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HbA_{1c} as a screening tool for detection of Type 2 diabetes: a systematic review

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Abstract

Aim To assess the validity of glycated haemoglobin A_{1c} (HbA_{1c}) as a screening tool for early detection of Type 2 diabetes.

Methods Systematic review of primary cross-sectional studies of the accuracy of HbA_{1c} for the detection of Type 2 diabetes using the oral glucose tolerance test as the reference standard and fasting plasma glucose as a comparison.

Results Nine studies met the inclusion criteria. At certain cut-off points, HbA_{1c} has slightly lower sensitivity than fasting plasma glucose (FPG) in detecting diabetes, but slightly higher specificity. For HbA_{1c} at a Diabetes Control and Complications Trial and UK Prospective Diabetes Study comparable cut-off point of ≥ 6.1%, the sensitivity ranged from 78 to 81% and specificity 79 to 84%. For FPG at a cut-off point of ≥ 6.1 mmol/l, the sensitivity ranged from 48 to 64% and specificity from 94 to 98%. Both HbA_{1c} and FPG have low sensitivity for the detection of impaired glucose tolerance (around 50%).

Conclusions HbA_{1c} and FPG are equally effective screening tools for the detection of Type 2 diabetes. The HbA_{1c} cut-off point of > 6.1% was the recommended optimum cut-off point for HbA_{1c} in most reviewed studies; however, there is an argument for population-specific cut-off points as optimum cut-offs vary by ethnic group, age, gender and population prevalence of diabetes. Previous studies have demonstrated that HbA_{1c} has less intra-individual variation and better predicts both micro- and macrovascular complications. Although the current cost of HbA_{1c} is higher than FPG, the additional benefits in predicting costly preventable clinical complications may make this a cost-effective choice.

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Keywords diabetes, diagnosis screening, HbA_{1c}, systematic review

Abbreviations ADA, American Diabetes Association; BMI, body mass index; CV, coefficient variation; DCCT, Diabetes Control and Complications Trial; DM, diabetes mellitus; FPG, fasting plasma glucose; FPLC: fast high-pressure liquid chromatography; Hb, haemoglobin; HbA_{1c}, glycated haemoglobin A_{1c}; IDF, International Diabetes Federation; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; ROC, receiver operating characteristic; UKPDS, UK Prospective Diabetes Study; WHO, World Health Organization

Introduction

The high prevalence of diabetes mellitus has emerged as a worldwide public health problem in the past 20 years. Type 2 diabetes is the most common form of diabetes, estimated to account for 85–90% of diabetes [1]. Impaired glucose tolerance (IGT) is a predictor for the subsequent development of Type 2 diabetes and is itself a risk factor for macrovascular disease. Type 2 diabetes is often asymptomatic in its early stages and can remain undetected for several years [2]. Increasing evidence shows that half of those with Type 2 diabetes are not aware that they have the condition. Early

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diagnosis of the condition is important as careful diabetes management can reduce long-term complications, such as blindness, kidney failure, cardiovascular disease and limb amputation [3–5].

There is no consensus on the most accurate screening test for detection of diabetes. The most widely used screening tests include the fasting plasma glucose (FPG) test and the oral glucose tolerance test (OGTT). Both these tests involve measurement of blood glucose. However, the measurement of both OGTT and FPG require patients to fast overnight for at least 8 h and confirmation of diagnosis using FPG requires the test to be repeated at least twice. Furthermore, studies have shown that the sensitivity of FPG for diabetes diagnosis is not as high as expected, with nearly one-third of individuals with diabetes remaining undetected [6]. OGTT is also costly, time-consuming and labour intensive and has low reproducibility that can add confusion and uncertainty to the confirmation of diabetes diagnoses [7]. The accuracy of FPG and OGTT may be reduced by patient non-adherence to fasting, laboratory error and/or use of certain medications [6].

The glycated haemoglobin (HbA_{1c}) test has been suggested as an alternative screening test for Type 2 diabetes. HbA_{1c} levels represent a 2–3-month average of blood glucose concentrations. The accuracy of HbA_{1c} analysis may be influenced by the presence of haemoglobinopathy or renal failure, as well as laboratory error and/or use of certain medications [6], but, compared with the OGTT, HbA_{1c} measurement is quicker and more convenient. HbA_{1c} can be measured at any time of the day regardless of the duration of fasting or the content of the previous meal. HbA_{1c} can also be analysed with a small blood sample using a portable device, although this is an expensive option currently [8]. There is also the potential for blood obtained from a finger prick to be sent to a central laboratory for analysis, allowing screening of individuals in remote areas [9,10].

HbA_{1c}, OGTT and FPG are equivalent as predictors of the development of retinopathy and nephropathy [11–14]. In recent years, the validity of HbA_{1c} as a screening tool for diabetes has also been examined, using OGTT as the gold standard and FPG as the comparison. These studies have been conducted in hospital and community settings in different countries, and in different ethnic groups. However, there is as yet no consensus on a suitable cut-off point for HbA_{1c} in the detection of diabetes. We conducted a systematic review of these primary cross-sectional studies to assess the validity of HbA_{1c} as a screening tool and determine the most appropriate cut-off point for the diagnosis of diabetes.

Research design and methods

Study design

A systematic literature search was conducted to identify published primary research with data on the accuracy of HbA_{1c} in the detection of Type 2 diabetes.

Search strategy

MEDLINE, PUBMED and EMBASE electron databases (1994–September 2004) were searched using the keywords 'diabetes mellitus', 'screening', 'diagnosis', 'HbA_{1c}', 'fasting plasma glucose test' and 'OGTT'. Search engines, such as 'Google' and 'Medscape', were also used to search for related articles. Reference lists of all publications including original studies, letters, commentaries, guidelines, and reviews were manually checked to identify studies not found through electronic searching.

Inclusion criteria

All peer-reviewed journal articles that related to the validation of HbA_{1c} as a tool for detection of diabetes and were published in English were examined. Papers were analysed further if FPG was used as a comparison, and the 75-g OGTT was used as the reference test. The reference test had to have been performed on at least 80% of the participants in whom $HbA_{1c}$was measured. The sensitivity and specificity of the HbA_{1c} and FPG test must either have been reported, or were possible to compute from the data provided. The diagnosis of diabetes must have been based on the diagnostic criteria according to American Diabetes Association (ADA; 1997) or World Health Organization (WHO; 1999) guidelines. All studies had to report Diabetes Control and Complications Trial (DCCT)-aligned HbA_{1c} results, or results that could be converted to DCCT-aligned results using published conversion regression models. If standardization information was not available from the published paper, authors or laboratories were contacted for confirmation of methods used.

Data extraction

Information on study quality, study characteristics and accuracy of HbA_{1c} results were extracted from each selected paper. Study participant characteristics including summary data on age, gender, diabetes risk factors and ethnicity were extracted. Prevalence of Type 2 diabetes in each study paper was extracted directly, or was calculated using the summary data reported in the article.

Information was also examined in relation to study design, verification methods and accuracy of the results, including the cut-off point used, sensitivity and specificity, likelihood ratios and the use of the receiver operating characteristic (ROC) curve analysis to identify the cut-offs. If the accuracy estimates were not provided, raw data were used to calculate these estimates.

Analysis

The methodological rigour and quality of studies were assessed by examining the sampling frame, recruitment methods and sample size, the test measurements of HbA_{1c} and FPG, and the method used for collecting blood samples. The adequacy of the test descriptions was assessed to determine if sufficient to allow potential replication, and whether there was 80% verification with the reference test. Finally, the accuracy of DCCT-aligned HbA_{1c} and FPG tests was compared against OGTT as the standard reference test, based on sensitivity (probability of detecting diabetes or IGT among those who have diabetes/IGT),

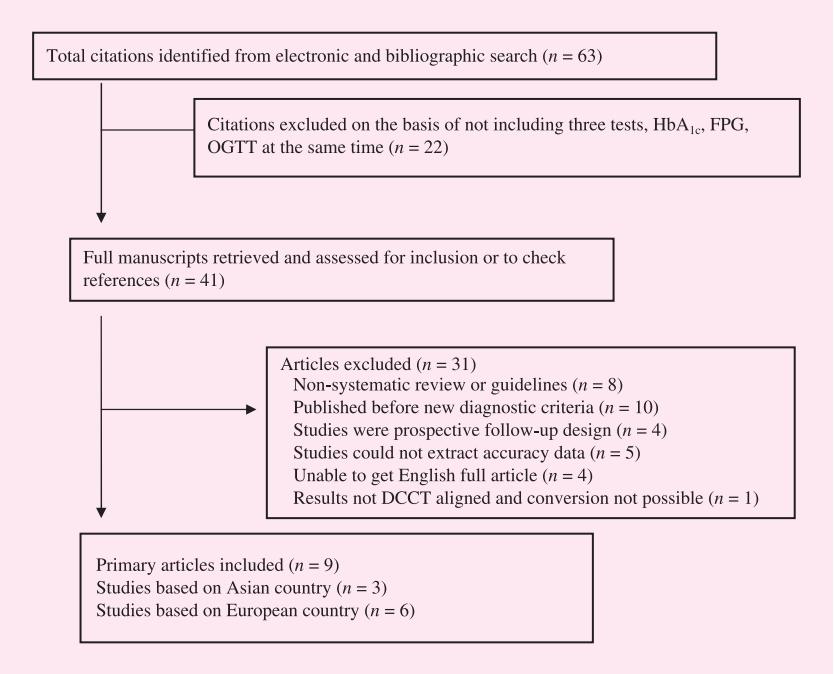

FIGURE 1 Studies selected for systematic review.

specificity (probability of a negative test among those without the condition), likelihood ratios [likelihood of having a certain test result (positive or negative) in those with the condition compared with the likelihood of the same test result in those without the condition], and ROC curve analysis. The advantage of using likelihood ratios is their stability in the face of changing disease prevalence, especially when considering the wide variation in the prevalence of diabetes observed between different ethnic groups. Likelihood ratios of more than 1 indicate increased probability of the target disorder [15].

Results

Search results

The initial search strategy identified 63 studies, nine of which were retained in the systematic review after applying the inclusion criteria (Fig. 1). Of the eligible studies, all were published between 1998 and 2004, and six were conducted in European countries and three in Asian countries (see Table 1). All nine studies differed in the population targeted, some were general population screening, others targeted those at increased risk of diabetes for screening. Recruitment methods also differed, with some community based [16–19], and others hospital or health institution based [20–24].

Methodological quality of studies and characteristics of participants

Methodological qualities and participants' characteristics for each study are described in Tables 1 and 2. Two community-based studies included random samples from the general population [16,17], whilst the others were based on sampling strategies that enriched the sample with participants with one or more risk factors for Type 2 diabetes [18,19]. All hospital-based studies included subjects at high risk for Type 2 diabetes.

All the studies used OGTT as the referenced standard test on all participants; however, there was variation between studies in the testing methods for HbA_{1c} and FPG. The majority used venous plasma, while two studies used capillary whole blood or capillary plasma samples [19,21]. In both studies, the OGTT cut-off values were adjusted for the use of capillary rather than venous blood.

For testing HbA_{1c}, most studies used DCCT-aligned methods. Saydan *et al.* [18], and Jesudason *et al.* [20] used the US national standard ion-exchange HPLC system (Bio-Rad Laboratories, Hercules, CA, USA). Jesudason *et al.* [20] also used a standard immunological method tested by a portable device DCA 2000 (Bayer Diagnostics, Mulgrave, Victoria, Australia). Tanaka *et al.* [22] used DCCT aligned-HPLC(723Ghb) by the Tosoh Corporation (Tokyo, Japan). Two other studies also used the HPLC method, confirmed to be DCCT standardized [16,24]. Tavintharan *et al.* used the DCCT-aligned DCA 2000 immunological method [2].

Mannucci *et al.* [17] and Wiener and Roberts [19] tested HbA_{1c} by the HPLC method using the HA8121 or HA-8140 machine [17,19] and their cut-off points were adjusted in this review using the Menarini Diagnostics (Florence, Italy) re-calibration formula [(measured value) = 1.06 (DCCT-aligned value)–1.51] [25]. Herdzik *et al.* [21] used the Pharmacia (Uppsala, Sweden) fast high-pressure liquid chromatography (FPLC) method, and this was converted to DCCT-aligned results using the Federation of Clinical Chemistry and Laboratory

Table 1 Studies examining the usefulness of HbA_{1c} as a screening tool for the early detection of Type 2 diabetes

First author, year and country	Settings	Recruitment methods	HbA_{1c} test method	Glucose method	Blood sample	Adequate test description	Verification with OGTT
Community-based studies							
Colagiuri 2004, Australia	Forty-two randomly selected urban and non-urban areas (census collector districts)	≥ 25 years of age, household survey followed by biomedical examination; 5604 had ≥ 1 risk factor in accordance with Australian screening guidelines	Boronate affinity HPLC, normal range: (3.9–6.2%) CV: not stated	Glucose oxidase	Venous plasma	Poor	100%
Mannucci 2003, Italy	A suburban community on the outskirts of Florence	30–70-year-old self-referred general population	HPLC (HA8121, Menarini Diagnostics; upper limit 5.5%) CV: not stated	Glucose oxidase (5.5–6.1)	Venous plasma	Adequate	100%
Saydan 2002, USA	National Center for Health (NHANES III participants)	Age 40–74, BMI ≥ 24 kg/m^2 with IGT	Ion-exchange HPLC (Bio-Rad Laboratories; CV 2%; normal range: 4.3–6.1%)	Hexokinase	Venous plasma	Poor	100%
Wiener 1998, UK	Two cities in the UK	GP-referred high risk of DM 13–92 years	Ion-exchange HPLC [Daiichi HA-8121(NMGH) or HA-8140(RLH), CV2%] Normal range: (3.8–5.5%)	Hexokinase	Capillary plasma	Adequate	100%
Hospital-based studies							
Jesudason 2003, Adelaide, Australia	Endocrine Test Unit at the Royal Adelaide Hospital	Volunteers with high risks of DM (obesity, family history of diabetes and gestational diabetes, symptoms of polyuria and polydipsia)	HPLC and DCA 2000 (4.3–6.1%) (4.2–6.3%)	Hexokinase	Venous plasma	Good	100%
Herdzik 2002, Poland	Outpatients clinic or the Department of Internal Medicine at Pomeranian Academy of Medicine	≥ 18 years of age, suspicion of having diabetes as a result of symptoms or having known risk factors for IGT	FPLC (Pharmacia FPLC System) CV (0.47–0.94%) Normal range: 3.8–5.2%	Oxidase	Capillary whole blood	Good	100%
Tanaka 2001, Japan	Saiseikai Central Hospital and Juntendo University Hospital in Tokyo	Suspected of having DM; excluded anaemia (Hb < 12 g/dl) and renal or hepatic dysfunction	HPLC (723Ghb III, Tosoh Corp.) Normal range: 4.3–5.8%	Not stated	Venous plasma	Poor	100%
Tavintharan 2000, Singapore	Volunteers attending the hospital (large proportions were nurses)	< 55 years of age, family history, hypertension, hyperlipidaemia, with BMI > 25 kg/m^2; excluded renal, liver, or cardiac disease and previous gastrectomy	DCA 2000 (CV 3.3%) Normal range: 4.2–6.5%	Technicon RA Systems analyser Interassay CV 2.0%	Venous plasma	Adequate	100%
Ko 1998, Hong Kong	Diabetes and Endocrine Center of Wales Hospital for diabetes screening	High risk for IGT (family history of diabetes, gestational diabetes, history of IGT and obesity)	Automated ion-exchange chromatographic method (Bio-Rad Laboratories; CV = 3.1%) normal range: 5.1–6.4%	Oxidase	Venous plasma	Adequate	100%

BMI, body mass index (kg/m^2); CV, coefficient of variation; DCA 2000: a portable device produced by Bayer (Bayer Diagnostics, Mulgrave, Victoria, Australia) for testing HbA_{1c} by immunological method; DM, diabetes mellitus; FPLC, fast high-pressure liquid chromatography, a kind of ion-exchange chromatography; IGT, impaired glucose tolerance; NGSP: National Glycohemoglobin Standardization Program, sponsored by the American Diabetes Association and others; NHANES III, the Third National Health and Nutrition Examination Survey.

Table 2 Community-based studies—participant characteristics, presence of baseline data, the prevalence of Type 2 diabetes and results of HbA_{1c} and FPG tests

Criterion	Colagiuri *et al.*		Mannucci *et al.**		Saydan *et al.*		Wiener and Roberts*		
Age (year)	> 25 years summary statistics not reported		52.2 ± 18.5 (30–70)		40–74		13–92		
No. and	10 447		1215		2844		401		
gender (M/F)	Not stated		567/648		Not stated		208/193		
Ethnicity	Australia national population		Italian		USA		UK		
Baseline data	No		Yes		No		No		
Prevalence of diabetes (%)	7.4		6.6		6.2 (ADA criteria)		44		
Results of tests									
HbA_{1c}									
Cut-off point (%)	≥ 5.3		> 6.6		≥ 5.5	≥ 6.0	> 6.9	> 7.4	> 7.6
Items	DM	IGT	DM	IGT	IGT		DM		
Sn (%)	78.7	42.0	M 98 F 100	M 59 F 55	60.0	16.7	64.0	50.6	41.0
Sp (%)	82.8	88.2	M 30 F 21	M 19 F 9	55.0	92.9	91.0	98.2	100
Likelihood ratio									
PLR	4.58	3.56	M 1.30 F 1.28	M 0.73 F 0.60	1.33	2.35	7.11	28.11	∞
NLR	0.26	0.66	M 0.31 F 0	M 2.12 F 4.86	0.73	0.90	0.40	0.50	0.59
FPG									
Cut-off point (mmol/l)	≥ 5.5	≥ 6.1	≥ 7.0	≥ 6.1	≥ 5.6	≥ 6.1	> 6.0	> 6.9	
Items	DM IGT		DM	IGT	IGT		DM		
Sn (%)	79.9 51.9	63.6 34.6	M 91 F 100	M 59 F 55	76.5	34.9	89.9	78.1	
Sp (%)	79.9	93.9	M 30.0 F 21.6	M 19.3 F 9.3	37.9	86.9	65.9	87.9	
Likelihood ratio									
PLR	3.98 3.9	10.4 ∞	1.30 1.28	0.73 0.60	1.23	2.66	2.64	6.45	
NLR	0.25 0.55	0.39 0.65	0.31 0	2.12 4.86					
ROC curve available	No		Yes (for IGT)		Yes		Yes		
Diagnose criteria	WHO (1999)		WHO (1999)		WHO (1999)		WHO 2-h OGTT		

ADA, American Diabetes Association; DM, diabetes mellitus; F, female; FPG, fasting plasma glucose; IFG, impaired glucose tolerance; M, male; NLR, negative likelihood ratio; OGTT, oral glucose tolerance test; PLR, positive likelihood ratio; ROC, receiver operating characteristic; Sn, sensitivity; Sp, specificity; WHO, World Health Organization.
*HbA_{1c} cut-off points converted to DCCT-aligned equivalent values, see Research design and methods.

Medicine (IFCC) Master Equations [27]. For testing FPG, two studies did not report the specific methods used [22,23], whilst the remaining studies used either hexokinase or glucose oxidase enzymatic methods for the FPG tests.

Results by participant characteristics

The age range of study participants varied across studies. Wiener and Roberts [19] included a sample with an age range from 13 to 92 years, while the other studies only included adults aged 18 years or older. The gender mix also varied across studies. In the studies by Tavintharan *et al.* [23] and Ko *et al.* [24], the majority of participants were female, 65 and 80%, respectively. Only three articles provided baseline data [17,21,24]. Participant risk profile and prevalence of Type 2 diabetes varied across studies, ranging from 6.2 to 44.0%. Apart from the exceptionally high diabetes prevalence (44%) reported for the UK enrolled participants [19], the prevalence in hospital-based studies (10–21%) was higher than in community based studies (6.2–7.4%).

Results by cut-off point

Sensitivity is the fraction of individuals at or above the HbA_{1c} cut-off point who have diabetes, whereas specificity is the fraction of individuals with an HbA_{1c} level below the cut-off

point who do not have diabetes. For calculations of sensitivity and specificity for IGT, individuals with undiagnosed diabetes were excluded. The ROC curve analysis was performed to assess the best predictive cut-off values for detecting new diabetes and IGT.

Most studies (seven of nine) used the ROC curve to identify the cut-off point for diagnosing Type 2 diabetes or IGT with FPG or HbA_{1c}. Different studies used different cut-off points and reported different sensitivities and specificities (Tables 2 and 3). Even when the same cut-off points were used, different results were reported by different investigators.

Three cut-off points 5.9, 6.1 and 6.2% of HbA_{1c} were advised as optimum cut-offs for detecting diabetes in at least two different studies. At a cut-off point of 5.9%, the sensitivity ranged from 76 to 95%, specificity 67 to 86% [22,23], and at a cut-off point of 6.1%, the sensitivity ranged from 78 to 81%, specificity 79 to 84% [23,24]. At a cut-off point of 6.2%, the sensitivity ranged from 43 to 81% and specificity 88 to 99% [20,21,23]. For FPG at a cut-off point of ≥ 6.1%, two studies showed the sensitivity ranged from 48 to 64% and specificity from 94 to 98% [19,23]. At a cut-off point of 5.6, the sensitivity ranged from 80 to 88%, and specificity from 79.2 to 85.8% [20,24].

At equivalent cut-off points, sensitivity was generally lower in detecting IGT for both HbA_{1c} and FPG tests when it came to their ability to detect IGT, and this was true in both community- and hospital-based studies. Some studies reported different results for the validity of HbA_{1c} and FPG. Wiener and Roberts reported higher specificity of HbA_{1c}, but higher sensitivity of FPG in diagnosing diabetes at different cut-off points [19]. Tavintharan *et al.* showed HbA_{1c} had higher sensitivity but lower specificity than FPG to diagnose diabetes at different cut-off points [23]. However, at certain cut-off points (7.0% HbA_{1c} and 6.1 mmol/l FPG), Tavintharan *et al.* reported the same sensitivity (48%) and specificity (98%) for HbA_{1c} and FPG [23].

Saydah *et al.* found that HbA_{1c} had a lower sensitivity, but higher specificity, for detecting diabetes among non-Hispanic White subjects than for all other ethnic groups [18]. Similar variation in test performance can be seen when comparing findings across studies based on people from Asian [22–24] and European backgrounds [19,20].

Jesudason *et al.* studied the use of HbA_{1c} and FPG to predict Type 2 diabetes and cardiovascular risk, and compared results for HbA_{1c} measured by HPLC and the portable DCA 2000 device [20]. They concluded that HbA_{1c} (HPLC) of < 4.7% and ≥ 6.2% predicted with certainty the absence or presence of Type 2 diabetes as defined by the OGTT. The corresponding cut-offs were < 5.0 and ≥ 6.8% for HbA_{1c} (DCA 2000). Cardiovascular risk increased at least 2.2 times at HbA_{1c} ≥ 6.2% (by HPLC), 1.8–2.2 times at HbA_{1c} 5.6–6.1%, 2 times at FPG ≥ 6.4 mmol/l, and 1.7–1.9 times at FPG 5.6–6.3 mmol/l. The DCA 2000 and HPLC methods for HbA_{1c} have shown a correlation with a constant bias of approximately 0.2%. Based on the ROC curve, DCA 2000 had higher values than HPLC. An HbA_{1c} cut-off point of 6.2% (DCA 2000) had similar accuracy as FPG ≥ 6.0 mmol/l for predicting Type 2 diabetes (sensitivity 72.7 and 74.1%; specificity 94.7 and 94.5%, respectively).

The DCA 2000 method was also used to test HbA_{1c} in a study conducted by Tavintharan *et al.* [23]. Both Jesudason *et al.* [20] and Tavintharan *et al.* [23] recommended HbA_{1c} with a cut-off level of 6.2% to be the best predictor for diabetes.

Saydah *et al.* [18] stratified by age and body mass index (BMI) for the sensitivity and specificity of fasting glucose and HbA_{1c} in identifying IGT among US participants between 40 and 74 years of age. They concluded that age and BMI influence the sensitivity of both tests in detecting IGT. Both tests had relatively higher sensitivity and specificity when used to screen people aged 60 to 74 years than for people between 40 and 59 years, but the differences were not substantial. Sensitivity also increased when BMI increased from ≥ 24 to ≥ 27, or to ≥ 30 kg/m^2.

Discussion

This systematic review did not find clear evidence to suggest that one test, HbA_{1c} or FPG, was superior to the other in screening for diabetes or IGT. On the whole, HbA_{1c} had slightly lower sensitivity but higher specificity than the FPG in detection of diabetes, but neither was effective in detecting IGT. This is not surprising as neither FPG or HbA_{1c} involve a glucose challenge.

It is rarely possible in screening tests to have both high sensitivity and specificity. In the case of diabetes, we have a relatively common disease and so efficiency of screening, and therefore the specificity of the test used, is arguably more important. For screening purposes, a test that produces a large number of false positives would pose major problems to the health department and may be costly in resource terms. However, to fully assess these tests, it is important to consider the relative abilities of these tests to not only detect both diabetes and IGT, but also to predict the long-term prognosis.

First, it is worth considering the choice of test that is used in this review as the 'gold standard' test for diagnosing diabetes. In individuals with Type 2 diabetes who have insulin resistance, the insulin secretory response can initially compensate for the insulin resistance. However, eventually, first-phase insulin secretion is lost, and second-phase secretion is impaired, causing postprandial hyperglycaemia, one of the earliest markers of disease progression. Abnormalities in hepatic, pancreatic and muscle metabolism all result from long-standing hyperglycaemia. By the time most patients experience symptoms significant enough to cause them to seek medical attention, Type 2 diabetes has often been present, unrecognized for years, hence the presence of complications at diagnosis in some subjects [28].

Therefore, from the view of the pathophysiology of Type 2 diabetes, postprandial hyperglycaemia (PPG) is one of the earliest markers of disease progression. FPG is influenced by

Table 3 Hospital-based studies—participant characteristics, presence of baseline data, the prevalence of Type 2 diabetes and results of HbA_{1c} and FPG tests

Criterion	Jesudason *et al.*						Herdzik *et al.**		Tanaka *et al.*		Tavintharan *et al.*					Ko *et al.*	
Age (year)	53.8 (19–88)						> 18 (20–82)		56		43.2 (37–50)					Mean 55	
No. and gender (M/F)	505 211/294						234 129/105		866 569/297		111 35%/65%					2877 565/2312 (19.6%/80.4%)	
Ethnicity	Australian						Polish		Japanese		Chinese Indian Malays Others	60%, 20%, 19%, 1%				Hong Kong Chinese	
Baseline data	No						Yes		No		No					Yes	
Prevalence of diabetes (%)	10.7						19		20.6		17					21	
Results of tests																	
HbA_{1c}																	
Cut-off point (%)	≥ 4.7	≥ 5.6	≥ 6.2	≥ 5.0	≥ 6.2	≥ 6.8	≥ 6.4	≥ 6.2	≥ 5.9	≥ 6.5	≥ 5.9	≥ 6.8	≥ 6.1	≥ 6.2	≥ 7.0	≥ 6.1	≥ 5.5
Items	DM	HPLC		DM	DCA 2000		DM	IGT	DM IGT		DM		DCA 2000			DM	DM IGT
Sn (%)	100	85.2	42.6	100	72.2	42.6	73.7	51.3	76 76	49 49	95	52	81	81	48	77.5	91.7 80.2
Sp (%)	10	80.5	99.1	11.1	94.7	99.6	93.2	95.8	86 77	98 98	67	97	84	88	98	78.8	43.6 48.8
Likelihood ratio																	
PLR	1.11	4.37	106.5	1.12	13.6	106.5	10.84	12.2	5.43 3.30	24.5 24.5	2.88	17.3	5.06	6.75	24	3.66	1.63 1.57
NLR	0	0.18	0.58	0	0.29	0.58	0.28	0.51	0.28 0.31	0.52 0.52	0.07	0.49	0.23	0.22	0.53	0.29	0.19 0.41
FPG																	
Cut-off point (mmol/l)	≥ 4.7	≥ 5.6	≥ 6.4				≥ 7.0	≥ 6.1	≥ 7.0		≥ 7.0		≥ 6.1	≥ 6.0	≥ 5.8	≥ 5.8	≥ 5.6
Items	DM						DM	IGT	DM		DM					DM	DM IGT
Sn (%)	100	9.6	59.3				77.7	62.6	52		19		48	52	52	85.1	88.4 64.4
Sp (%)	23.1	85.8	99.1				100	100	100		100		98	98	90	84.4	79.2 87.7
Likelihood ratio																	
PLR	1.3	5.61	65.89				∞	∞	∞		∞		24	26	5.2	5.46	4.25 5.24
NLR	0	0.24	0.41				2.23	3.74	4.8		8.1		0.53	0.49	0.53	0.18	0.15 0.41
ROC curve available	Yes						Yes		No		Yes					No	
Diagnose criteria	WHO 1999						WHO 1999		WHO 1999		WHO (2-h OGTT) and ADA (1997)					WHO 2-h OGTT	

*HbA_{1c} cut-off points converted to DCCT-aligned equivalent values, see Research design and methods.
ADA, American Diabetes Association; DM, diabetes mellitus; NLR, negative likelihood ratio; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; PLR, positive likelihood ratio; ROC, receiver operating characteristic; Sn, sensitivity; Sp, specificity; WHO, World Health Organization.

insulin secretion, carbohydrate absorption and hepatic and peripheral glucose metabolism. Elevated FPG levels result from unrestrained hepatic glucose production secondary to resistance to insulin action in the liver, and every diabetic individual has their own internal and external causal factors that trigger this disease. The difficulty is in preventing and treating long-term micro- or macrovascular complications which magnify the economic cost of diabetes.

HbA_{1c} is related to both elevated OGTT and FPG [10], and the various complications [29]. HbA_{1c} can therefore be used for assessing the risk of complications of diabetes as well as for monitoring glycaemic control. Both the DCCT (Type 1 diabetes) [30] and UK Prospective Diabetes Study (UKPDS; Type 2 diabetes) [31] studies have shown a reduction in the risk of complications in diabetic patients associated with a reduction in HbA_{1c}. In the DCCT, a sustained reduction in HbA_{1c} from 9.0 to 7.0% over 6.5 years led to a reduction in the risk of developing retinopathy of 76%, 39% risk reduction for microalbuminuria and 60% for neuropathy. The risk of developing any microvascular complication of diabetes was reduced by 25% in the UKPDS, where HbA_{1c} was lowered from 7.9 to 7.0% over 10 years through intensive diabetes management.

Some studies in this review reported similar sensitivities and specificities for both HbA_{1c} and FPG, whereas others demonstrated different sensitivities and specificities for the same cut-off points. There are many confounders that potentially influenced the results of the primary studies, making the comparison of HbA_{1c} and FPG among studies difficult and complex.

First, different ethnic groups were found to have different sensitivity and specificity for HbA_{1c} which may be related to genetic differences in the concentration of haemoglobin (Hb), the rates of glycation and the lifespan or amount of red blood cell. Anand *et al.* [15] stratified their findings according to the three ethnic groups included in their study population, East Asian, Chinese and European, and reported ethnic variation in the sensitivity and specificity for both HbA_{1c} and FPG tests. Similar ethnic differences in sensitivity and specificity at equivalent cut-off points were observed across the studies in this review.

Genetic variants (e.g. HbS trait, HbC trait) and chemically modified derivatives of haemoglobin (e.g. carbamylated Hb in patients with renal failure, acetylated Hb in patients taking large amounts of aspirin) can affect the accuracy of HbA_{1c} measurements. Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g. recovery from acute blood loss, haemolytic anaemia) will falsely lower HbA_{1c}. For example, iron-deficiency anaemia is reported to increase test results. In some patients with haemoglobinopathies such as D Punjab, sickle cell disease, very high fetal haemoglobin or abnormal red cell turnover, HbA_{1c} measurement may not be appropriate. Only two of the studies included in this review considered the possible influence of these factors, such as anaemia, renal or hepatic dysfunction, and excluded affected patients [22,23].

The lack information provided on the detection and exclusion of variant haemoglobins in the majority of studies is a limitation of this review, however, haemoglobinopathies are unlikely to be present in numbers that will impact on the overall findings, given the ethnicity of the source populations and the sample sizes (1.7% prevalence found in the UK screening programme [8]). Clinically silent variants have now also been raised as a potential influence on HbA_{1c} analysis [32]. However, this observation was based on four cases drawn from a Caucasian population of about 500 000, so the overall chances of inclusion of undetected variant haemoglobin cases in the studies under review remain extremely small. Therefore, variant haemoglobins, clinically important or silent, are unlikely to impact on the diagnostic test outcomes reported here. However, the potential presence of variant haemoglobin will need to be considered when these tests are applied at the individual level, especially for individuals and/or populations at high risk of variant haemoglobins.

Second, variation in the prevalence of risk factors may also have affected the results of the primary studies. Only two studies recruited from the general population through random or self-referred sampling [16,17], whilst the others enrolled participants with at least one major risk factor for Type 2 diabetes. The exceptionally high diabetes prevalence (44%) reported for the UK community-based sample [19] would likely have been related to referral bias, as this study included only those patients referred for OGTT. Only three studies [17,21,24] provided data on gender distribution, and one of these studies examined the estimates stratified by gender [17]. This study observed a higher sensitivity but a lower specificity among females than males in diagnosing diabetes for both FPG and HbA_{1c}. Saydah *et al.* [18] demonstrated higher sensitivity and specificity of fasting glucose and HbA_{1c} for the 60–74 age group compared with the 40–59 year-old group. Other studies with a wide age distribution did not provide age-specific sensitivity and specificity estimates.

It is known that variation in collection, storage and processing time of blood can influence the results of blood glucose assays. It is recommended, therefore, that blood for fasting plasma glucose analysis should be collected after the individual has fasted overnight and that plasma be separated from the cells within 60 min. If whole blood is used, the sample should be kept at 0–4 °C or centrifuged or assayed immediately. Glycolytic inhibitors such as sodium fluoride do not inhibit glycolysis initially and the glucose levels will be 0.5–1.0 mmol/l lower than if glucose was measured immediately [33]. Such methodological detail ideally should have been compared between the studies in this review, but this level of detail was not provided in any of the reviewed studies.

The studies in this review reported different optimum DCCT-aligned cut-off points for HbA_{1c} for the early detection of Type 2 diabetes. However, three studies observed the same cut-off point of ≥ 6.2% as optimum [19,20,23]. Ko *et al.* suggested HbA_{1c} ≥ 6.1% as the optimum cut-off point in their cross-sectional study [24]. Further follow-up of their cohort

with IGT over 18 months provided further confirmation of 6.1% as the optimum cut-off point for early detection of diabetes as further cases were detected [34]. These findings are consistent with previous follow-up studies that also identified a cut-off point of ≥ 6.1% for HbA_{1c} as the optimum cut-off point for detection of diabetes [35,36].

In 1994, McCane *et al*. examined the relationship between diabetes complications and concomitant results of OGTT, fasting glucose and HbA_{1c}, and reported that all three significantly predicted the development of retinopathy and nephropathy [13]. In 2000, Ito *et al*. showed a high correlation between all the three measures and again HbA_{1c} ≥ 6.1% was reported as the optimum cut-off point [12].

The consistency of results is not surprising given that FPG, OGTT and HbA_{1c} tests reflect different underlying aspects of the same pathology of hyperglycaemia. The FPG depends on baseline insulin secretion and hepatic glucose output, whilst the OGTT, especially the 2-h glucose value, is influenced by peripheral insulin resistance, and HbA_{1c} is a combination of glucose and protein. All these test outcomes are potentially useful for directly predicting increased morbidity and premature mortality.

Glucose can react with many different proteins and cause structural alterations and subsequently impair protein and tissue function. According to Jesudason *et al*. [20], HbA_{1c} has much lower intra-individual coefficient variation (CV) than OGTT and FPG, and HbA_{1c} may better reflect the risk of long-term micro- and macrovascular complications than OGTT and FPG. One study in the USA has shown one-quarter of those with previous IGT revert to normal glucose tolerance [37]. In another follow-up study, Little *et al*. [38] reported that 68% of those with elevated HbA_{1c} (> 6.1%) at baseline progressed to diabetes compared with 28% of those with normal HbA_{1c} at baseline, suggesting that, in subjects with IGT, HbA_{1c} may be useful in determining who will likely progress to diabetes [39].

Earlier studies also reported HbA_{1c} ≥ 7.0% as the diagnostic criterion to trigger pharmacological intervention for diabetes [40,41]. Data from Peters *et al*. showed that using an HbA_{1c} > 7.0% as a cut-off to diagnose diabetes mellitus (DM) had a 99% sensitivity, and a specificity of 99.6% [39]. The results of the DCCT showed that an HbA_{1c} of 7.0% was associated with an increased risk of microvascular complications, such as diabetic retinopathy and nephropathy. For macrovascular complications, a new 6-year large prospective study showed that HbA_{1c} (ranging from 5.0 to 6.9%) significantly predicted all-cause mortality, coronary and cardiovascular disease independent of age and other classic risk factors [41]. In order to minimize the risk of developing complications, International Diabetes Federation (IDF) 2005 guidelines advise that people with diabetes should maintain a DCCT-aligned HbA_{1c} < 6.5%.

The findings of this review combined with previous studies [12,35,36,38,42] suggest the optimum cut-off point of HbA_{1c} to be ≥ 6.1% or ≥ 6.2% for the diagnosis of diabetes. McCarter *et al*. [29] showed that HbA_{1c} has genetic inter-individual biological variation, but low intra-individual variation which will be useful in the early detection of diabetes and prediction of risk of long-term complications. HbA_{1c} therefore is not only a marker of hyperglycaemia via measuring the mean blood glucose (MBG), but also reflects the risk of both long-term macro- and microvascular diabetic complications. HbA_{1c} results arguably have the advantage of being more meaningful for clinical workers, and even for non-diabetic patients.

As in the studies included in this review, all HbA_{1c} results should be reported according to an international standardized reference. Studies using non-DCCT standardized methods should report DCCT-aligned results, or DCCT traceable results, and best practice may be to include conversion equations to facilitate cross-study comparisons. The future basis for international standardization will likely be the reference system developed by the IFCC Working Group on HbA_{1c} Standardization. The IFCC method is more specific and accurate than other methods as there is no interference from abnormal haemoglobins such as HbS and HbC, and no interference from acetylated or carbamylated Hb. Excellent inter- and intra-laboratory precision has been demonstrated [42].

An international working group was established in 2004 (ADA/EASD/IDF Working Group of the HbA_{1c} Assay) to facilitate standard global reporting. This group also recommend that the IFCC method be the gold standard reference method for calibration of all instruments and methods used in assaying HbA_{1c} [33]. This group continues to advocate for consistency in existing methods and reporting. Other new approaches are also being considered based on mean blood glucose equivalents [8]. These methods need to be linked to existing methods including HbA_{1c}, and their diagnostic value determined in relation to diabetes, as well as the influence of other cofactors, such as age, sex and ethnic group.

As stated previously, the sample sizes in the reviewed studies make it unlikely that they will have included participants with variant haemoglobins in numbers that would be likely to influence the review findings. However, HbA_{1c} results are likely to be influenced by the presence of variant and abnormal haemoglobins [8], so the conclusions in relation to cut-off levels from this review may or may not be relevant to this subgroup. Therefore, the diagnoses of diabetes should not be based on HbA_{1c} alone for those identified as being at increased risk of haemoglobinopathy, including mildly anaemic individuals or those from an ethnic background.

Conclusions

The following conclusions can be drawn from this systematic review. First, HbA_{1c} and FPG are both equally effective as screening tools in early detection of Type 2 diabetes, but neither of the tests is effective in detecting IGT. An OGTT is therefore still required to diagnose IGT. However, previous studies have demonstrated a low intra-individual variation for HbA_{1c} and that this test is a good predictor for both the micro- and

macrovascular complications of diabetes. Currently, the cost of HbA_{1c} is higher than FPG, but the additional benefits of the HbA_{1c} test in predicting costly clinical complications may make this a cost-effective choice.

Second, this review reinforces the need for the standardization of HbA_{1c} measurements worldwide to allow meaningful comparison of results across laboratories. The current advisable method is ion-exchange HPLC or immunological assay based on the DCCT/UKPDS where the normal range is approximately 4.0–6.1%. The cut-off for HbA_{1c} is recommended as either ≥ 6.1% (≥ 2 SD above the normal mean) or ≥ 6.2%, as found to be the optimum cut-off point in most studies. In future, it is likely that the IFCC reference method will become the international standard [33], but population-specific cut-off points should also be considered, as the optimum cut-offs have been shown to vary with ethnicity, age, gender and the population prevalence of diabetes.

Finally, further investigation of the validity of all three tests in predicting diabetes-related mortality and morbidity, especially in relation to macrovascular disease, is urgently needed to draw firm conclusions on the best screening tool for Type 2 diabetes.

Competing Interests

None to declare.

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