models, diabetes was dichotomized to No (0; HbA1c level <6.5) and Yes (1; HbA1c level ≥6.5).

Statistical Analyses:

Differences in the characteristics of participants by diabetes category were analysed using the χ^2 test for categorical data and ANOVA for continuous data. We examined the association between diabetes and risk factors i.e., age, gender, residence area, education, BMI, family history of diabetes, smoking, systolic and diastolic blood pressure, using univariate and multivariate logistic regression models. Multivariate regression analysis included all these variables.

Taking OGTT as the standard on a sub subsample, the diagnostic accuracy summary statistics (sensitivity, specificity, ROC area, positive and negative predictive value) for the diagnosis of diabetes using HbA1c were determined. All statistical analyses were performed using Stata version 14 (StataCorp, College Station, Texas). Statistical significance was defined as $p<0.05$ and analysis were adjusted for the cluster design.

Patient and Public Involvement statement:

Patients were not involved in the study conception or design. There was consultation with interested representative public bodies but not with individual members of the public.

Results:

Of the 22,500 participants, 3,644 (16%) were anaemic and therefore excluded from the study. Out of the remaining, 18,856 participants aged 20 and above, were examined from 378 clusters of which 216 were rural generating a response rate of 84%. The mean age was 45.23 years (standard deviation [SD] 13.97 years). Most of the participants 10,116 (53.55%) were men, 4,148 (21.96%) were hypertensive and those with higher blood pressure were advised to check their blood pressure by visiting their doctors. Majority of the participants, 13,834 (73.24%), had no formal education and 1,209 (6.40%) had graduated. 6,010 (31.81%) had a family history of type-2 diabetes. Overall on WHO cut-off 345 (1.83%) were underweight, 6,839 (36.20%) normal-weight, 8,038 (42.55%) overweight, 2,864 (15.16%) class I obese, 633 (3.35%) class II obese, and 172 (0.91%) class III obese. On waist circumference (WC) cut-off (n=12865) 8,574 (66.64%) were normal-weight, 2,318 (18.02%) were overweight and 1,974 (15.34%) were obese. On central obesity cut-off (WHR, n=12865) 4,271 (33.20%) were normal weight, 7,467

(58.04%) were overweight and 1,127 (8.76%) were obese. Mean systolic blood pressure was 126.30mmHg (SD 14.2) and diastolic blood pressure was 83.24mmHg (SD 10.2).

Overall, 3,201 subjects (16.98%, 95%CI 16.44, 17.51) had type-2 diabetes based on HbA1c screening. Pre-diabetes was present in 2,057 subjects 10.91% (95% CI 10.46, 11.36).The mean HbA1c level of the entire cohort (n=18856) was 5.62% (SD 1.96), among known type-2 diabetes (n=2179) had 8.68% (SD 2.70) and newly diagnosed type-2 diabetes (n=1577) had 8.56% (SD2.08). The prevalence of diabetes differed significantly by age, education, BMI, WC, WHR, family history, and blood pressure (Table 1) (Figure 1). The prevalence of diabetes was highest in age 51-60 years (26.03%, p<0.001), no formal education (17.73%, p<0.001), class 3 obese (32.19%, p<0.001), and with a positive family history of diabetes (31.34%, p<0.001) (Figure 1). There were also statistically significant differences in diabetes prevalence by gender (female 17.85%, p=0.01), rural/urban (rural 19.09%, p<0.001) and that smoking status (p=0.008).

On univariate logistic regression analysis, there was a significant association between age, gender, education, BMI category, family history, blood pressure and type 2 diabetes (p<0.005) (Table 2).

On multivariate logistic regression, there was significantly higher risk of diabetes with age (adjusted Odds Ratio [OR] 2.03, 3.39, 4.87 and 4.93, p< 0.001, aged 31-40 years, 41-50 years, 51-60 years and 61 years and above respectively, compared to age 20-30 years), BMI (adjusted OR 1.54, 2.13, 2.44, p-value <0.001 for class 1, class II and class III obese respectively, compared to normal weight) with evidence of a dose-response relationship. Similarly, there was a significantly higher risk of diabetes with lower educational attainment (adjusted OR 1.83, 1.39, 1.57, no formal education, primary and secondary education respectively, compared to graduates). There was a significantly higher risk of diabetes in people with a positive family history (adjusted OR 3.94, 95% CI 3.6, 4.3 p<0.001), than with no family history of diabetes. There was no significant association with smoking and rural/urban area.

Among 1,029 participants who were tested for 2-hour OGTT, in addition to HbA1c, the mean 2-hour OGTT was 200.26 (SD 91.7), and the median was 178 (inter-quartile range 100). Taking the OGTT as the gold standard, HbA1c recommended cut-off for diabetes showed a sensitivity of 84.7% (95% CI 80.8, 88) and Specificity of 87.2% (95% CI 84.3, 89.8%), ROC area 0.86 (95% CI 0.84, 0.88), positive predictive value 81.9% (95% CI 77.9, 85.4), and negative predictive value 89.3 (86.5, 91.6) (Table 3).

Discussion:

This is the first community based national study done in the region based on HbA1c and with the eligible 18,856 subjects from Pakistan this is the largest study to date from that country. The prevalence of type-2 diabetes across Pakistan was 16.98% (95%CI 16.44, 17.51) and pre-diabetes was 10.91% (95% CI 10.46, 11.36). This is higher than found in the only previous national survey conducted in1999 (n=5433) using OGTT. There was a significantly higher risk of type-2 diabetes with increasing systolic blood pressure, age, BMI, WC, WHR with evidence of a dose-response relationship. Similarly, there was a significant inverse relationship of type-2 diabetes with the level of formal education. The risk of diabetes increased 2.68 times with a prior family history. The HbA1c level had good sensitivity and specificity level for the diagnosis of type-2 diabetes compared to a 2-hour OGTT level and is therefore valid in community settings for screening purposes.

We have noted the findings of Basit et al[15]. The methodology for that study was based on 75g OGTT for glucose handling as opposed to HbA1c used in our study. While the prevalence of diabetes and pre-diabetes is different in the two studies, the point that both studies make is that both diabetes and pre-diabetes are much more prevalent than previously thought.

The previous national prevalence study conducted in 1999 used OGTT where almost 80% of the subjects were women as the test was conducted in the morning time [4]. In contrast, our study screening was done all through the day so that working men had an equal opportunity to be part of the study. They constituted 50% of the study population. OGTT is the gold standard for the type 2 diabetes screening but because of the length of time which is required for the test and the fact that the person must be fasting, it is very difficult to perform in many community settings in Pakistan. Also, the high temperatures in South Asia make it difficult to keep the sample stable for transportation to the laboratory. To minimize the effect of temperature and transportation errors on HbA1c, all tests were conducted in the field.

Recently a study carried out in 15 states of India showed that the prevalence of type-2 diabetes ranged between 4%-13.6% and showed variation due to age, male sex, obesity and family history using capillary FBG for diagnosis[16]. A capillary blood sample for epidemiological studies is not an ideal test but the authors acknowledged the logistic hindrance in carrying out venous sample test in the field. A high prevalence in another Indian study was reported ranging from 12.1-14% for diabetes using OGTT on a sample size of 11,216 subjects[17].

A study conducted in Bangladesh was based on capillary fasting level found a prevalence of type-2 diabetes of 4.3% in a rural setting[18]. Risk factors were positive family history for diabetes, age, high BMI and low socio-economic status, similar to our study. Although these are geographically distant areas, the risk factors showed commonality in both studies, which suggests these risk factors as an important tool for mass screening[19].

The age sex-standardized prevalence of type-2 diabetesfor Sri Lankans was 10.3% based on OGTT[20]. The risk factors were almost the same as seen in our study. The investigators found dysglycaemia in almost 21.8%participants and predicted that this would lead to a higher prevalence of T2DM in the years to come. Another study from Sri Lanka indicated a prevalence of 14.2% basis of FBG[21]. FBG as well as, OGTT blood glucose levels, may not be reproducible in an epidemiological survey if the individual are changing lifestyle in terms of diet and exercise. The use of HbA1c in our study makes our study more scientific, addressing issues pertaining to sampling errors in the local environment.

The Asian population is known to have a significantly higher risk of developing diabetes and its related complications as predicted by IDF projections[1]. It makes it very important from a public health point of view to identify high-risk individuals at an early stage. The HbA1c test has been used successfully in community settings[22]. A national health survey New Zealand in 2008-9 used HbA1c to identify high-risk individuals with diabetes and pre-diabetes[23]. A study in Japan revealed that a combination of tests including FBG and HbA1c yields more diabetes cases compared to any of these tests alone[24].

WHO experts have accepted HbA1c as a diagnostic tool provided quality assurance tests are in place and there are no conditions present, which preclude its accurate measurement. The New Zealand Society for the study of Diabetes and the Australian Diabetes Society have already endorsed HbA1c as a test for the diagnosis of diabetes[25 26].Recently a prevalence study done in Korea concluded that FBG testing results in underestimation of diabetes and pre-diabetes[27]. This study suggested the use of standardized HbA1c as a diagnostic tool for diagnosis of type-2 diabetes.

We found that HbA1c level had a good sensitivity and specificity level for diagnoses of diabetes compared to the OGTT. HbA1c has the advantage of being a simple test and less time consuming, making it an ideal test for community surveys in our populations. In Pakistan, as there is no effective primary care (general/ family practice) structure most of the population does not undergo primary screening for diabetes. Sometimes as the diagnosis is note made, people may present to tertiary care with complications.

Thus there is a strong case for applying HbA1c for screening purposes in the community setting. There will always be an argument about the cost of the test and whether this to be used for screening purposes. However particularly those at social disadvantage need to be undergo screening to improve the diagnosis timely treatment of diabetes[28]. Early diagnosis will also reduce diabetes-related complications.

Strengths and limitations:

Our study has the strength that we carried out HbA1c on all participants and OGTT on a subsample. Furthermore this is the largest ever national prevalence study of type-2 diabetes mellitus from Pakistan and the first community based national study to use HbA1c as the diagnostic tool.

Limitations are the relatively low number (n=1,027) of 75gOGTT. Nevertheless the specificity and sensitivity of HbA1c vs OGTT was good. We had to exclude 16% of recruited participants because of anaemia.

The central Government of Pakistan has developed and agreed on Non- Communicable Diseases (NCD) National Action Plan including diabetes however, it was never implemented. After the 18th Amendment in the constitution of Pakistan in 2010, provinces are responsible for making and implementing their own health policies and the role of central Government is limited to coordination among the different provinces[29]. There is a dire need that based on the agreed NCD National Action Plan each province should build their capacity for implementing it at both primary and secondary level. Pakistan is a signatory to the Sustainable Development Goals 2030 document which outlines among its goals, increasing access to universal health coverage, increasing coverage of health insurance program and adopting a family medicine approach. When implemented, these will not only be major steps towards prevention and control of diabetes but all non-communicable diseases.

Conclusions:

This national diabetes prevalence study is the first one in the region using HbA1c identified a huge population of type-2 diabetes and pre-diabetes group. The prevalence of type-2 diabetes and pre-diabetes is much higher than previously thought in Pakistan. Comprehensive strategies need to be developed to incorporate screening, prevention and treatment of type-2 diabetes at community level. Those who are obese, with no formal education, older, family history of diabetes and hypertensive merit close attention and timely intervention.

HbA1c is an applicable test in community settings in developing countries and it has a good correlation with 2-hour OGTT. Our findings have the potential to influence policy in developing countries and induce a shift towards the prevention and control of non-communicable diseases.

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Contributors AHA, ZUH conceived the study. AHA, SM, FQ, AS, AJ, OI, AR, IA, HA collected the data. ZJ, NS study design and data monitoring. ZUH, AHA, SF, AH, conducted the analysis and wrote the manuscript. All authors reviewed the manuscript. AHA and ZUH contributed equally.

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Competing interest None declared

Patient Consent Written informed consent granted. The data is fully anonymised and neither the patient nor anyone else could identify the patient.

Ethical approval The ethical approval was granted from the Khyber Medical University ethical board (DIR/KMU-EB/SP/000395).

Data sharing statement No additional data are available

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Table 1 Characteristics of the participants by diabetes categories (n=18,856)

	Non-Diabetic N (%)	Pre-Diabetic N (%)	Diabetic N (%)	*P-value*
	13,598 (72.11)	2,057 (10.91)	3,201 (16.98)	
Age (years)				
20-30	2,772 (20.37)	218 (10.60)	176 (5.52)	<0.001
31-40	3,503 (25.74)	425 (20.66)	511 (16.01)	
41-50	3,802 (27.94)	654 (31.79)	1,033 (32.37)	
51-60	1,955 (14.37)	412 (20.03)	833 (26.10)	
61 & above	1,576 (11.58)	348 (16.92)	638 (19.99)	
Gender				
Male	7363 (54.15)	1099 (53.43)	1638 (51.17)	0.010
Female	6235 (45.85)	958 (46.57)	1563 (48.83)	
Education				
No formal education	9,853 (72.41)	1516 (73.70)	2,439 (76.43)	<0.001
Primary	1936 (14.23)	354 (17.21)	452 (14.16)	
Secondary	818 (6.01)	99 (4.81)	184 (5.77)	
Graduation	1001 (7.36)	88 (4.28)	116 (3.64)	
Area				
Urban	9,117 (67.00)	1,213 (58.97)	1,932 (60.55)	
Rural	4,491 (33.00)	844 (41.03)	1,259 (39.45)	<0.001
Family History				
Negative	10325 (75.93)	1210 (58.82)	1320 (41.24)	<0.001
Positive	3273 (24.07)	847 (41.18)	1881 (58.76)	
Smoking				
Never	12682 (93.26)	1893 (92.03)	2937 (91.75)	0.008
Ex-Smoker	275 (2.02)	49 (2.38)	91 (2.84)	
Current smoker	641 (4.71)	115 (5.59)	173 (5.40)	
Systolic BP (mean ±SD)	124.98	128.17	130.77	0.020
Diastolic BP (mean ±SD)	82.75	84.13	84.84	0.006
BMI				
Underweight (<18.5)	279 (2.05)	31 (1.51)	35 (1.09)	<0.001
Normal (18.5-<25)	5137 (37.78)	668 (32.47)	1019 (31.83)	
Overweight (25-<30)	5884 (43.27)	850 (41.32)	1288 (40.24)	
Obese1 (30-<35)	1844 (13.56)	396 (19.25)	621 (19.40)	
Obese 2 (35-<40)	372 (2.74)	83 (4.04)	178 (5.56)	
Obese 3 (≥40)	82 (0.60)	29 (1.41)	60 (1.87)	
Waist circumference *				
Normal Weight	6,787 (70.88)	645 (51.68)	1,142 (55.93)	<0.001
Over weight	1,676 (17.50)	285 (22.84)	357 (17.48)	
Obese	1,113 (11.62)	318 (25.48)	543 (26.59)	
Waist to Hip ratio *				

Normal Weight	3,219 (33.62)	394 (31.57)	658 (32.22)	
Over weight	5,528 (57.73)	768 (61.54)	1,171 (57.35)	
Obese	828 (8.65)	86 (6.89)	213 (10.43)	0.002

* n for Waist circumference & waist to hip ratio is 12865

Table 2 Logistic regression analysis of the participant characteristics associated with having diabetes (HbA1c≥6.5 % DCCT aligned /48 mmol/mol IFFC units) (n=18,856)

	Univariate		**Multivariate**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Age				
20-30	1		1	
31-40	2.21 (1.8, 2.6)	<0.001	2.03 (1.7, 2.4)	<0.001
41-50	3.93 (3.3,4.6)	<0.001	3.39 (2.9, 4.0)	<0.001
51-60	5.97 (5.0, 7.1)	<0.001	4.87 (4.1, 5.8)	<0.001
61 & above	5.63 (4.7, 6.7)	<0.001	4.93 (4.1, 6.0)	<0.001
Gender				
Male	1		1	
Female	1.12 (1.0,1.2)	0.003	1.04 (0.9, 1.1)	0.334
Education				
No formal education	2.02 (1.6,2.4)	<0.001	1.83 (1.5, 2.3)	<0.001
Primary	1.85 (1.49, 2.3)	<0.001	1.39 (1.1 , 1.8)	0.006
Secondary	1.89 (1.47,2.4)	<0.001	1.57 (1.2 , 2.0)	0.001
Graduation	1		1	
Area				
Urban	1		1	
Rural	1.26 (1.16, 1.34)	<0.001	1.08 (0.9, 1.2)	0.084
Family History of diabetes				
Negative	1		1	
Positive	3.98 (3.6,4.3)	<0.001	3.94 (3.6, 4.3)	<0.001
Smoking				
Never	1		1	
Ex- Smoker	1.39 (1.1, 1.8)	0.006	1.13 (0.9 , 1.5)	0.323
Current Smoker	1.13 (0.9,1.3)	0.14	1.06(0.9, 1.3)	0.571
Systolic BP	1.02 (1.021, 1.026)	<0.001	1.01 (1.01, 1.02)	<0.001
Diastolic BP	1.01 (1.01, 1.02)	<0.001	0.99 (0.9 , 1.0)	0.310
BMI				
Underweight (<18.5)	0.64 (0.4, 0.9)	0.001	0.71 (0.5, 1.0)	0.077
Normal (18.5-<25)	1		1	
Overweight (25-<30)	1.08 (0.9,1.2)	0.06	1.06 (0.9, 1.1)	0.182
Obese1 (30-<35)	1.57 (1.4,1.7)	<0.001	1.54 (1.3, 1.7)	<0.001
Obese 2 (35-<40)	2.22 (1.8,2.6)	<0.001	2.13 (1.7, 2.6)	<0.001
Obese 3 (≥40)	3.07 (2.2,4.2)	<0.001	2.44 (1.7, 3.5)	<0.001
Waist circumference *				

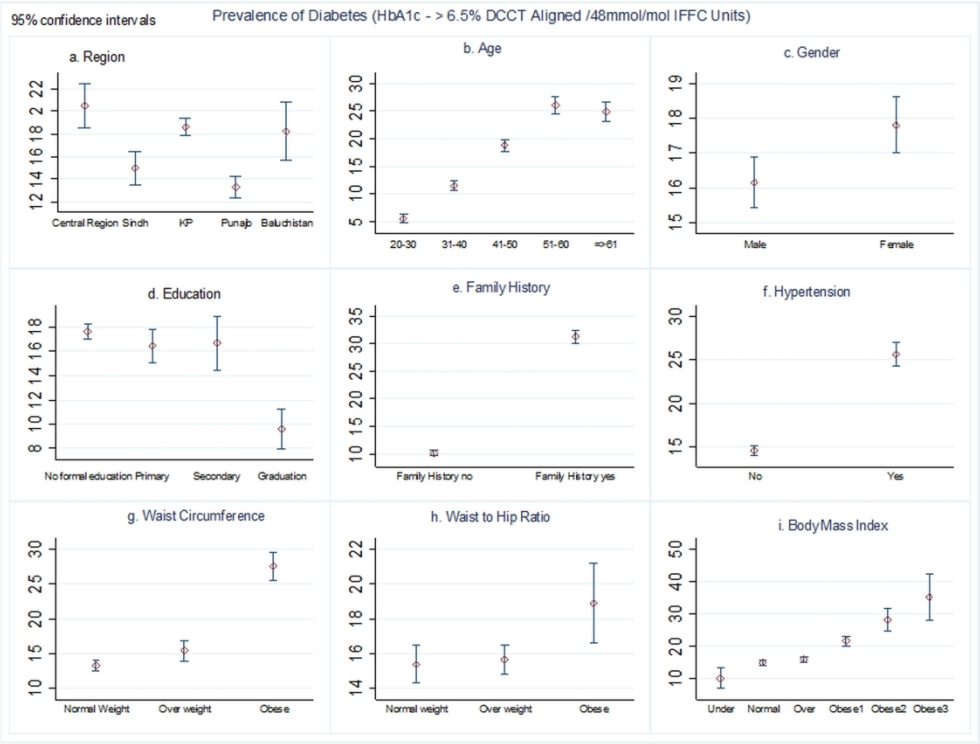
Normal Weight	1		1	
Over weight	1.18 (1.04, 1.34)	0.01	0.98 (0.8, 1.1)	0.774
Obese	2.46 (2.19, 2.77)	<0.001	1.86 (1.6, 2.2)	<0.001
Waist to Hip Ratio *				
Normal Weight	1		1	
Over weight	1.02 (0.92, 1.13)	0.40	0.8 (0.7, 0.9)	<0.001
Obese	1.27 (1.07, 1.51)	0.005	1.13 (0.9, 1.4)	0.205

* n for WC &WHR is 12865.The following variables were included in the multivariate regression: Age, gender, education, residence area, family history of diabetes, smoking, systolic and diastolic blood pressure and BMI.

Table 3: Summary statistics for diabetes diagnosed by HbA1c compared to diabetes diagnosed by 2hrs OGTT (n=1,027)

			[95% Confidence Interval]	
Prevalence	Pr(A)	40.6%	37.6%	43.7%
Sensitivity	Pr(+|A)	84.7%	80.8%	88.0%
Specificity	Pr(-|N)	87.2%	84.3%	89.8%
ROC area	(Sens. + Spec.)/2	0.86	0.84	0.88
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	6.62	5.36	8.18
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	0.18	0.14	0.22
Odds ratio	LR(+)/LR(-)	37.62	26.34	53.73
Positive predictive value	Pr(A|+)	81.9%	77.9%	85.4%
Negative predictive value	Pr(N|-)	89.3%	86.5%	91.6%

Figure 1: Prevalence of diabetes (diagnosed by HbA1c≥6.5 % DCCT aligned /48 mmol/mol IFFC units) by regions of Pakistan, age, gender, education, family history of diabetes, hypertension, waist circumference, waist to hip ratio and body mass index (n=18,856).



Prevalence of diabetes (diagnosed by HbA1c≥6.5 % DCCT aligned /48 mmol/mol IFFC units)

246x187mm (300 x 300 DPI)

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	3
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	6-7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	7-8
Bias	#9	Describe any efforts to address potential sources of bias	8
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8
	#12b	Describe any methods used to examine subgroups and interactions	8
	#12c	Explain how missing data were addressed	8
	#12d	If applicable, describe analytical methods taking account of sampling strategy	8
	#12e	Describe any sensitivity analyses	8
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8-9
	#13b	Give reasons for non-participation at each stage	8
	#13c	Consider use of a flow diagram	6
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8-9

	#14b	Indicate number of participants with missing data for each variable of interest	8-9
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
	#16b	Report category boundaries when continuous variables were categorized	9
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9
Key results	#18	Summarise key results with reference to study objectives	9
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	11
Generalisability	#21	Discuss the generalisability (external validity) of the study results	11
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12