The use of HbA1C in the diagnosis of diabetes mellitus type 2 in high risk subjects

Hasni Ibrahim[1], Adibah Hanim Bt Ismail[1], Shaiful Bahari Ismail[2], Wan Mohamed Wan Bebakar[3]

Family Medicine Department School of Medical Sciences [1], Medical Department School of Medical Sciences [2], USM, 16150 Kubang Kerian, Kelantan, Malaysia.

Abstract

We determined the specificity and sensitivity of HbA1c and its cut off level in the diagnosis of type 2 diabetes mellitus among high risk subjects. A cross-sectional study was conducted at Family Medicine Clinic, Hospital Universiti Sains Malaysia (HUSM) from March 2005 till January 2006. All subjects, age more than 35 years old with one or more risk factors for diabetes together with capillary blood glucose ≥5.6 mmol/l were included in this study. They were instructed to fast overnight for 10-12 hours with 3 days on normal diet and physical activity. Venous blood for glycosylated haemoglobin (HbA1c) and oral glucose tolerance test (OGTT) were done on appointment basis. Four hundreds and two asymptomatic high risk subjects were eligible in this study. HbA1c level was measured by using Cation-exchanged, high performance liquid chromatograph (HPLC) method via D10 HbA1c Program from BioRad with interassay coefficient of variations (CV) of 1.6-1.9%. Of 402 patients, 32.6% (n=131) had type 2 diabetes mellitus based on OGTT. HbA1c of 6.4% gave an optimal sensitivity of 68% and specificity of 90% to predict either FPG of 7.0 mmol/L or 2HPP of 11.1 mmol/L. With HbA1c cut off level of 6.4%, the positive predictive value to detect diabetes was 76% and negative predictive value was 86%. The likelihood ratio that HbA1c $\geq$ 6.4% to come from diabetes patient was 6.8 times. This study shows that HbA1c is a highly specific and convenient alternative to fasting plasma glucose and glucose tolerance test for the diagnosis of type 2 diabetes mellitus in high-risk group.

Keywords: Glycosylated haemoglobin, fasting plasma glucose, 2 hours postprandial test, oral glucose tolerance test

Introduction

Diabetes mellitus is a global epidemic and the most common non-communicable disease that affect more than 150 million people world-wide. It has become epidemic in a number of countries in Asian-Pacific region, particularly in newly industrialized nations. In Malaysia the prevalence of diabetes has steadily increased over the years. Kelantan is one of the states in Malaysia has shown that the prevalence of diabetes was 10.5%.[1]

The usefulness of glycosylated haemoglobin (HbA1c) in the screening and diagnosis of diabetes has been widely debated, and it is criticized primarily for its lack of sensitivity and for the confounding aspects of assay and reference-range standardization and of inadequate quality control.[2,3] Indeed large epidemiological studies have suggested that HbA1c testing is less sensitive than fasting plasma glucose (FPG) measurement in terms of its diagnostic capabilities.[4,5] However data from the study by Perry et al in 2001 which was obtained in a well defined at risk population, and data from previous reports obtained in more general populations indicate that the combination of FPG and HbA1c measurement is more predictive than either parameters alone.[6]

Methodology

This was a cross sectional study, to determine HbA1c as a diagnostic test for type 2 diabetes mellitus in a high risk group of patients attending Family Medicine Clinic, Hospital Universiti Sains Malaysia (HUSM) from March 2005 till January 2006. All subjects who fulfilled the inclusion and exclusion criteria were instructed to fast overnight from 10 pm or 10-12 hours and 3 days on normal diet (more than 150 g of carbohydrate daily) and usual physical activity. Venous blood for HbA1c and OGTT were withdrawn from forearm area on appointment day.

Inclusion Criteria

1. Subject with age 35 years or older
2. Subject with one or more risk factors for developing type 2 diabetes mellitus; BMI > 23kg/m², history of gestational diabetes mellitus (GDM), history of big baby > 4.0 kg, history of type 2 diabetes mellitus in first degree relatives, Hypertension (>140/90), hyperlipidaemia and dyslipidaemia (HDL < 0.9 or Triglyceride > 2.3 mmol/L).
3. Positive screen using capillary blood glucose ≥ 5.6 mmol/L

Exclusion Criteria

1. Previous diagnosis of diabetes.
2. Pregnancy
3. History of haemolytic anaemia, haemoglobinopathies and renal failure.

Study protocol

The protocol for this study was approved by the School of Medical Sciences Research and Ethical Committee. All subjects gave written informed consent and OGTT was performed following the WHO guideline.
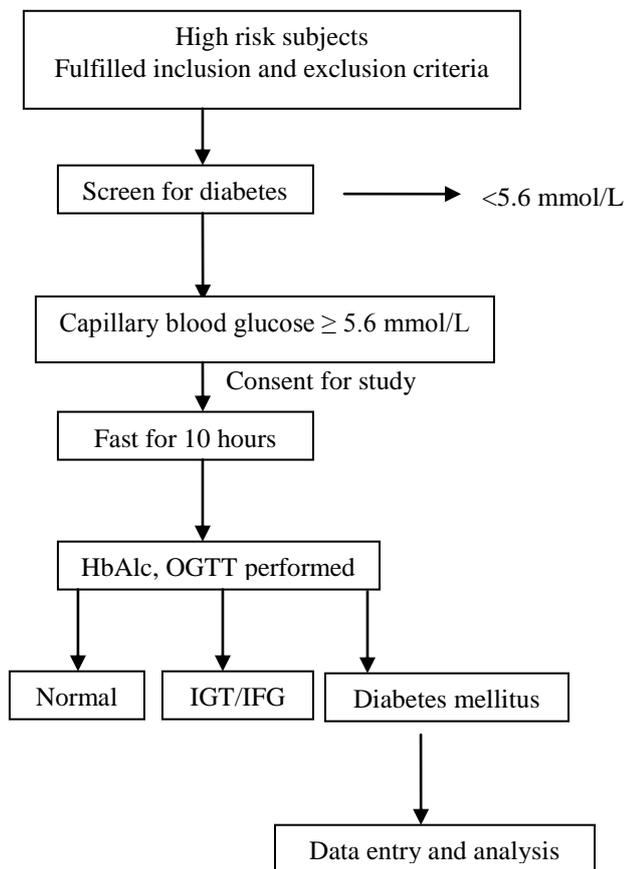

Fig 1: Protocol of the Study

Statistical analysis

All the data were analyzed using statistic program for Social Sciences (SPSS) Program Software version 11.5. WHO's criteria for diagnosis of diabetes with oral glucose tolerance test (OGTT) was used as the "gold standard" (Table 1). The results were summarized with receiver operating characteristic (ROC) analysis to examine the sensitivity and specificity of HbA1c cutoff level. The ROC curve plots the sensitivity against 1 minus the specificity at all possible HbA1c cut off levels. The bigger the area under the curve (AUC), the better the predictive value of HbA1c. An AUC value of 0.500 would indicate no predictive value, whereas a value of 1.000 would indicate perfect predictive value with no false positive or false negative. Table 2 summarizes the classification of subjects for the ROC analysis.

Results

The sensitivity and specificity of HbA1c in detecting diabetes based on OGTT at each cut off level were shown by the area under the curve that was 0.870. HbA1c of 6.4% has moderate sensitivity (68%) and highly specificity (90%). With HbA1c cut off level of 6.4%, the positive

Table 1: Diagnostic values for type 2 Diabetes Mellitus / Glucose Intolerance- OGTT (WHO, 1999 and ADA 2003)

OGTT Plasma Glucose Values (mmol/L)		
category	0-hour	2-hour
Normal	< 5.6	< 7.8
IFG	5.6-6.9	-
IGT	-	7.8-11.0
DM	≥7.0	≥ 11.1

Table 2: Classification of subjects for ROC analysis

	Plasma glucose	
	Normal and classification IFG/IGT (FPG < 7.0 or 2h-PP < 11.1 mmol/l	Diabetes (FPG ≥ 7.0 or/and 2h PP ≥ 11.1 mmol/l
HbA1c < cut off level	TN	FN
HbA1c ≥ cut off level	FP	TP

FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Table 3: The sensitivity and specificity at each cut off of HbA1c based on OGTT

HbA1c*, %	Sensitivity (%)	Specificity (%)
5.9	84	74
6.0	81	78
6.1	78	83
6.2	74	85
6.3	71	88
6.4	68	90
6.5	64	91

*This cut off and above values, result as DM.

predictive value to detect diabetes was 76% and negative predictive value was 86%. The likelihood ratio that HbA1c ≥ 6.4% to come from patient with diabetes mellitus was 6.8 times (Table 3).

Discussion

The American Diabetic Association recommended using the FPG level as the preferred test for screening and diagnosis of diabetes because the OGTT is construed as being cumbersome and highly variable.[7] Indeed, surveys have shown that practicing physician seldom obtain an OGTT to diagnose diabetes.[8] Because epidemiological data suggest that an FPG level of 7.0 mmol/l closely corresponds to post glucose challenges level of 11.1 mmol/l, the expert committee recommended an FPG level of 7.0 mmol/l (confirmed on a separate occasion) as the diagnostic criteria for diabetes.[9] However, many individuals demonstrate abnormal responses to glucose challenge before the onset of fasting hyperglycaemia. Indeed, numerous reports have indicated that up to 50% of patients with diabetes who were diagnosed by OGTT criteria would have been missed by current FPG criteria.[10,11,12]

Table 4: Predictive value and Likelihood ratio for cut off level of HbA1c to detect type 2 diabetes

HbA1c	Normal	DM	Total	Predictive values	Likelihood ratio
≥ 6.4	27	88	115	PPV 76%	6.8
< 6.4	246	41	287	NPV 86%	
Total	273	129	402		
	Sensitivity	Specificity			
	68%	90%			

Abbreviations: DM= Type 2 diabetes. PPV= positive predictive value, NPV= negative predictive value.

Thus, improved methods to detect diabetes are particularly needed for high-risk individuals. Several studies have examined the relationship between glycosylated haemoglobin (GHb) and OGTT.[13,14] Results of these studies are difficult to compare because each used different criteria for diagnosis of diabetes based on OGTT results as well as different methods for measuring GHb. In general, studies have shown that GHb is correlated with various parameters of OGTT. With a highly precise high performance liquid chromatography (HPLC) method in population with high prevalence of diabetes (32.6% in this study), the author found that HbA1c was highly specific (90%) and moderately sensitive (68%) in identifying subjects with diabetes (as diagnosed by OGTT). The specificity is almost similar to the study by Little *et al.,* 1988 (91% specificity) where HbA1c was used as a screen for a diabetes in 381 adults from a population with a high prevalence of type 2 diabetes (Pima Indians). The study also gave a higher sensitivity of 85% as compared to 68% in this study.[15]

In other study by Simon *et al.*, OGTT and measurement of HbA1c were performed in 333 outpatients with high prevalence of diabetes mellitus (7.5%).[16] The sensitivity was 60% and specificity was 90.9% with HbA1c cut off level of 6.0%. This was comparable to results in this current study. However the predictive value for a positive diagnosis was only 34.9%. Hence they suggest using combination of HbA1c and FPG to diagnose diabetes mellitus that gave sensitivity of 40%, but high specificity of 99.4% and high positive predictive value of 83.3%.[16] It seems better for diagnosis to use test with a high degree of specificity and a high predictive value for a positive diagnosis than test with a good sensitivity but poor specificity.

The positive predictive value in this study was good, 76% and negative predictive value was 86%. This means that among those individuals with an HbA1c ≥6.4%, 76% would be diagnosed with diabetes based on OGTT measurement. This is higher than a study by Rohlfing et al, 2000 that gave a positive predictive value of 42% using HbA1c cut off level of 6.1%.[17] Therefore HbA1c is a highly specific and convenient alternative to OGTT for diagnosis of diabetes in high-risk group.

References
1. Mafauzy M, Mokhtar N, Wan Mohamad WB, and Musalmah M. Diabetes mellitus and associated cardiovascular risk factors in Northeast Malaysia. Asia Pac J Public Health 1999; 11:16-19.
2. Harris MI, Eastman RC. Early detection of undiagnosed non-insulin-dependent diabetes mellitus. JAMA 1996; 276:1261-1262.
3. Goldstein DE. Isn't it time to retire the oral glucose tolerance test for diabetes screening and diagnosis? Diabetes Care 1998; 21:1215–1216.
4. Modan M, Halkin H, Karasik A, Lusky A. Effectiveness of glycosylated hemoglobin, fasting plasma glucose and a single post load plasma glucose level in population screening for glucose intolerance. Am J Epidemiol 1984;119: 431-444.
5. Hanson RL, Nelson RG, McCance DR, Beart JA, Charles MA, Pettitt DJ, et al. Comparison of screening tests for non-insulin-dependent diabetes mellitus. Arch Intern Med 1993; 153: 2133-2140.
6. Perry RC, Shanker RR, Naomi Fineberg, Janet McGill, Baron AD. HbA1c Measurement Improves the Detection of Type 2 Diabetes in High-Risk Individuals With Nondiagnostic Levels of Fasting Plasma Glucose. The early Diabetes Intervention Program (EDIP). Diabetes Care 2001; 24: 465-471.
7. American Diabetes Association. Screening for type 2 diabetes. Diabetes Care 1998; 21 (Suppl.1):S20-S22.
8. Orchard TJ. From diagnosis and classification to complications and therapy. Diabetes Care 1994; 17:326-338.
9. Mc Cance DR, Hanson RL, Charles MA, Jacobsson LTH, Petitt DJ, Bennett PH, et al. Comparison of test for glycosylated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ 1994; 308: 1323- 1328.
10. Ko GTC, Chan JCN, Yeung VTF, Chow CC, Tsang LWW, Li JKY, et al. Combined use of a fasting plasma glucose concentration and HbA1c or fructosamine predicts the likelihood of having diabetes in high risk subjects. Diabetes Care 1998; 21:1221- 1225.
11. Barrett Connor E, Ferara A. Isolated post challenge hyperglycaemia and the risk of fatal cardiovascular disease in older women and men: The Rancho Bernardo Study. Diabetes Care 1998; 21:1236 -1239.

12. Manucci E, Bardini G, Ognibene A, Rotella CM. Screening for diabetes in obese patients using the new diagnostic criteria. Diabetes Care 1998; 21:468-469.
13. Albut EC, Nattrass M, Northam BE. Glucose tolerance test and glycosylated haemoglobin measurement for diagnosis of diabetes mellitus- an assessment of the criteria of the WHO Expert Committee on Diabetes Mellitus 1980. Ann Clin Biochem 1985; 22:67-73.
14. Dods RF. Bolmey C. Glycosylated haemoglobin assay and oral glucose tolerance test compared for detection of diabetes mellitus. Clin Chem 1979; 25:764-768.
15. Little RR, England JD, Wiedmeyer HM, McKenzie EM, Pettitt DJ, Knowler WC, et al. Relationship of glycosylated hemoglobin to oral glucose tolerance. Implications for diabetes screening. Diabetes 1988; 37:60-64.
16. Simon D, Coignet MC, Thibult N, Senan C, Eschwege E. Comparison of glycosylated hemoglobin and fasting plasma glucose with two-hour postload plasma glucose in the detection of diabetes mellitus. Am J Epidemiol 1985; 122: 589-593.
17. Rohlfing CL, Little RR, Wiedmeyer HM, England JD, Madsen R, Harris MI, et al. Use of GHb (HbA1c) in screening for undiagnosed diabetes in the U.S population. Diabetes Care 2000; 23:187-191.