



GLYCATED HEMOGLOBIN (HbA1c); SCREENING FOR UNDIAGNOSED DIABETES IN HEALTHY INDIVIDUALS IN SARGODHA

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ABSTRACT... HbA1c gives an integrated index of glycemia over the entire 120 days life span of red blood cells. Therefore, measuring HbA1c would be appropriate in diagnosing a disease characterized by chronic hyperglycemia and a gradual progression to complications.

Objectives: our primary objective was to evaluate the use of HbA1c as screening test for undiagnosed diabetes (WHO criteria of Fasting plasma glucose (FPG) of $\geq 7\text{mmol/l}$ (126mg/dl)) in healthy asymptomatic individuals in Pakistani population. **Study Design and Methodology:** A cross sectional population survey was carried on asymptomatic, healthy individuals without past history of diabetes. Venous blood was obtained to measure fasting plasma glucose (fasting > 8 hours) and Hb A1c. **Place and Duration of Study:** Khan lab Sargodha from July 2013 to March 2014. **The test:** It was performed by using Nycocard HbA1c in vitro diagnostic medical device for quantitative determination of glycosylated hemoglobin in whole blood. **Results:** In our sample size of 775, the lowest HbA1c was found to be 5% and Highest 13.2%. Arithmetic means was 6.7565%, while the median value was 6.2% and standard deviation 1.3323. When using FPG only, the detection rate of diabetes was 32.65% (female, 14.71%; male, 17.94%). When HbA1c was included as a diagnostic test, the detection rate increased to 40% (female, 18.84%; male, 21.16 %). An additional 7.6% of participants were diagnosed with diabetes when using HbA1c criteria. ROC (A receiver operating characteristic) curve was used for analysis. At HbA1c cutoff of $\geq 6.5\%$ it demonstrated sensitivity of 98.02% (95% CI) and specificity of 88.12% (95% CI) for detection of undiagnosed diabetes mellitus in healthy asymptomatic individuals in Pakistani population. Area under the ROC curve was 0.981354 with significance level P (Area=0.5) 0.0001. **Conclusions:** Our study reveals that HbA1c is a highly specific and convenient alternative to fasting plasma glucose for screening of diabetes mellitus in Pakistani population. A large scale survey should be carried out to set our own national standardizations.

Key words: HbA1c, Glycated hemoglobin, Diabetes mellitus, sensitivity, specificity, Sargodha.

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INTRODUCTION

Glycated hemoglobin (HbA1c) is the result of an irreversible non-enzymatic glycation of the beta chain of hemoglobin A. It was initially identified as "unusual" hemoglobin in diabetes over 40 years ago¹. HbA1c gives an integrated index of glycemia over the entire 120 days life span of red blood cells. About 50% of HbA1c is formed in the month prior to sampling and 25% in the month before that². Therefore, measuring HbA1c would be appropriate in diagnosing a disease characterized by chronic hyperglycemia and a gradual progression to complications. The potential utility of HbA1c in diabetes care was first time mentioned in the 1985 WHO report³. Now it's

measurement has become the gold standard for assessment of glycaemic control.

The major obstacle to implement specific guidelines for diabetes care was a lack of comparability of HbA1c test results among methods and laboratories. National Glycohemoglobin Standardization Program (NGSP) was implemented in 1998 to standardize the results. By the end of 2002, 97 % of laboratories used an NGSP- certified method. The reports are now traceable to (1993 Diabetic Control and Complication trial) DCCT/ UKPDS (United Kingdom Prospective Diabetic Study) GHb/ HbA1c results⁴. In March 2009, WHO updated the

1999 and 2006 reports recommending the use of HbA1c as a diagnostic test with a cut point of 6.5% for diagnosing diabetes⁵. The American Diabetes Association (ADA) has also affirmed the decision of an international expert committee to use HbA1c test to diagnose diabetes with a threshold of $\geq 6.5\%$ ⁶. There were thirty different methods to measure HbA1c with significant variability of results between different laboratories as well as on the same sample⁷. Now the commonly used HbA1c assay methods by the laboratories include high performance liquid Chromatography, Boronate Affinity and Immunoassays.

International federation of clinical chemistry (IFCC) has recommended the use of SI units, i.e., mmol HbA1c/mol of total hemoglobin in place of percentage^{7,8}. This has been agreed by The ADA, European Association for the Study of Diabetes (EASD) and International Diabetes Federation (IDF) that, in the future, HbA_{1c} is to be reported in the International Federation of Clinical Chemistry (IFCC) units. In New Zealand HbA1c measurement is now only reported in SI units of mmol/mol⁹. Health professionals will need to familiarize with new term and units. An eAG (estimated average glucose)⁷ can also be calculated by HbA1c values given in DCCT percentage.

MATERIAL AND METHODS

This was a cross sectional study conducted on residents of Sargodha city and its surroundings. Awareness posters, briefly describing the significance of this test were displayed at selected clinics. Only those individuals who volunteered were enrolled. Informed consent was taken from all those who participated in the study. The idea was to select the individuals representing all fractions of the population with diverse socioeconomic background. Those included in the study were healthy individuals; age ≥ 20 years without past history of diabetes, with or without risk factors like a family history positive for diabetes, a history of gestational diabetes or obesity. These included both the healthy persons as well patients suffering from co-morbid conditions not specifically affecting the measurement of HbA1c.

After taking the consent, the initial assessment involved the filling of a questionnaire, followed by clinical examination along with the previous investigations was reviewed to evaluate the diseases that interfered with the measurement of HbA1c. Data regarding age, sex and co-morbid conditions including, chronic kidney and liver disease was recorded. A battery of tests was also performed to exclude the factors affecting the HbA1c levels. The criteria of exclusion included the non-willing individuals, Patients suffering from diseases that interfered with the measurement of HbA1c, like severe anemia, chronic renal failure and severe liver disease. Hemoglobinopathies are known to affect the HbA1c results. However the assay we have used is not interfered by the abnormal hemoglobins.

Biochemical measurements (HbA1c and FPG)

Venous blood was collected from each participant after overnight fasting (minimum of 8 hours fast) in the laboratory. The samples were processed and analyzed on the same day. Fasting glucose was measured by using "GOD-PAP": enzymatic photometric test. Glycated hemoglobin was measured as HbA1c by using automatic analyzer; NycoCard READER II which is a boronate affinity assay. The results are traceable to the IFCC reference method for measurement of HbA1c.

Data entry and analysis

Based on WHO recommendations and ADA criteria a cutoff value of 126 mg % was used. Subjects with fasting glucose ≥ 126 mg % were classified as having diabetes, whereas subjects with fasting glucose < 126 mg % were classified as not having diabetes. Statistical analyses were performed using MedCal for Windows, version 12.5.0.0 (MedCal Software, Ostend, Belgium). To evaluate the validity (accuracy) of Hba1c as a diagnostic test, the data was analyzed by ROC (a receiver operating characteristic) curve.

RESULTS AND DISCUSSION

Our sample size was 775, analysis of it revealed 354 (45.68%) females and 421(54.32%) males. The minimal age in the sample was 20 years while the maximum age was 87 years. The median age

Characteristics	HbA1c < 6.5%				HbA1c ≥ 6.5%			
	FPG < 126 mg/dL		FPG ≥ 126 mg/dL		FPG < 126 mg/dL		FPG ≥ 126 mg/dL	
No. (%)	460 (59.35)		5 (0.65)		62 (8)		248 (32)	
	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)
	204 (26.32)	256 (33.03)	4 (0.52)	1 (0.13)	36 (4.65)	26 (3.35)	110 (14.19)	138 (17.81)
Age, years								
20-30	42 (5.42)	44 (5.68)	2 (0.26)	0	13 (1.68)	2 (0.26)	13 (1.68)	10 (1.29)
31-40	45 (5.81)	63 (8.13)	0	0	12 (1.55)	3 (0.39)	25 (3.23)	42 (5.42)
41-50	71 (9.16)	88 (11.35)	1 (0.13)	1 (0.13)	4 (0.52)	9 (1.16)	36 (4.64)	43 (5.55)
51-60	36 (4.64)	53 (6.84)	0	0	6 (0.77)	6 (0.77)	25 (3.22)	26 (3.35)
>60	10 (1.29)	8 (1.03)	1 (0.13)	0	1 (0.13)	6 (0.77)	11 (1.42)	17 (2.20)

Table-I. HbA1c and FPG and their relation with different age groups and sex.

of the sample was 44 years. HbA1c and FPG and their relation with different age groups and sex have been summarized in Table I.

The median serum level of HbA1c was 6.2% with sample levels ranging from 5% to 13.2%. A total of 60 % had level < 6.5% and 40% had level ≥ 6.5%. The mean HBA1c in females was 6.73%, while in males it was 6.78%. F-test (comparison of

standard deviation) was 1.1214 with significance level, P= 0.264, while T test assuming equal variance (two tailed probability P=0.6036). (Table III, Fig 3)

The mean HBA1c in diabetic patients was 8.25% (SD = 1.30), while in non-diabetic individuals it was 6.03% (SD=0.46) (Table II). An increasing level of HbA1c is observed with increasing age

Age group (years)	HbA1c					
	All individuals		Diabetic patients		Non-diabetics	
	Mean ± SD		Mean ± SD		Mean ± SD	
All age groups (years)	6.7565±1.3323		8.25±1.30		6.03±0.46	
	Female	Male	Female	Male	Female	Male
	6.73±1.29	6.78±1.37	8.14±1.33	8.34±1.28	6.06±0.48	6±0.44
20-30	6.47±1.20	6.27±0.87	8.09±1.54	7.8±0.97	6.02±0.54	5.93±0.33
31-40	6.61±1.10	7.10±1.51	7.98±1.28	8.30±1.24	6.07±0.25	5.90±0.31
41-50	7.27±1.73	6.69±1.35	9.02±1.16	9.02±1.06	5.96±0.31	6.09±0.37
51-60	7.30±1.55	6.88±1.20	8.80±1.22	8.38±0.97	6.18±0.11	6.45±0.88
61-70	6.70±1.46	7.69±1.75	8.65±0.51	8.15±0.0	5.60±0.00	6.7±0.00
>71	8.43±2.51	9.38±2.21	8.43±2.51	9.38±2.21	00±00	00±00

Table-II. HbA1c levels in different age groups.

both in diabetic and non-diabetic individuals. (Table II). Fig 3 shows the comparison of mean HbA1c in female and male individuals.

ROC (A receiver operating characteristic) (Fig 1) curve was used for analysis. At HbA1c cutoff of ≥6.5% it demonstrated sensitivity of 98.02% (95% CI) and specificity of 88.12% (95% CI) for detection

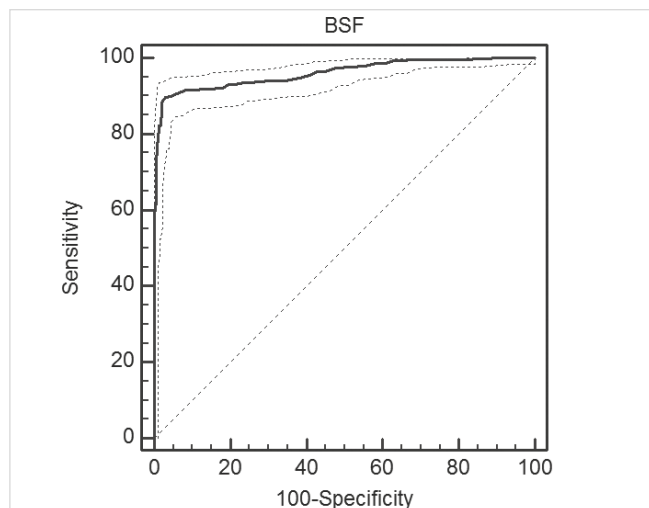


Fig-1. ROC curve for HbA1c, as a diagnostic indicator for diabetes mellitus (Fasting blood sugar ≥ 126)

of undiagnosed diabetes mellitus in healthy asymptomatic individuals in Pakistani population. Area under the ROC curve was 0.981354 with significance level P (Area=0.5) 0.0001. Correlation between HbA1c and FPG is shown by scatter diagram (Fig. 2). Correlation coefficient r It is 0.8872 significance level of $p < 0.0001$ (95% CI for r).

The use of HbA1c as diagnostic test was recommended in mid 1980s. Poor assay standardization and its availability prevented its wide spread acceptance¹⁰. In 2009 a

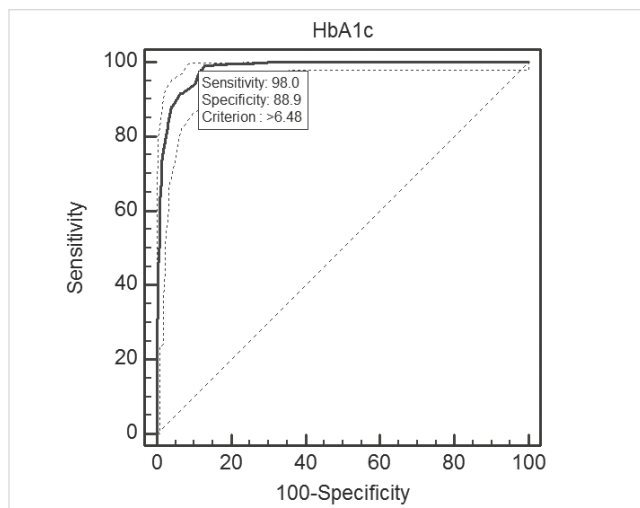


Fig-1. ROC curve for HbA1c, as diagnostic indicator for diabetes mellitus (Fasting blood sugar ≥ 126 mg/dl)

threshold level of ≥ 48 mmol/mol ($\geq 6.5\%$) was recommended by an international expert committee¹¹. This recommendations has been adopted by American diabetic association (ADA) and by WHO^{12,13}, Australia¹⁴. New Zealand Society for the Study of Diabetes (NZSSD) also strongly favours HbA1c as diagnostic test in preference to OGTT testing¹⁵.

The use of HbA1c for screening and diagnosing diabetes is evidence based. A recent review of 63 published papers by Bennet et al¹⁶ did not found any evidence to suggest that FPG is superior to

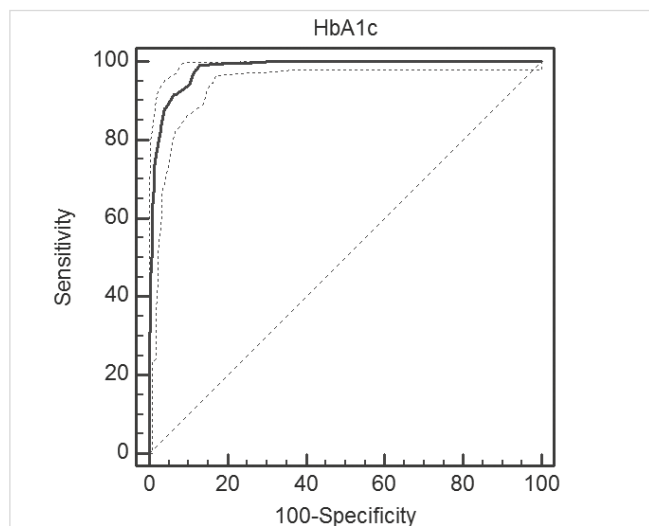


Fig-2. ROC curve for HbA1c, as diagnostic indicator for diabetes mellitus (HbA1c $\geq 6.5\%$)

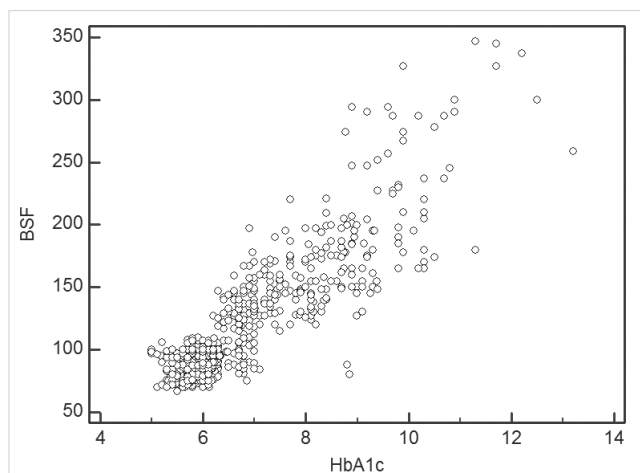


Fig-2. Scatter plot according to HbA1c and FPG.

HbA1c in screening for diabetes with OGTT as reference standard. HbA1c had a slightly higher specificity and lightly lower sensitivity, than FPG for detection of diabetes.

In USA the analysis of Third National Health and Nutrition Examination Survey (NHANES III) 1988-94 by Rohlfing et al¹⁷ for the sensitivity and specificity of HbA1c in the diagnosis of diabetes based on FPG showed that HbA1c provided a specific and convenient approach to screening for diabetes and suggested a value of 6.1% or greater, 2SD above the mean in the normal NHANES III population. Similar results are obtained by analysis of 1999-2004 NHANES data by Buell et al¹⁸. They found that at cut point 5.8 % or greater of HbA1c yielded the highest sum of sensitivity (86%) and specificity (92%).

To evaluate the sensitivity and specificity we have used the ROC (A receiver operating characteristic) curve for analysis. At HbA1c cutoff of $\geq 6.5\%$ it demonstrated sensitivity of 98.02% (95% CI) and specificity of 88.12% (95% CI) for detection of undiagnosed diabetes mellitus in healthy asymptomatic individuals in Pakistani population. Area under the ROC curve was 0.981354 with significance level P (Area=0.5) 0.0001. In Japanese people the area of the ROC for HbA1c was almost the same as that for FPG (0.856Vs.0.902) respectively, suggesting similar value for both the tests¹⁹. In our study the area of Roc for HbA1c and FPG was 0.981354 and 0.960874 respectively (fig 1, fig 2).

Racial and ethnic differences independent of blood glucose has been observed. The diabetes prevention program and the ADOPT subgroup found that African-Americans had an HbA1c 0.4-0.7 greater than the Caucasians²⁰. The significance of this difference remains unclear. We study is too small to claim our national standards. However it does reflect HbA1c levels in Pakistani population.

	Female	Male
Mean	6.7294	6.7793
Standard Deviation	1.2910	1.3671
NO. Of cases	354	421

Table-III. Comparison of means HbA1c (t-test)

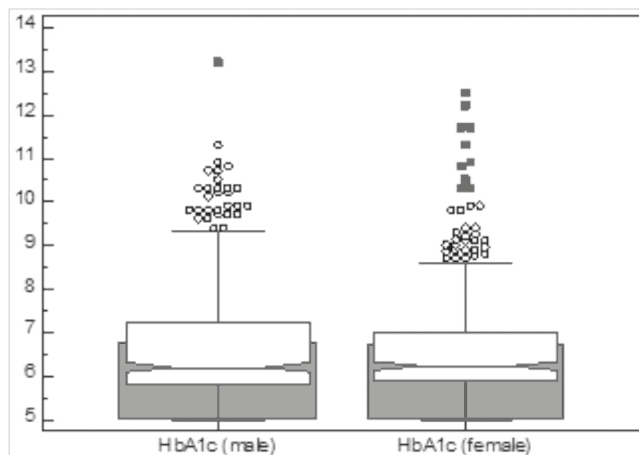


Fig-3. Comparison of means HbA1c (t-test)

The study conducted by Jeon JY et al²¹ in Korean population revealed 2.1% of subjects with a HbA1c $\geq 6.5\%$ and FPG < 126 mg/dL and 1.1% with a HbA1c $< 6.5\%$ and FPG ≥ 126 mg/dL. However, our study shows 8% of subjects with HbA1c $\geq 6.5\%$ and FPG < 126 mg/dL and for 0.65% with a HbA1c $< 6.5\%$ and FPG ≥ 126 mg/dL. The difference is probably due to difference in the selection of the individual undergoing the tests. Our study does not truly representation of all section of our population.

The vast majority of younger individuals (age < 40 years) of our sample were healthy individuals who do not have any suspicion of diabetes, they were undergoing detailed medical examination under compulsion such as for employment. While the more elder group include those individuals who had suspicion of diabetes but were never diagnosed before as diabetic.

When using FPG only, Jeon JY et al²¹ detected diabetes in 10.5% (female, 8.5%; male, 12.5%) ; In our study the detection rate is 32.65% ((253 out of 775) (female, 14.71%; male, 17.94%). When HbA1c was included as a diagnostic test, the detection rate by Jeon JY et al (15) was 12.4% (female, 8.5%; male, 12.5%). In our study the detection rate increased to 40% (310 out of 775) (female, 18.84%; male, 21.16 %).

In above mentioned study the difference in detection rate is 1.9% while in our study an

additional 7.6% of participants were diagnosed with diabetes when using HbA1c criteria. Small sample size and different criteria of selection of the individuals could explain these differences.

Participants with HbA1c $\geq 6.5\%$ and fasting plasma glucose < 126 were older in the above mentioned study while in our study this trend is seen in relatively younger age group. Similarly the prevalence of diabetes has been reported to be increased with increasing age²¹. While in our study the highest rate of detection of diabetes is observed to be between 31 to 50 years.

CONCLUSIONS

We concluded that using fasting glucose level only may result in an underestimation of diabetes. HbA1c is an acceptable complementary diagnostic test for diabetes in Pakistani population. However, national standardization is needed to order to use HbA1c as a diagnostic method of diabetes, since our sample is too small to have definitive conclusion.

However the clinicians need to be aware of its availability and cost effectiveness.

There are clear advantages for HbA_{1c} over glucose (and in particular OGTT) as a diagnostic test for diabetes

LIMITATIONS OF THE STUDY

Small sample size, financial constraints were the main reason. Increasing sample size with inclusion of more pockets of population would help to establish national standards in Pakistani population.

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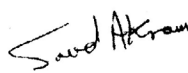

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AUTHORSHIP DECLARATION

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