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Original article

Use of HbA1c to diagnose type 2 diabetes mellitus among high risk Sri Lankan adults

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ABSTRACT

Aim: Even though, glycosylated hemoglobin (HbA1c) was found to be effective in predicting diabetes especially in Caucasians there is limited evidence of its diagnostic utility in high risk Sri Lankan adults. This study aimed to determine the optimal HbA1c cut-off points for detecting diabetes in a high risk population in Sri Lanka.

Materials and methods: This community based study consisted of 254 previously healthy adults with history of diabetes in one or more first-degree relatives. Fasting plasma glucose (FPG) , glucose tolerance test (GTT) and HbA1c were measured in all and GTT was used as a reference to diagnose diabetes. Receiver operating characteristic curve was created to find the optimum HbA1c cut-off value to predict diabetes.

Results: Prevalence of diabetes was 12.2% (n = 31) with FPG and 16.1% (n = 41) with GTT. Prevalence rose to 27.6% ($P < 0.01$) when HbA1c with cut-off of $\geq$6.5% was used as the diagnostic test. The ROC curves showed the HbA1c threshold of 6.3% provided the optimum balance between sensitivity (80.5%) and specificity (79%). In compared to GTT, FPG had only a modest sensitivity (65%) in diagnosing diabetes in this high risk population.

Conclusion: Our study showed that optimum HbA1C cut-off for detecting diabetes was 6.3% and it had better sensitivity, but lower specificity than FPG. This study further showed that the prevalence of diabetes would become double if HbA1c is used over FPG to screen this high risk population.

1. Introduction

Glycosylated hemoglobin (HbA1c) provides a reliable measure of chronic hyperglycemia, and correlates well with the risk of long-term complications of diabetes mellitus (T2DM) [1]. In recent past HbA1c has also been recommended for the purpose of diagnosis of abnormalities in glucose tolerance including pre-diabetes and diabetes. Both the American Diabetes Association (ADA) and the World Health Organization (WHO) have recommended HbA1c level of $\geq$6.5% and 5.7% as the diagnostic cut-off for diabetes and pre-diabetes respectively [1,2].

HbA1c as diagnostic test offer several advantages over fasting blood sugar. Firstly, HbA1c is less affected by day-to-day variation in plasma glucose and secondly it doesn't require fasting and dietary preparations [3]. It is also now formally endorsed in many countries as a diagnostic test for T2DM. However, debates still continue regarding its applicability for diagnosis of diabetes. Even though, HbA1c is found to be effective in predicting diabetes especially in Caucasians there is limited evidence of its diagnostic utility in South Asians.

Many factors including hemoglobin level can directly influence the levels of HbA1c. Asians are known to have comparatively lower hemoglobin levels than the Caucasians [4] and it can lead to change of the HbA1c levels. In addition, there are significant ethnic disparities in the correlation between HbA1c and ambient blood glucose levels [4]. This may be related to genetic differences in the concentration of hemoglobin, the rates of glycation, and the life span of red blood cells. Furthermore, variety of genetic or disease-related factors which are comparatively common among South Asians can affect HbA1c giving rise to false results [4]. Hemoglobinopathies which are also common among South Asians may also affect HbA1c measurements due to altered amino acids on binding

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sites of immunoassays for HbA1c [4]. Potentially these factors could influence the diagnostic performance of Hba1c among South Asians. Diabetes in South Asians is also different to Caucasians in many ways. South Asians tend to develop diabetes at relatively younger age and at relatively lower body mass index than the Caucasians [5]. They also tend have more complications particularly macrovascular complications [5]. In addition there are many people with undiagnosed diabetes or at-risk with impaired glucose tolerance in countries like Sri Lanka. Considering these differences in the HbA1c test itself and characteristics of Sri Lankan population, more evidence and data are needed to evaluate HbA1c cut-off points in diagnosing diabetes and pre-diabetes.

This study aimed to determine the optimal HbA1c cut-off points for detecting diabetes in high risk population in Sri Lanka and to study the sensitivity and specificity of cut-off levels of HbA1c recommended by the ADA against the gold standard oral glucose tolerance test (GTT).

2. Methods

This was a community based cross- sectional study carried out in sub-urban locality in Southern Sri Lanka. The study population consisted of males and females aged 20 years or more with no previous history of diabetes, but having history of diabetes among first degree relatives.

A two stage cluster sampling procedure was used to recruit individuals for this study. In the first stage, five divisional secretariats were selected purposely to represent different geographical areas. In the second stage, eligible individuals were selected randomly using voters list available in the divisional secretariats offices. Selected individuals were invited to attend the Diabetes Research Unit of Faculty of Medicine, Galle for interview and investigation. Participants were asked to fast for 8–10 h prior to the blood investigations. Data were obtained using predesigned questionnaires on cardiovascular risk factors, socioeconomic factors, and family history. All selected individuals who consented for the study were subjected to FBS, glucose tolerance test and HbA1c measurements. Collection of blood samples was carried out by qualified medical laboratory technicians using standard protocols. All laboratory tests were quality controlled and abnormal results were repeated and confirmed. Plasma glucose measurements were carried out with an automated analyser using the glucose oxidase method at Faculty of Medicine, University of Ruhuna. HbA1c assays were carried out in a reputed laboratory using standard high performance liquid chromatographic method (Boehringer Mannheim, Germany). T2DM was made according to the American Diabetes Association (ADA) criteria when any of the followings were met or exceeded: Fasting blood sugar ≥126 mg/dL, two hours value of OGTT ≥200 mg/dL and HbA1c value ≥6.5%. Impaired glucose state was defined as FBS 100–126 mg/dL, 2-h value of OGTT 140–199 mg/dL and HbA1c value 5.5–6.5%.

The minimum sample size required for the study was 196 individuals and was calculated based on the estimated prevalence of diabetes mellitus in Sri Lanka as 15% with an absolute precision of 5% in an infinite population. With a 1.3 design effect for cluster design, required sample size for the present study was estimated at 228 individuals. Considering 10% of dropouts 254 individuals were recruited for the study.

2.1. Statistical analysis

Prevalence of T2DM was calculated as percentages and 95% CI for percentage. Characteristics of study sample were expressed as percentages for categorical variables, and as mean and SD for continuous variables. Groups were compared using *t*-test, and chi square test. For the analysis, ADA's criteria for diagnosis of diabetes with 2-h glucose values of GTT was used as the "gold standard". ROC curve was performed to assess the discriminative capacity of HbA1c for detection of diabetes. Sensitivity and specificity were calculated. The Pearson correlation coefficient was used to check the association between HbA1c and GTT.

2.2. Ethics statement

Ethical clearance for the present study was obtained under the study on "Incidence of glycemic abnormalities in relation to total fiber and energy intake in Sri Lankan adults—A Prospective observational community based study", from the Ethical Review Board of the Faculty of Medicine, University of Ruhuna, Sri Lanka. Informed written consent was obtained from all individuals prior to data collection.

3. Results

The mean age of the study population was 50.3 (12.1) years, and 48.6% were females. The mean FBS, GTT (2 h glucose value) and HbA1c were 102 mg/dL, 145 mg/dL and 6.1% respectively (Table 1).

Of the total number of 254 subjects, 12.2% ($n = 31$) were diagnosed with diabetes based on FPG criterion of ≥126 mg/dL and 16.1% ($n = 41$) were diagnosed based on GTT (two h value of ≥200 mg/dL). Significantly higher number of subjects ($n = 70$, 27.6%) were diagnosed with diabetes based HbA1c criteria of ≥6.5% ($P < 0.01$) (Table 2). Nearly 10% of subjects (25/254) were detected to have diabetes based on all three tests. Twenty seven (11%) were diagnosed to have diabetes by both FPG and GTT. Out of 41 subjects diagnosed with diabetes based on GTT criterion, 8 were found to be non-diabetic based on both FPG and HbA1c criteria. Out of 70 subjects diagnosed with diabetes based on HbA1c, 34 (48%) were found to be non-diabetic based on both FPG and GTT. Overall less than half of the subjects diagnosed as T2DM by HbA1c fulfilled the criteria for diabetes based on FPG (35%) and GTT (41%). Interestingly, all patients detected to have diabetes based on FPG, were found to have diabetes either by HbA1c or GTT (Fig. 1).

The prevalence of pre-diabetes based on FPG and GTT was 27%, and 19% respectively. With the HbA1c, the prevalence of pre-diabetes rose 39% and it was significantly higher than the both GTT and FPG ($p < 0.01$) (Table 2).

Considering GTT as the gold standard, HbA1c at cut-off value of ≥6.5% had 78% sensitivity and 82% specificity. In comparison, FPG had a comparatively lower sensitivity (65%), but had a higher specificity (98%) (Table 3). When FPG and HbA1c were used in combination, the sensitivity improved to 82% and the specificity remained at 82%.

The diabetic group with Hba1c >6.5% were further categorized into two groups based on 2-h value of GTT (presence of T2DM or not) (Table 4). Results showed that both age and BMI were not different between subjects with or without T2DM. However there

Table 1
Descriptive statistics of study participants.

	Mean	SD
Age	50.5	(12.0)
Weight[a]	59.7	(12.6)
Height[b]	163.3	(82.4)
FBS	101.9	(29.0)
Hba1c	6.1	(1.1)
GTT	145.5	(80.1)
BMI[c]	23.7	(4.9)

[a] Kilograms.
[b] Entimeters.
[c] kg/(meters)^2.

Table 2
Prevalence of diabetes according to FPG, GTT and HbA1c.

Total = 254	Number of subjects with T2DM	%	Number of subjects with pre-diabetes	%
FPG	31	12.2	68	26.7
GTT	41	16.1	49	19.2
HbA1c	70	27.6	99	38.9

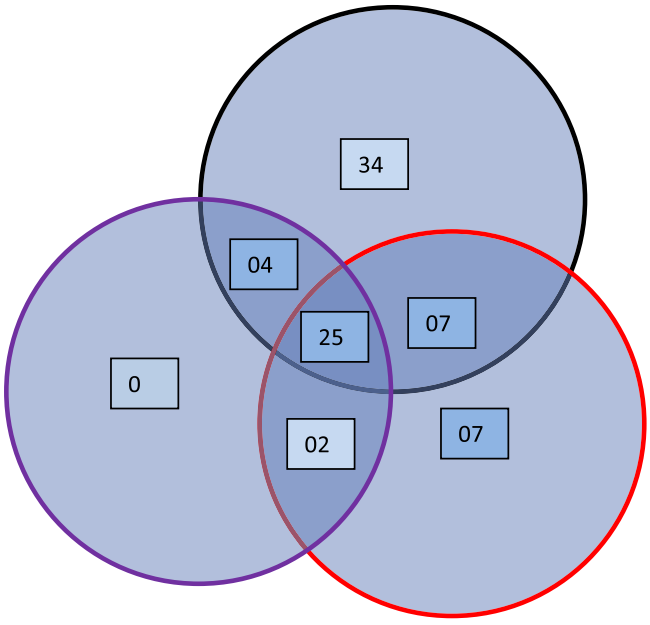

Fig. 1. FPG: fasting plasma glucose, GTT: glucose tolerance test, DM: diabetes mellitus. DM was diagnosed by GTT when 2-h value ≥ 11.1 mmol/l (red circle), by FPG when value ≥ 7.0 mmol/l (purple circle) and by HbA1c when value of ≥6.5% (black circle).

Table 3
Detection of T2DM by GTT, HbA1c and FPG.

		GTT		Total
		DM	No DM	
Hba1c	DM	32	38	70
	No DM	9	175	184
		DM	No DM	
FBS	DM	27	4	31
	No DM	14	209	223
Total		41	213	
FBS or HbA1c	DM	34	38	72
	No DM	7	175	182
Total		41	213	

Table 4
Age, BMI and FPG of individuals diagnosed to have diabetes mellitus based on HbA1c (total 70).

GTT	total	Age (sd)	p	BMI (sd)	p	FPG (sd)	p
DM	41	54.8 (11.5)	0.42	25.6 (7.7)	0.106	153 (45.4)	<0.001
No DM	29	51.6 (11.8)		23.2 (4.05)		98 (26.0)	

is significant difference of mean FBS values between the two groups even though all of them have T2DM based on Hba1c.

ROC curve was performed to assess the discriminative capacity of HbA1c for detection of T2DM. The area under the ROC curve for the ability of HbA1c to predict T2DM based on GTT was 0.911 (SE 0.02) (Fig. 2). The best cut off point of HbA1c value to predict T2DM occurred at 6.35 (sensitivity of 80.5%, specificity 79%). When the lower cut-off value of 6% was used, the sensitivity improved to 95%, but it was at the expense of reduced specificity(67%).

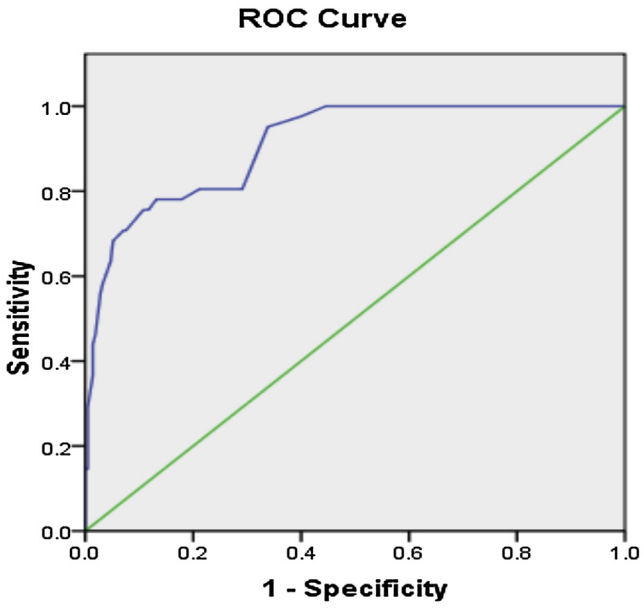

Fig. 2. ROC plot for T2DM detected by GTT (reference variable) and HbA1c (classification variable).

HbA1c had a positive and significant correlation with GTT ($r = 0.78$, $P < 0.001$) and FBS ($r = 0.70$, $P < 0.001$) (Fig. 3).

4. Discussion

Traditionally the diagnosis of diabetes mellitus was based on fasting plasma glucose (FPG) or, much less frequently GTT. However, there is a practical need to replace FPG and GTT with a simpler and reliable test that doesn't require any special preparation. As HbA1c fulfills these two criteria and hence drawn more attention as a potential diagnostic tool of diabetes mellitus. This study was conducted to evaluate the sensitivity and specificity of HbA1c in diagnosing diabetes among high risk individuals (having strong family history of diabetes) in Sri Lanka. The main findings of this study included

1. The prevalence of newly diagnosed diabetes and pre diabetes vary depending on the test used with significantly higher prevalence reported with HbA1c compared to FPG and GTT.
2. There is significantly higher number of undiagnosed diabetes among the study population with prevalence of undiagnosed diabetes as high as 12% with FPG and 26% with HbA1c.
3. As screening test HbA1c had better sensitivity than FPG. However, FPG had a very high specificity (97%).
4. When both HbA1c and FPG are performed as screening tools for diabetes, the sensitivity of detecting diabetes improved slightly,

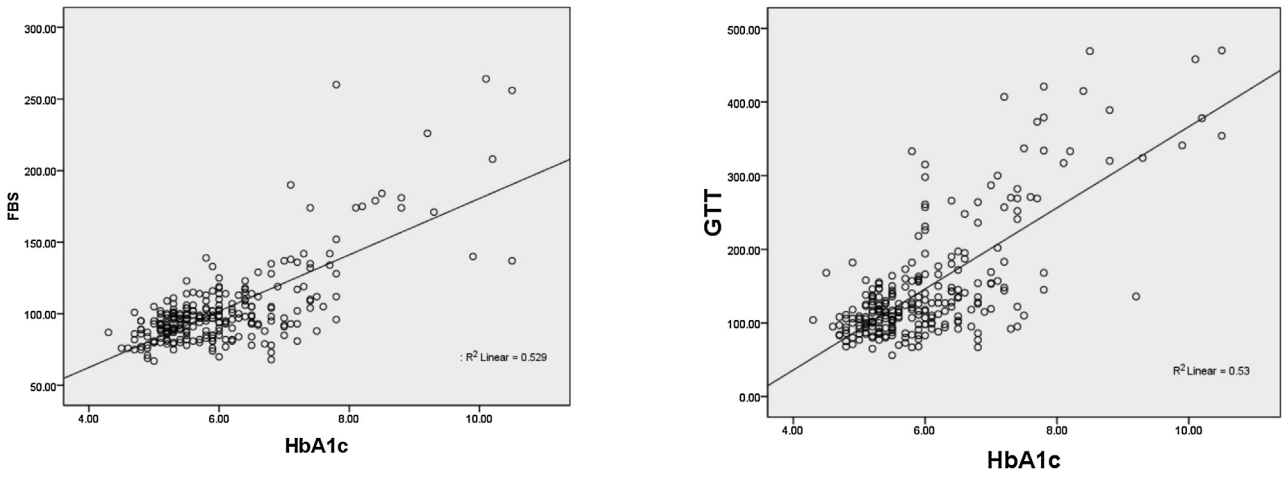

Fig. 3. Scatterplot diagram showing association of HbA1c with FPG and GTT.

however, the specificity remained same in compared to Hb1c alone.

5. The best cut off point of HbA1c value to predict DM occurred at 6.35% (sensitivity of 80.5%, the specificity 79%).

In our study, there was a marked disparity in the prevalence of diabetes between FPG, GTT and HbA1C. According to HbA1c the prevalence of newly diagnosed diabetes was 27.6% and it was more than double the prevalence based on FPG (12.2%). Many recent studies conducted in different settings reported similar findings of HbA1C showing a higher prevalence of diabetes and pre-diabetes than FPG [6–8]. The Danish Inter99 Study showed that the A1c cutoff of 6.5% increased the prevalence of diabetes by 60% compared with the use of GTT [8]. Another study conducted in South India showed diabetes prevalence of 6.1% (n = 134) with FPG, 10.1% with the GTT, and 12.8% with HbA1c [6]. Studies conducted in Africa and Europe also have shown similar disparity with higher prevalence of diabetes recorded with HbA1c [6,9–11] Reasons for the marked contrast of the prevalence between the tests are probably related to different sensitivity of the tests and the cutoff values used.

Many previous studies conducted in Asia particularly in Indian subcontinent suggest lower cut off for HbA1c than the ADA recommended cutoff of 6.5% [2,12,13]. Two large Indian studies revealed a HbA_{1c} cut-point of ≥6.0% and ≥6.1% to be optimally sensitive and specific for detecting diabetes in South and North Indian population respectively. However, the cutoff value of 6.3% found in our study was similar to the cutoff value detected in another study by Mohan et al. (6.4%) [12]. It is also revealed that many factors including racial and ethnic differences, body mass index (BMI), and age influence the diagnostic cutoff value of HbA1c [7]. HbA1c cut off point of 6.3% found in our study had sensitivity of 80.5% and specificity of 79%. One of the study conducted in Hong Kong involving a preselected high-risk population reported a lower sensitivity of 77.5% and a specificity of 78.8% for HbA1c cutoff of 6.1% [14]. Overall the sensitivity and specificity of HbA1c found in our study is similar to many studies conducted in other settings. Interestingly, studies involving in different ethnic groups within the same country also have revealed different HbA_{1c} cut-offs for diabetes[10]. The reason for such differences could be inter-individual variability of glycosylation of hemoglobin and the other known and unknown characteristics unique to these populations. Therefore, we suggest that further studies are required to evaluate these differences in HbA1c cutoff before recommending a common and universal HbA_{1c} cut-off point to diagnose diabetes.

Even though many studies suggest HbA1c cut-off of 6.5% is more sensitive than FPG, National Health and Nutrition Examination Survey (NHANES) data indicate the opposite. NHANES revealed HbA1c cut point of 6.5% (48 mmol/mol) is less sensitive than FPG as it identifies one third fewer cases of undiagnosed diabetes than FPG. Similar finding of lower prevalence of diabetes by HbA_{1c} was observed in few other major epidemiological studies [15,16]. The most studies which revealed HbA1c to be less sensitive than glucose based tests (FPG) are done on low risk population. In contrast many studies conducted in high risk population (Asian ethnicity [6,12], individuals with obesity [3], individuals with established coronary artery disease or peripheral vascular disease [17]) showed a better sensitivity of HbA1c compared to FPG. Therefore, selecting an appropriate screening test for diabetes may have to be based on the underlying risk of diabetes in given population.

It is also important to note that the FPG has only a modest sensitivity (65%) in diagnosing diabetes in this high risk population. Around 35% of individuals with diabetes would miss treatment if FPG is used as a screening or diagnostic test in these individuals. Therefore, the FPG which is widely accepted as a good diagnostic test [18] may not be appropriate as the sole screening tool for diabetes in a high risk population like this (first degree relatives of patients with diabetes). Some studies have reported better sensitivity and specificity when FPG and HbA1c are used in combination. A study conducted in Swedish population suggests that a combination of HbA_{1c} and FPG can detect a higher number of diabetes compared to each of these tests alone [19]. However, our study did not show any significant difference between HbA1c alone or in combination with FBS.

Other important observation in our study is the significantly higher incidence of newly diagnosed diabetes. With FPG the incidence of diabetes was 12.2%, and with GTT and HbA1c it was 16.1% and 27.6% respectively. As we excluded individuals with diabetes at the time of recruitment the true prevalence of diabetes in this high risk population (adults with family history of diabetes) should be alarmingly high. Therefore, we strongly recommend regular screening and preventive strategies for the individuals with family history of diabetes.

The present study had some limitations. The optimal HbA1c cut-off value of 6.3% given in this study is based on discriminative capacity of HbA1c to detect diabetes diagnosed by GTT. Ideally the cut-off value has to be based on discriminative ability of HbA1c to detect glycaemic point at which the complications related to hyperglycemia start to occur. Furthermore, due to the cross-

sectional nature of this study, we couldn't evaluate the ability of A1C to predict future micro and macrovascular complications related to diabetes.

In conclusion, our study showed that optimum HbA1C cut-off for detecting diabetes was 6.3% and It had better sensitivity, but lower specificity than FPG. This study further showed that the prevalence of diabetes would become double if HbA1c is used over FPG to screen this high risk population. There is also alarming high prevalence of undiagnosed diabetes among previously healthy adults with family history of diabetes.

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Author contributions

Conceived and designed the experiments: HMMH. Performed the experiments: HMMH, MUD, TPW. Analyzed the data: HMMH, NPW Contributed materials/analysis tools: HMMH, MUD. Wrote the paper: HMMH, NPW.

Disclosure

We would like to declare that we have no conflicts of interest in this work

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