Glycated haemoglobin A1c compared to fasting plasma glucose and oral glucose tolerance testing for diagnosing type 2 diabetes and pre-diabetes: a meta-analysis

by

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Declaration

I, Mrs. Jing Shao, hereby declare that the dissertation which I hereby submit for the degree Master of Science in Clinical Epidemiology at the School of Health Systems and Public Health, in the Faculty of Health Sciences, of the University of Pretoria is my own work and has not previously been submitted by me for a degree at another university.

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Prof. Paul Rheeder Date

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Summary

Title: Glycated haemoglobin A1c compared to fasting plasma glucose and oral glucose tolerance testing for diagnosing type 2 diabetes and pre-diabetes:

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Degree: MSc (Clinical Epidemiology)

Background

In 2010, glycated haemoglobin A1c (HbA1c) was officially recommended as a screening tool to diagnose type 2 diabetes mellitus (T2DM) and pre-diabetes, with cut-off points 6.5% and 5.7% to 6.4% respectively. The implications of using the HbA1c criterion, compared to the general diagnostic criteria: fasting glucose test (FPG) and oral glucose tolerance test (OGTT), is however still being debated.

Objectives

The objectives of this study were to evaluate and compare the pooled prevalence of type 2 diabetes mellitus (T2DM) and pre-diabetes, as measured by the Haemoglobin A1c (HbA1c) test, or the fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT). Secondly, to determine and compare the diagnostic test characteristics (sensitivity, specificity) of these tests.

Methods

Published papers, with a cross sectional study design, were selected for a systematic review and meta-analysis. The search strategy was an electronic review of journal articles listed on MEDLINE, PubMed and Google scholar between 1996 and 2012. Reference lists were checked, journals were hand searched and experts were contacted when necessary. Initially all studies related to the validation of HbA1c as a tool to detect pre-diabetes or T2DM in humans, published in English, were examined.

Studies were excluded if they did not meet the above mentioned criteria, and/or were conducted with pregnant women. Further analysis was done if FPG or OGTT was compared to HbA1c. The diagnosis of diabetes had to have been based on ADA or WHO criteria. These criteria are: HbA1c 5.7%-6.4% for pre-diabetes and >=6.5% for T2DM; FPG 5.6mmol-7mmol/l for pre-diabetes and >=7mmol/l for T2DM; OGTT 7.8mmol-11.1mmol/l for pre-diabetes and >=11.1mmol/l for T2DM). The OGTT and FPG tests were used as the reference tests and the prevalence reflected as a positive or negative proportion.

The sensitivity and specificity of HbA1c >=6.5% among cases defined by OGTT or FPG should have been reported, or it was possible to calculate these from the data provided. Study results relating to diagnostic accuracy were extracted and synthesized using multivariate random effects meta-analysis methods. This study focused on patients who were suspected of having T2DM, from two sub-groups (a community-based group and a high-risk group) to compare the detection rate of HbA1c with FPG and OGTT.

Sensitivity analysis was also conducted if heterogeneity of pooled studies was observed. The reasons for heterogeneity were explored and the results analysed (using random effects models), with and without the heterogeneous studies.

Results

The prevalence of OGTT versus HbA1c was compared in a total of 10 studies, with 26172 participants in community based populations. It was found that the prevalence of positive cases was highest when the OGTT was used. In comparison with OGTT, there were 5.2% less cases, and the odds of a diagnosis of T2DM was 0.5 times less likely. The differences in prevalence and odds ratio were statistically significant as the p value was less than 0.05. Using the “Numbers Need to Screen” (NNS) and “Absolute Difference in Prevalence” (ADP) calculation, there was 1 more diabetic case for every 19 people screened. This 5.2% of difference in prevalence is therefore also clinically significant.

The prevalence of T2DM when HbA1c and FPG tests were used, was compared in a total of 11 studies, with 51338 participants from the community based populations. Using HbA1c, there were 2.5% less positive cases and the odds of diagnosis of T2DM was 0.77 times less likely than with FPG testing. The differences of prevalence and odds ratio were not statistically significant as the p value was 0.05.

In the high risk populations, a total of 7 studies, with 16033 participants, were included, in comparing the prevalence of T2DM using HbA1c and OGTT. Using OGTT there were 3.3% more cases than when HbA1c was used. However, the odds ratio for diagnosis using HbA1c rather than OGTT was found to be 0.79, which is not statistically significant as the p value was 0.38. So there was no difference between the prevalence estimated by both test methods.

A total of 3 studies, with 3395 participants from the high risk populations, were included when the prevalence of positive cases, using HbA1c and FPG, were compared. Using HbA1c, there were 4.8% more positive cases, and the odds of diagnosing T2DM was 1.43 times more likely than that of FPG. It was found that the differences in prevalence and odds ratio were statistically significant as the p value was 0.01.

In addition to pre-diabetes, a total of 4 studies, with 8659 participants, included testing for pre-diabetes using HbA1c and OGTT in community based populations. Using HbA1c as a diagnostic method, there were 22.4% less positive cases, and the diagnosis of pre-diabetes was 0.18 times less likely than with OGTT testing.

An assessment of HbA1c diagnostic accuracy test was done in community-based and high-risk populations, with OGTT as the reference test. According to the results, using HbA1c only could detect approximately 69% of positive cases in community based populations and 56% of T2DM in high risk populations. The diagnostic odds ratio was 31.12 in the community-based subgroup. This means that for the T2DM patients, the odds for diagnosis using HbA1c>=6.5%, among subjects with T2DM, is 31 times higher than HbA1c>=6.5%, among subjects without T2DM. In the high-risk subgroup, the diagnostic odds ratio was 10.23.

Analyses showed that results varied across studies and should preferably not be pooled, due to heterogeneity. Heterogeneity could be due to variables like age, BMI, ethnicity and cardiovascular profile. Therefore a sensitivity analysis was applied (dropping heterogenous studies) if heterogeneity was found in the analysis of prevalence, odds ratio or HSROC curve.

Conclusion

It was concluded that the OGTT test would still be the preferred screening test for both T2DM and pre-diabetes in community-based populations, as it was found to be more sensitive. Both the OGTT and HbA1c tests have the same ability to detect T2DM in high risk populations. The FPG test would not be the preferred choice for screening T2DM in either community-based or high-risk populations, compared to the HbA1c test. However, when sensitivity analysis was applied to the subgroup of HbA1c and FPG in community based populations, it was found that FPG became more sensitive than HbA1c, when the high odds ratio studies were removed from the original data. The heterogeneity in the analysis could possibly be explained by age, BMI, ethnicity and the cardiovascular profile.

Keywords Type 2 diabetes, HbA1c, OGTT, FPG, diagnosis screening, systematic review, meta-analysis, prevalence, sensitivity, specificity

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Chapter 1

Introduction

Diabetes has become a global epidemic. After cardiovascular and cerebrovascular diseases, infectious disease and cancer, diabetes has become the fourth highest cause of morbidity and mortality.[1] The most common form of diabetes is type 2 diabetes mellitus (T2DM) which contributes to 80% of all diabetes in most countries[1] with prevalence increasing rapidly in both developed and developing countries. At present, diabetes is not curable, so early detection, diagnosis, and treatment of diabetes are important elements in preventing diabetic complications and improving short- and long-term outcomes in patients.[2,3]

Over the past three decades, fasting plasma glucose (FPG) and oral glucose tolerance testing (OGTT), have mainly been used for diagnosing and detecting diabetes. Recently, Haemoglobin A1c (HbA1c) was proposed by both the American Diabetes Association (ADA) (2012)[4] and an International Expert Committee (IEC) (2009)[5] as an optional assay for diagnosing and detecting T2DM (with a cut-off point of 6.5%) and pre-diabetes (with cut-off point from 5.7% to 6.4%). However, the implications of using the HbA1c criterion and the cut-off point of 6.5% are still disputed.[2,3]

1. 1 Defining the research problem

Although both ADA and IEC recommend HbA1c >=6.5% as the optimal test for the diagnosis of T2DM, the threshold for HbA1c when diagnosing T2DM is still in

dispute, as WHO reported that subjects with HbA1c <6.5%, could still be diagnosed using glucose-based criteria.[6] In addition, there is some debate about which of these tests should be used to diagnose pre-diabetes, as the IEC have suggested a range of HbA1c between 6.0% and 6.4%[5] and ADA have recommended a range between 5.7% and 6.4%.[4]

This systematic review study has been conducted on published papers about the accuracy of diagnostic tests for T2DM. Diagnostic accuracy studies aim to determine the quality assessment of the index test for detecting the target results. A series of patients are tested using the specified test (or tests), known as the "index test(s)" as well as a reference test. The results of the index test(s), which include sensitivity, specificity, positive and negative predict values, positive and negative likelihood ratios, diagnostic odds ratios and receiver operating characteristic curves (ROC), are then compared to the results of the reference test. The reference standard should be the best available method to determine whether or not the patient has the target results.[7]

A systematic and comprehensive search of the literature was used to review eligible primary studies. First of all, a clear and explicit description of the diagnostic test and its accuracy estimates, the target condition and study design should be included in the electronic search strategy.[8] Secondly, references to the primary studies, narrative studies and systematic review, which could be missed by electronic searches, have to be manually reviewed.[9] Publication bias may be more prevalent in diagnostic accuracy studies, as they are often based on routinely collected data.[8]

1.2 Literature overview and motivation for study

1.2.1 Definition of diabetes

The American Diabetes Association (ADA) defines diabetes as ‘‘a group of metabolic diseases characterized by hyperglycaemia, resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels”.[10] Pre-diabetes is defined as a lesser form of glucose tolerance, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT).[11,12]

1.2.2 Epidemiology of diabetes

Without effective prevention and control programmes, diabetes and pre-diabetes are likely to continuously increase globally.[11,12] It is estimated that the prevalence of diabetes (both type 1 and type 2 diabetes) among all age groups worldwide was 2.8% in 2000 and will be 4.4% by 2030.[13] The most common form of diabetes is type 2 diabetes (T2DM). It accounts for over 85% to 90% of cases of diabetes in most countries and has been recognized as a global epidemic, with its prevalence increasing at a rapid rate in both developed and developing countries.[14,15] This form of diabetes is associated with older age, obesity, a family history of diabetes, a previous history of gestational diabetes, physical inactivity, and ethnicity,[14,15] and can remain undetected for up to seven years.[2]

Approximately 10 million people in Africa have diabetes, which is becoming the fourth leading cause of death in developing countries.[16] The population that suffers from IGT exceeds 24% in many African countries.[17]

1.2.3 Diagnosis of diabetes

Up to the present we do not have any cure for diabetes. Accordingly, early detection, diagnosis, and self-glucose monitoring are considered to be the most important elements in preventing diabetic complications and improving short- and long-term outcomes in patients.[2] Current diagnostic methods of diabetes focus on blood glucose concentration, including FPG and OGTT.[18]

1.2.3.1 Current diagnostic methods for diabetes

Fasting plasma glucose (FPG)

Because of its convenience and lower cost, FPG is preferred as a diagnostic test for diabetes and pre-diabetes. It is used to measure plasma glucose in a person who has not eaten anything for at least eight-hours.[19,20] Test criteria for FPG are shown below in Table 1.

Table 1 Fasting plasma glucose test criteria[19,20]

Plasma Glucose Result	Diagnosis
Less than 5.5mmol/L(WHO criteria)	Normal
Between 5.6mmol/L and 7mmol/L(ADA criteria) Between 6.1mmol/L and 7 mmol/L(WHO criteria)	Pre-diabetes(Impaired Fasting Glucose, person has high risk of developing type2 diabetes but does not have it yet)
>= 7mmol/L(WHO criteria)	Diabetes (needs repeat test unless unequivocal symptoms)

The FPG test requires patients to fast overnight for at least eight hours and needs to be repeated once to confirm the status of patients.[1] Unfortunately, in high risk populations, such as overweight and obese patients, using the current FPG cut-off point criteria, nearly 70% of diabetic patients remain undetected compared to

OGTT in Cosson E et al study.[21] This study was a cross-sectional study which was conducted in overweight and obese patients in northern suburb of Paris.

Oral glucose tolerance test (OGTT)

Compared with FPG, OGTT is more sensitive in diagnosing pre-diabetes, but it is less convenient to administer. As in the FPG, the OGTT test also requires at least an eight-hour fast. The plasma glucose level is measured immediately before and two hours after a person drinks a liquid containing 75 grams of glucose dissolved in water.[19,20] The Test criteria for the OGTT are shown in Table 2.

Table 2 Test criteria for the oral glucose tolerance test[19,20]

2-hours Plasma Glucose Result	Diagnosis
<= 7.7mmol/L	Normal
Between 7.8mmol/L and 11.1mmol/L (WHO criteria)	Pre-diabetes (Impaired Glucose Tolerance, person has high risk of developing type2 diabetes, but does not have it yet)
>= 11.1mmol/L (WHO criteria)	Diabetes (need to repeat test again on a future day)

The OGTT shows a higher sensitivity for diagnosing diabetes, compared with FPG. However, it is more costly, time-consuming, labor intensive and has low reproducibility. This results in uncertainty about the diagnosis, which is often inconclusive.[22]

1.2.3.2 New criterion for diagnosis of diabetes

The HbA1c reflects the average blood glucose concentration over a period of two to three months.[19] It was first mentioned to be potentially useful for diabetes care, in the 1985 WHO report.[23] This test has been suggested as a screening test for T2DM, and pre-diabetes, at different cutoff points.[1]

Background of HbA1c as diagnostic criteria of diabetes and pre-diabetes

In order to demonstrate the benefits of intensive glycemic control on long-term microvascular and neurologic complications of type 1 diabetes mellitus, the Diabetes Control and Complications Trial (DCCT) and its follow-up study, Epidemiology of Diabetes Intervention and Complications (EDIC), conducted over 20 years (from 1983 up to 2005) used consistent measurement with the HbA1c assay (a precise high-performance liquid chromatography (HPLC) method).[24]

After the DCCT trial, the International Federation of Clinical Chemistry (IFCC) established a Working Group (WG) to achieve uniform international standardisation of HbA1c in 1995. This WG concluded its findings in 2010.[25] The IFCC-WG developed two methods, which were approved by the IFCC in July 2001. These two methods were mass spectrometry (MS) and capillary electrophoresis (CE).

After cleaving haemoglobin into peptides with a proteolytic enzyme, the specific glycated and non-glycated N-terminal peptides of the beta-chain were measured by the HPLC, using either MS or CE.[26] This standardisation aimed to make the accuracy and precision of the HbA1c assay at least as good as those of previous glucose assays using prescribed measurement methods.

In 2005, the WHO, with the International Diabetes Federation (IDF), assembled an expert meeting, to review and evaluate the recommendation that theHbA1c test be used for the diagnosis of diabetes mellitus.[27] In 2010, HbA1c was officially recommended as a screening tool, and 5.7% to 6.4% and 6.5% were proposed as the cut-off points for diagnosis of pre-diabetes and T2DM.[6] The HbA1c, as a screening tool to diagnose diabetes, can be performed at any time during the day and does not need patients to fast. It reflects long-term glucose levels and is well

standardised and reliable in some countries.[6] It is affordable and can be used in low-income countries.[21]

However, there were some negative aspects. This test was found to be affected by a variety of genetic, haematologic and illness-related factors, such as vitamin B12 deficiency, chronic renal failure and increased erythrocyte lifespan, which increased HbA1c in plasma, causing an over-estimation of the diagnosis of diabetes. Chronic liver disease, decreased erythrocyte lifespan, and hypertriglyceridaemia, also decreased HbA1c in the blood and under-estimated the diagnosis of diabetes.[28] In addition, HbA1c was unavailable in many countries, even though it had been internationally standardised. There was also some indication that the precision of laboratory measurement should be improved to match international standards.[28]

Another publication, analysed the advantages and disadvantages of using HbA1c as screening test for T2DM in South Africans.[29] It was found that there were several limitations . First of all, the precision of HbA1c measuring methods, standardisation and quality in the laboratory was questioned. Secondly, it was found that ethnicity, age, renal failure, HIV infection, and other blood factors could affect the precision of HbA1c measurement. Last but not least, the cost of the HbA1c test indicated that it was not the best choice when screening patients for T2DM.

1.2.4 The diagnostic odds ratio (DOR)

For single well-designed studies, results can be influenced by study design, participant selection, disease spectrum and analytical methods. In contrast, diagnostic meta-analysis is seen to be the most acceptable method for combining

data from multiple studies, to compare a diagnostic test with a reference test method.[30]

Diagnostic test accuracy, measured as sensitivity or specificity, compares how well each test distinguishes participants with T2DM from those without. However, using only sensitivity or specificity does not represent the test's discriminatory performance, as high sensitivity may accompany low specificity. Thus simple pooling of sensitivity or specificity may be inappropriate. This approach also ignores threshold differences.[31]

Odds ratios are related to prevalence surveys, as well as sensitivity and specificity in classic epidemiological studies. Normally, odds ratio describes the strength of the association between two binary data values.[32] The diagnostic odds ratio (DOR), which is defined as the ratio of the odds of the test being positive if the subject has a disease, relative to the odds of the test being positive if the subject does not have the disease[31] was considered a better option for this study. It was used in the meta-analysis to provide a statistic, to compare different tests, as it clarifies the ability of each test to detect the status of positive cases in subjects who had T2DM and those who did not.

The value of a DOR ranges from 0 to infinity: higher values indicate a better discriminatory test performance. If the DOR equals one, the test does not have the power to distinguish participants with T2DM or pre-diabetes from those without these disorders. If the DOR is lower than one, it means that the test is inappropriate compared to the reference test.[33]

As mentioned previously, for this study, the reference test was OGTT, which was compared with HbA1c, to detect T2DM in community based and high risk populations.

1.3 Motivation

Since HbA1c was approved by ADA and WHO, this test has not only been widely used as indicator of glycemic control, but was also accepted as an important method for diagnosis of diabetes, because of its high sensitivity for early diagnosis.[34,35]

However, some publications still question the efficacy of this test, in comparison with glucose concentration tests.[36,37] These publications have assumed that glucose-based criteria, in particular the OGTT, represent the gold standard for diagnosing diabetes.

1.4 Aim and objectives

1.4.1 Aim

The aim of this study was to use a systematic review of existing literature and meta-analysis to compare HbA1c, FPG and OGTT, for diagnosing T2DM and pre-diabetes in community based and high risk populations. The OGTT and FPG were used as a reference tests to compare the prevalence and Odds Ratio (OR) of positive cases of T2DM and pre-diabetes, with those diagnosed using HbA1c. The sensitivity and specificity of HbA1c and OGTT were also compared.

1.4.2 Objectives

1) To evaluate the prevalence and prevalence differences of T2DM and pre-diabetes as measured by the HbA1c, compared with FPG and OGTT from cross-sectional studies; and

2) To determine and compare the diagnostic test characteristics of the above for T2DM and pre-diabetes from cross-sectional studies.

Chapter 2

Methods

2.1 Research design and methods

2.1.1 Study design

A systematic literature search was conducted to identify published primary research on the diagnosis of pre-diabetes or T2DM with data on the HbA1c, FPG and OGTT.

2.1.2 Search strategy

MEDLINE, PubMed and Cochrane electronic databases (from 1996 to August Week 3 2012) were searched using the keywords “diabetes mellitus”, “fasting glucose”, ”glucose tolerance test”, and “diagnosis”. Google scholar, as a search engine, was also used to search for related studies. The reference lists of all articles were manually reviewed, and the full texts of potentially relevant articles were also retrieved.

2.1.3 Inclusion criteria

All the primary studies that were related to the validation of the HbA1c as a tool to detect pre-diabetes or T2DM on human beings and were published in English, were examined, and the HbA1c measurement method had to be the same as DCCT trial methodology in all the studies. The approach is summarised below:

- **Type of studies**: All the studies were cross-sectional studies.
- **Type of participants**: Either community based participants or high risk participants.
- **Type of tests**: FPG, OGTT or HbA1c. HbA1c was the index test and the selected articles were analysed further, if FPG or OGTT was performed on at least an 80% sample size as the reference tests. The diagnosis of diabetes must have been based on the ADA or the WHO criteria, which were an HbA1c of between 5.7% and 6.4% for the pre-diabetes criterion and >=6.5% for the T2DM criterion; FPG was 5.6-7mmol/l for the pre-diabetes and >=7mmol/l for T2DM; and OGTT was 7.8-11.1mmol/l for the pre-diabetes and >=11.1mmol/l for T2DM.

The sensitivity and specificity of the HbA1c >=6.5% among diabetes cases defined by OGTT or FPG must either have been reported, or it was possible to calculate them from the data provided.

Studies were excluded if they did not meet the above mentioned criteria, and /or were conducted on pregnant women.

The full-text articles of abstracts selected were retrieved and reviewed according the same inclusion and exclusion criteria.

In order to avoid selection bias, the search strategy of this study was also conducted by an independent second reviewer, according to the inclusion and exclusion criteria. Discrepancies were resolved between the main author and the independent reviewer.

2.1.4 Data extraction

Information on study design, study characteristics, sample size, individual study participant characteristics such as age, sex, HbA1c testing methods, and plasma glucose concentration measurement were extracted.

The prevalence estimates of T2DM and pre-diabetes diagnosed by HbA1c, FPG and OGTT were extracted from every individual study. and if the original study did not directly express the prevalence, it was worked out using the raw data, including sensitivity, specificity, predictive values and likelihood ratios. For each study, 95% confidence intervals (CI) of the prevalence estimate also were extracted. The pre-test prevalence of the T2DM was set at 6%.[37]

Accuracy estimates such as sensitivity, specificity, true positives, false positives, true negatives, false negatives were extracted, or calculated using the raw data reported in each study.

2.1.5 Analysis

This study was based on a systematic review with meta-analysis.

First of all, the prevalence and prevalence differences of T2DM and pre-diabetes were evaluated as measured by the HbA1c, then compared with FPG and OGTT from cross-sectional studies. Secondly, the diagnostic test characteristics of the above tests were determined and compared for T2DM from cross-sectional studies. Unfortunately there was insufficient data on pre-diabetes for analysis.

The selected studies were assessed by examining the study design (cross-sectional studies), study period, participant recruitment methods and sample

size, the test methods of HbA1c and plasma glucose concentration and the method used for collecting blood samples.

2.2 Statistical analysis Methodology

2.2.1 Methodology for comparing the prevalence

Diagnostic meta-analysis of the individual studies was conducted using R software.[38] Proportions (prevalence) of T2DM and pre-diabetes, as defined by HbA1c, FPG or OGTT, were transformed into a quantity (the Freeman-Turkey variant of the arcsine square root-transformed proportion) to suit the usual fixed- and random-effects model. The pooled prevalence was calculated as the back-transformed weighted mean of the transformed proportions, using inverse arcsine variance weights for the fixed-effects model and DerSimonian-Laird weights for the random-effects model. Heterogeneity and homogeneity between the studies were also assessed usingthe Cochran Q test ($p>0.1$), combining the data using a fixed-effects method. The random-effects method was used if heterogeneity was found. The Higgins I^2 statistic was used to quantify inconsistencies across the studies included in the meta-analysis. The closer the I^2 value was to 100%, the more likely it was that true heterogeneity existed, and therefore the less reliable the combined estimate became. Then the random effect model was used to get the pooled prevalence for each measurement method. The exact binomial confidence intervals for individual studies were calculated as well.

2.2.2 Methodology for prevalence odds ratios and pooled prevalence odds ratio

The "Metabin" [38] in R was used to calculate fixed and random effect estimates (risk ratio, odds ratio, risk difference or arcsine difference) for meta-analyses with binary outcome data. In this study, the "metabin" command was used to get the pooled prevalence odds ratio (OR) between HbA1c and OGTT or FPG, with the outcome of T2DMor pre-diabetes, in the community-based and high-risk populations. The Mantel-Haenszel method was used to get pooled prevalence OR. As a common concept, if the pooled prevalence OR is statistically significant, then the difference of prevalence is also statistically significant. In order to check the clinical significance, the NNS and the ADP were calculated. The ADP was calculated as the difference of pooled prevalence, and the NNS was calculated as 100/ADP.

2.2.3 Methodology for assessing diagnostic accuracy (HSROC curve analysis)

The "Metandi" command in STATA 11.0,[39] performs meta-analysis of diagnostic test accuracy studies, in which both the index test under study and the reference test (OGTT) are dichotomous. In this study, the index test, was defined as HbA1c >=6.5% indicating T2DM. The reference test was defined as OGTT. This was done because the selected publications used OGTT as the gold standard when calculating sensitivity and specificity of HbA1c. Unfortunately, there was not enough data captured, for comparing FPG and HbA1c, regarding pre-diabetes.

Participant characteristics (community based or high risk populations), in the included studies, were also considered.

A 2x2 table was drawn for each single study. Then four variables which were true positives, false positives, false negatives and true negatives were calculated according to the existing data from the single study.

The analysis used both the bivariate model[40] and the hierarchical summary receiver operating characteristic (HSROC) model.[41] The bivariate model models the sensitivity and specificity more directly. It assumes that the logit (log-odds) transformations have a bivariate normal distribution between studies.

The HSROC model assumes that there is an underlying ROC curve in each study with parameters alpha and beta, which characterize the accuracy and asymmetry of the curve. The 2×2 table for each study then arises from dichotomizing at a positivity threshold, theta. The parameters alpha and theta are assumed to vary between studies; both are assumed to have normal distributions as in conventional random-effects meta-analysis.

Empirical Bayes estimates, which can be used to obtain posterior predictions of the sensitivity and specificity in each study (mu), were also conducted in the current study.[42] The best estimate of the true sensitivity and specificity in each study could be given by Empirical Bayes estimates, and these estimates would be “shrunk” toward the summary point compared with the study-specific estimates.[42]

2.2.4 Exploring heterogeneity

If heterogeneity of pooled studies was observed, the reasons for heterogeneity were explored and the results analysed (using random effects models) with and without these studies.

2.2.5 Publication bias analysis (funnel plot)

In this study, funnel plots were used for assessing publication bias.[43]

The funnel plot assumes that the results from small studies will scatter widely at the bottom of the graph with the spread narrowing among larger studies.[43]

A symmetrical inverted funnel normally indicates the absence of publication bias. Conversely, if there is publication bias, the plots will be skewed and asymmetrical.[43]

Chapter 3

Results

3.1 Selection criteria

A flow chart for the selection process is provided in Figure 1. In the systematic review and meta-analysis, 20 papers (which included 23 studies) were included.

Studies selected for systematic review are shown in Figure 1.

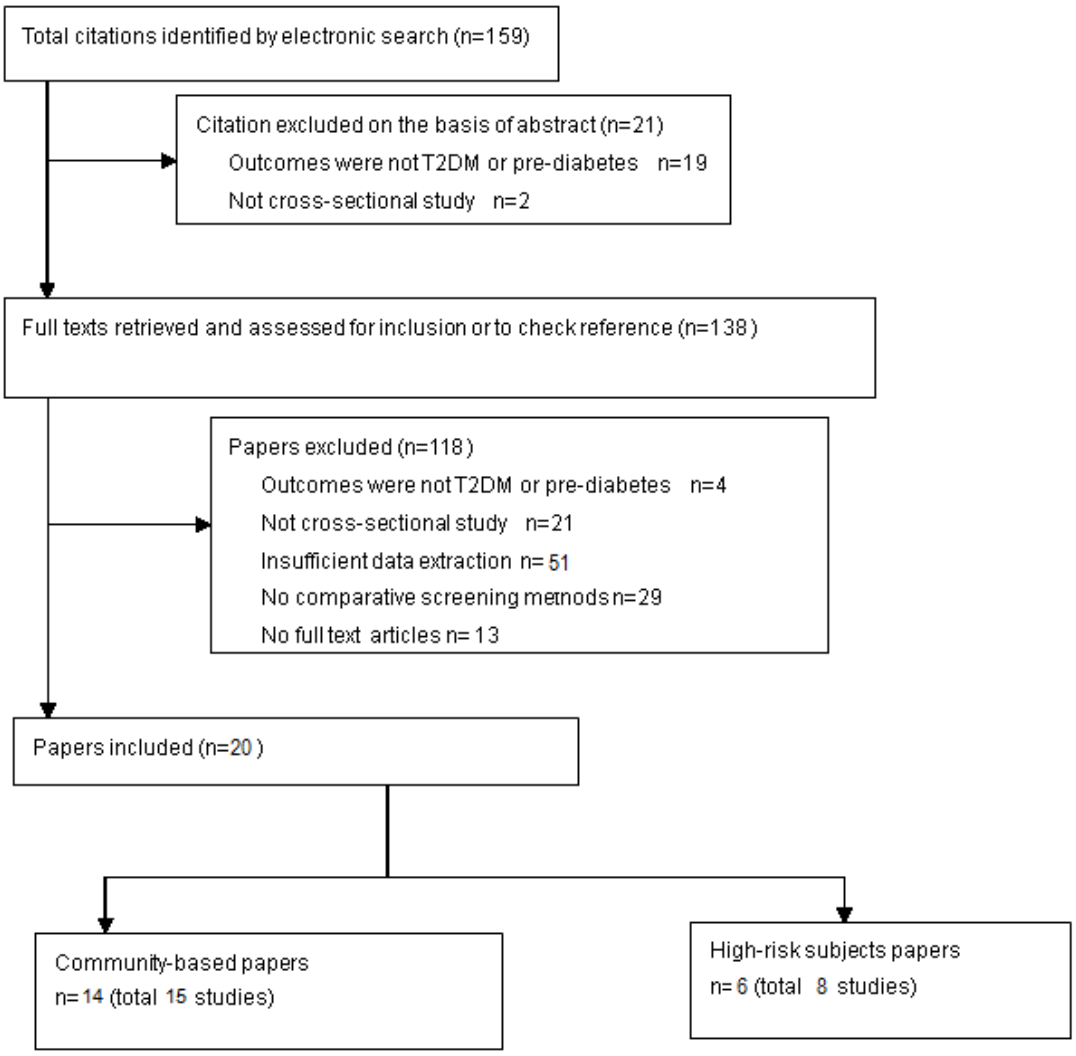

Figure 1. Search strategy (selection process)

3.2 Pooled prevalence from individual studies, odds ratios and pooled odds ratio of comparing HbA1c with OGTT or FPG in both subgroups

The publications selected for analysis are summarized in Table 3 and Table 4.

All the individual studies were cross-sectional studies, as specified in the selection criteria. Table 3 shows the studies from community based populations, and Table 4 show the high risk populations.

Table 3 Characteristics of the community based population studies

Study name	Study year	Diabetes (D) or pre-diabetes(PD)	Sample size	HbA1c test method	Reference test
Arab study [44]	2011	D&PD	482	HPLC	OGTT/FPG
South Africa study [45]	2011	D	819	HPLC	OGTT/ FPG
TOPICS 2 [46]	2011	D	26884	HPLC	FPG
Peru study [47]	2011	D	964	HPLC	FPG
ARIC study [48]	2011	D	12485	HPLC	FPG
NHANES III study [48]	2011	D	691	HPLC	FPG
Asian Indian study [49]	2011	D	461	SYNCHRON CX5	FPG
Shanghai study, China [50]	2010	D	4886	HPLC	OGTT
SIGT combined study, Georgia [51]	2010	D&PD	4706	HPLC	OGTT
Filipino Americans study [52]	2010	D	933	HPLC	OGTT/FPG
Qingdao study, China [53]	2010	D	2332	HPLC	OGTT/FPG
Greenland and Danish combined study, Denmark [54]	2010	D	7957	HPLC	OGTT
New Hoorn study [55]	2010	D&PD	2753	HPLC	OGTT
Beijing study, China [56]	2009	D&PD	903	HPLC	OGTT
Non-pregnant subjects study, U.K.[57]	1998	D	401	HPIEC	OGTT/FPG

Table 4 Characteristics of the high risk population studies

Study name	Study year	Diabetes(D) or pre-diabetes(PD)	Sample size	HbA1c test method	Reference test
High-risk Southeast Asian study [58]	2012	D	511	HPLC	OGTT/FPG
Italian Caucasians study [59]	2011	D	1019	HPLC	OGTT
Edmonton study [60]	2011	D	3163	HPLC	OGTT
Marshfield study [60]	2011	D	271	HPLC	OGTT
Spokane study [60]	2011	D	1358	HPLC	OGTT
Silent diabetes study [61]	2011	D	1015	HPLC	OGTT
LEADER study, UK [62]	2010	D	8696	HPLC	OGTT
dysglycemic states in older adults [63]	2010	D	1865	HPLC	FPG

Table 5 shows the number of cases and sample sizes of T2DM defined by HbA1c compared with OGTT in community based populations.

Table 5 Frequencies of cases and sample size of T2DM defined by HbA1c compared with OGTT as index test in community based populations

Study name	Diabetes defined by HbA1c>=6.5%			Diabetes defined by OGTT>=11.1mmol/L		
	Number of cases (N)	Sample size (S)	N/S %	Number of cases(N)	Sample size(S)	N/S%
Arab study (ADA criteria)[44]	12	482	2.5%	52	482	10.8%
South Africa study[45]	84	819	10.3%	147	819	17.9%
Shanghai study, China (WHO criteria)[50]	239	4886	4.9%	301	4886	4.7%
SIGT combined study, Georgia(ADA criteria)[51]	108	4706	2.3%	273	4706	5.8%
Filipino Americans study[52]	83	933	8.9%	145	933	15.5%
Qingdao study, China[53]	253	2332	10.8%	278	2332	11.9%
Greenland and Danish combined study, Denmark[54]	769	7957	9.7%	402	7957	5.1%
New Hoorn study[55]	55	2753	2%	107	2753	4%
Beijing study, China[56]	58	903	6.4%	100	903	11.1%
Non-pregnant subjects,UK[57]	52	401	13%	178	401	44.4%

A statistical analysis was done in R software with “metaprop” option. As mentioned under methodology, proportions (prevalence) of T2DM (shown in Table 5), as defined by HbA1c and OGTT in community based populations, were transformed into a quantity (the Freeman-Turkey variant of the arcsine square root-transformed proportion). The pooled prevalence was calculated as the back-transformed

weighted mean of the transformed proportions. The Cochran Q test (p>0.1) was used to assess heterogeneity and homogeneity between the studies. The random-effects method was used if heterogeneity was found. The Higgins I^2 statistic was used to quantify inconsistencies across the studies included in the meta-analysis.

The results are shown in Table 5.1.

Table 5.1 Prevalence of T2DM defined by HbA1c and OGTT in community based populations

Measure -ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	6%(5.7%-6.3%)	6.5%(4.2%-9.3%)	98.7% (98.4%-99%)	<0.0001	1713	26172
OGTT	7%(6.7%-7.4%)	11.7%(8.3%-15.6%)	98.8%(98.4%-99%)	<0.0001	1983	26172

According to the results, both the I^2 values of HbA1c and OGTT were close to 100%, so the random effect model was used to get the pooled proportions for HbA1c and OGTT (Table 5.1). A total of 26172 people were included as a community based study population. Using HbA1c as the diagnostic method, 1713 people were diagnosed with type 2 diabetes. Using OGTT as the diagnostic method for diabetes, 1983 people were diagnosed with type 2 diabetes. The difference in prevalence, shown by the two tests, was 5.2%.

In Table 5.2, the ORs were calculated for T2DM defined by HbA1c and OGTT in community based populations, where OGTT was defined as the reference test in each individual study.

Then in Table 5.3, the ORs are pooled to compare HbA1c with OGTT in community based populations. From Table 5.3 it can be seen that the random effect model was considered, in preference to the fixed effect model, as I^2 was 97.5% (nearly 100%).

Table 5.2 The pooled prevalence Odds Ratios of HbA1c compared with OGTT in community based populations

Study name	OR	95% CI	%W (fixed)	%W(random)
Arab study (ADA criteria)	0.21	0.11-0.4	2.87	8.80
South Africa study	0.52	0.39-0.70	7.48	10.08
Shanghai study, China (WHO criteria)	0.78	0.66-0.93	12.40	10.30
SIGT combined study, Georgia(ADA criteria)	0.38	0.30-0.48	15.12	10.22
Filipino Americans study	0.53	0.40-0.71	7.49	10.08
Qingdao study, China	0.90	0.75-1.08	14.05	10.31
Greenland and Danish combined study, Denmark	2.01	1.77-2.28	20.58	10.39
New Hoorn study	0.50	0.36-0.70	5.94	9.97
Beijing study, China(HbA1c>=6.5%)	0.55	0.39-0.77	5.30	9.94
Non-pregnant subjects study, U.k.	0.19	0.13-0.27	8.78	9.90

Table 5.3 Pooled OR of HbA1c compared with OGTT in community based populations

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity-	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.54	0.34-0.86	0.01	97.5% (96.5% - 98.2%)	<0.0001

Using HbA1c as a diagnostic method was shown to be 0.55 times as likely to diagnose T2DM, than OGTT in these community based populations. In another words, using HbA1c decreases the odds of diabetes detection by 55%, compared to OGTT. The p value, as well as the confidence interval, confirmed this to be statistically significant of OR. In absolute terms, the difference is that 5.2% more cases are detected by OGTT. So for every 19 people screened by OGTT, 1 more case of T2DM will be detected compared to HbA1c (NNS). The NNS confirmed the clinical significant of this difference prevalence.

The p value of heterogeneity test is much less than 0.05, which confirms heterogeneity among the studies. As shown in Table 5.2, the Arab study and UK

Non-pregnant study could contribute to the heterogeneity (low OR) as could the Greenland and Danish Study (high OR). So a sensitivity analysis was conducted excluding these studies.

Analysis without the Greenland and Danish study

Table 5.1.1 Prevalence of T2DM defined by HbA1c and OGTT in community based populations without the Greenland and Danish study

Measure-ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	4.7%(4.4%-5%)	6.2%(3.8%-9%)	97.9%(97.2%-98.5%)	<0.0001	944	18215
OGTT	8%(7.6%-8.4%)	12.6%(8.5%-17.5%)	98.8%(98.4%-99%)	<0.0001	1581	18215

Table 5.3.1 Pooled OR of HbA1c compared with OGTT in community based populations without the Greenland and Danish study

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity-	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.47	0.34-0.64	<0.0001	91.9%(86.8%-95%)	<0.0001

After checking the original results, the pooled OR is still in the order of 0.5, so it does not change much from the original analysis.

Analysis without the Arab and the Non-pregnant studies

Table 5.1.2 Prevalence of T2DM defined by HbA1c and OGTT in community based populations without the Arab and the UK Non-pregnant studies

Measure-ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	6%(5.7%-6.3%)	6.4%(3.9%-9.5%)	98.7%(98.3%-99.1%)	<0.0001	1649	25289
OGTT	6.6%(6.3%-6.9%)	9%(6.5%-11.9%)	98.1%(97.4%-98.6%)	<0.0001	1753	25289

Comparing with original results, the absolute difference in prevalence is lower as 2.6%.

Table 5.3.2 Pooled OR of HbA1c compared with OGTT in community based populations without the Arab and the UK Non-pregnant studies

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity-	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.68	0.42-1.08	<0.0001	97.3%(96.1%-98.2%)	<0.0001

The pooled OR is higher as 0.68 but still lies in the 95% CI of original result. So the result of sensitivity analysis does not change much.

Table 6 shows the frequencies of cases and sample sizes of T2DM defined by HbA1c, compared with FPG in community based populations.

Table 6 Frequencies of cases and sample sizes of T2DM defined by HbA1c compared with FPG in community based populations

Study name	Diabetes defined by HbA1c>=6.5%			Diabetes defined by FPG>=7.0mmol/L		
	Number of cases (N)	Sample size(S)	N/S%	Number of cases	Sample size	N/S%
Arab study[44]	12	482	2.5%	52	482	10.8%
South Africa study[45]	79	819	9.6%	117	819	14.3%
TOPICS 2[46]	719	26884	2.7%	724	26884	2.7%
Peru study[47]	34	964	3.5%	9	964	1%
ARIC study[48]	460	12485	3.7%	840	12485	6.7%
NHANESIII study[48]	31	691	4.5%	29	691	4.2%
Asian Indian study[49]	26	461	5.6%	48	461	10.4%
Shanghai study, China (WHO criteria)[50]	239	4886	4.9%	173	4886	3.5%
Filipino Americans study[52]	83	933	8.9%	61	933	6.5%
Qingdao study, China[53]	253	2332	10.8%	278	2332	11.9%
Non-pregnant study[57]	52	401	13%	155	401	38.7%

Using the same methods, the pooled prevalence was calculated in R software and is expressed in Table 6.1. The odds ratios of individual studies, calculated using R software, are shown in Table 6.2. The pooled OR, where HbA1c is compared with FPG in community based populations, is reflected in Table 6.3.

Table 6.1 The prevalence of T2DM (defined by HbA1c compared with FPG) in community based populations

Measure-ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	3.6%(3.5%-3.8%)	5.9%(4.3%-7.6%)	97.8%(97%-98.3%)	<0.0001	1988	51338
FPG	4.3%(4.2%-4.5%)	8.4%(5.6%-11.6%)	99.1%(98.9%-99.3%)	<0.0001	2486	51338

As the results shown, I^2 values of both HbA1c and FPG were close to 100%, so the random effect model was used to get the pooled proportions for HbA1c and FPG.. A total of 51338 people were included as community based study populations. Using HbA1c as the diagnostic method, 1988 people were diagnosed with T2DM. Using FPG as the diagnostic method for diabetes, 2486 people were diagnosed with T2DM. The difference in prevalence, shown by the two tests, was found to be 2.5%. The P value for the heterogeneity test of HbA1c and FPG was less than 0.1, confirming heterogeneity.

Table 6.2 The Odds Ratios of HbA1c compared with FPG in community based populations

Study name	OR	95% CI	%W (fixed)	%W(random)
Arab study	0.21	0.11-0.40	2.15	7.30
South Africa study	0.64	0.47-0.87	4.49	9.62
TOPICS 2	0.99	0.89-1.10	29.93	10.46
Peru study	3.88	1.85-8.13	0.37	6.61
ARIC study	0.53	0.47-0.60	34.36	10.43
NHANESIII study	1.07	0.64-1.80	1.18	8.18
Asian Indian study	0.51	0.31-0.84	1.92	8.34
Shanghai study	1.40	1.15-1.71	6.99	10.15
Filipino Americans study	1.40	0.99-1.97	2.36	9.37
Qingdao study, China	0.90	0.75-1.08	10.53	10.22
Non-pregnant study, U.K.	0.24	0.17-0.34	5.73	9.31

Table 6.3 The pooled OR comparing HbA1c with FPG in community based populations

Test method	OR of random effect model	95% CI of OR	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
FPG	reference	-	-	-	-
HbA1c	0.77	0.56-1.05	0.1	94.8%(92.4%-96.4%)	<0.0001

In Table 6.3, using the HbA1c as diagnostic method was found to be 0.77 times as this OR was not statistically significant as p value was 0.1. Therefore, there was no difference between prevalence defined by both HbA1c and FPG in community based populations.

Again heterogeneity was observed in Table 6.2. Sensitivity analysis was conducted by removing the lower OR studies (the Arab and the UK Non-pregnant study), and the higher OR studies (the Peru, Shanghai and Filipino-American studies).

Analysis without the Arab and the Non-pregnant studies

Table 6.3.1 The prevalence of T2DM (defined by HbA1c compared with FPG) in community based populations without Arab and Non-pregnant studies

Measure-ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	3.6%(3.4%-3.8%)	5.7%(4.1%-7.5%)	98%(97.25-98.5%)	<0.0001	1924	50455
FPG	4.2%(4%-4.3%)	6.1%(3.9%-8.7%)	98.9%(98.6%-99.2%)	<0.0001	2279	50455

Table 6.3.1 The pooled OR comparing HbA1c with FPG in community based populations without Arab and Non-pregnant studies

Test method	OR of random effect model	95% CI of OR	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
FPG	reference	-	-	-	-
HbA1c	0.97	0.73-1.30	0.84	93.7%(90.2%-96%)	<0.0001

The odds ratio now is much closer to 1 but the confidence interval is much wider and the result still not statistically significant.

Analysis without the Peru, Shanghai and Filipino-American studies

Table 6.4.2 The prevalence of T2DM (defined by HbA1c compared with FPG) in community based populations

Measure-ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	3.4%(3.3%-3.6%)	6%(4.1%-8.3%)	98.2%(97.5%-98.7%)	<0.0001	1632	44555
FPG	4.5%(4.3%-4.7%)	10.9%(6.8%-15.8%)	99.3%(99.2%-99.5%)	<0.0001	2243	44555

Table 6.3.2 The pooled OR comparing HbA1c with FPG in community based populations

Test method	OR of random effect model	95% CI of OR	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
FPG	reference	-	-	-	-
HbA1c	0.57	0.41-0.80	0.001	94.4%(91.1%-96.5%)	<0.0001

Compared with the original results, the new result does change to statistically significant when the higher OR studies are removed from the analysis. The absolute difference in prevalence was 4.9%, and the NNS was 20. So for every 20 people screened by FPG, there was 1 more T2DM case to be found compared to HbA1c. This was also clinically significant.

The frequencies and sample sizes of T2DM, defined by HbA1c compared with OGTT in high risk populations, are shown in Table 7.

Table 7 Frequencies of cases and sample sizes of T2DM defined by HbA1c compared with OGTT in high risk populations

Study name	Diabetes defined by HbA1c>=6.5%			Diabetes defined by OGTT>=11.1mmol/L		
	Number of cases(N)	Sample size(S)	N/S%	Number of cases(N)	Sample size(S)	N/S%
High-risk Southeast Asian study[58]	261	511	51.1%	302	511	59.1%
Italian caucasians study[59]	115	1019	11.3%	131	1019	12.9%
Multicenter study Edmonton study[60]	381	3163	12%	568	3163	18%
Multicenter study Marshfield study[60]	43	271	15.9%	78	271	28.8%
Multicenter study Spokane study[60]	430	1358	29.7%	242	1358	17.8%
Silent diabetes study[61]	42	1015	4.1%	143	1015	14.1%
LEADER study, UK (WHO criteria)[62]	502	8696	5.8%	291	8696	3.3%

Table 7.1 shows the pooled prevalence and Table 7.2 reflects the ORs, as calculated using R software. The pooled OR of HbA1c, compared to OGTT in high risk populations, can be seen in Table 7.3.

Table 7.1 Prevalence of T2DM defined by HbA1c and OGTT in high risk populations

Measurement method	Fixed effect model prevalence(95%CI)	Random effect model prevalence(95%CI)	I^2 for heterogeneity between studies(95%CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	9.8%(9.4%-10.3%)	16.7%(8.5%-26.9%)	99.5%(99.4%-99.6%)	<0.0001	1774	16033
OGTT	9.2%(8.7%-9.6%)	20%(9.7%-32.8%)	99.6%(99.5%-99.7%)	<0.0001	1755	16033

Same as before, the random effect model was used to get the pooled proportions for HbA1c and OGTT, which were 16.7% and 20%. In high risk populations, a total of 16033 people were included in the analysis. Using HbA1c as diagnostic method, 1774 of these people were diagnosed with T2DM. Using OGTT as the diagnostic method, 1755 people were diagnosed with T2DM. The difference of prevalence, defined by these two methods, was 3.3%.

Table 7.2 The Odds Ratios of HbA1c compared with OGTT in high risk populations

Study name	OR	95% CI	%W (fixed)	%W(random)
High-risk Southeast Asian study	0.72	0.56-0.93	10.51	14.36
Italian caucasians study	0.86	0.66-1.13	8.27	14.28
Multicenter study Edmonton study	0.63	0.54-0.72	35.54	14.70
Multicenter study Marshfield study	0.47	0.31-0.71	4.67	13.51
Multicenter study Spokane study	2.14	1.78-2.56	11.76	14.60
Silent diabetes study	0.26	0.18-0.38	9.75	13.86
LEADER study, UK (WHO criteria)	1.77	1.53-2.05	19.50	14.68

Table 7.3 The pooled OR of HbA1c compared with OGTT in high risk populations

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.79	0.48-1.32	0.38	97.5% (96.4%-98.3%)	<0.0001

The pooled OR is 0.79, lower odds when using HbA1c to detect T2DM in high risk populations, compared to OGTT. However, as the p value shown, the pooled OR was not statistically significant. Therefore, the difference of prevalence, 3.3% was not statistically significant. So in high risk populations, HbA1c and OGTT have the same ability to indicate T2DM. Again heterogeneity is observed. Table 7.2 clearly shows that the Spokane study and the LEADER study were contradictory to the other studies. The sensitivity analysis was done without Spokane and LEADER study.

Analysis without the Spokane and the LEADER studies

Table 7.5.1 The prevalence of T2DM (defined by HbA1c compared with OGTT) in high risk populations

Measure-ment method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	12.9%(12%-13.7%)	16.7%(6.9%-29.7%)	99.2%(98.9%-99.4%)	<0.0001	842	5979
OGTT	19.7%(18.7%-20.7%)	25.2%(13.7%-38.7%)	99.1%(98.7%-99.3%)	<0.0001	1222	5979

Table 7.3.1 The pooled OR comparing HbA1c with OGTT in high risk populations

Test method	OR of random effect model	95% CI of OR	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.56	0.40-0.77	0.0004	87.3%(72.6%-94.1%)	<0.0001

When both high OR studies were removed from the analysis, the pooled OR became statistically significant as the p value shows. The ADP in prevalence is 8.5%, and NNS is 12. Both ADP and NNS also confirm that the difference in prevalence is clinically significant. This result differs from the original result.

Table 8 lists the frequencies of cases and sample sizes of T2DM defined by HbA1c, compared with FPG in high risk populations.

Table 8 Frequencies of cases and sample sizes of T2DM defined by HbA1c compared with FPG in high risk populations

Study name	Diabetes defined by HbA1c>=6.5%			Diabetes defined by OGTT>=11.1mmol/L		
	Number of cases(N)	Sample size(S)	N/S%	Number of cases(N)	Sample size(S)	N/S%
High-risk Southeast Asian study[58]	261	511	51.1%	187	511	36.6%
Italian Caucasians study[59]	115	1019	11.3%	92	1019	9%
Dysglycemic States in older adults[63]	58	1865	3.1%	51	1865	2.7%

The pooled prevalence can be seen in Table 8.1, and the ORs in Table 8.2.

Table 8.1 Prevalence of T2DM based on HbA1c and FPG in high risk populations

Measurement method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	9.9%(8.9%-10.9%)	18%(1.5%-46.8%)	99.7%(99.5%-99.8%)	<0.0001	434	3395
FPG	7.6%(6.7%-8.5%)	13.2%(1.5%-33.8%)	99.5%(99.2%-99.6%)	<0.0001	329	3395

Again, the random effect model was used to get pooled proportions for HbA1c and OGTT. A total of 2376 people were included in the analysis. Using HbA1c as the diagnostic method, 319 people were diagnosed with T2DM. When FPG was used, 237 people were diagnosed with T2DM. Using HbA1c as a diagnostic method resulted in 4.8% more positive cases.

Table 8.2 The Odds Ratios of HbA1c comparing with FPG in high risk populations

Study name	OR	95% CI	%W (fixed)	%W(random)
High-risk Southeast Asian study	1.81	1.41-2.32	41.29	38.46
Italian Caucasians study	1.28	0.96-1.71	36.84	34.72
Dysglycemic States in older adults	1.17	0.79-1.71	21.87	26.82

The p value of heterogeneity test was 0.09 and 95% CIs of ORs were overlapped, which confirmed that there was no heterogeneity between these studies, and the selected individuals were from the same population.

The pooled OR is shown in Table 8.3.

Table 8.3 The pooled OR of HbA1c comparing with FPG in high risk populations

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
FPG	reference	-	-	-	-
HbA1c	1.43	1.08-1.88	0.01	59.3%(0%-88.4%)	0.09

As the results shown, using HbA1c as diagnostic tool increased the odds for detecting DM by 43% compared to FPG in high risk populations. And this difference was statistically significant, as the p value of OR was 0.01. So this 43% of more cases determined by HbA1c than FPG was statistically significant.

The frequencies of pre-diabetic cases, as well as the sample sizes, as defined by HbA1c and OGTT in community based populations, are reflected in Table 9.

Table 9 Frequencies of cases and sample sizes of pre-diabetes defined by HbA1c and OGTT in community based populations

Study name	Diabetes defined by HbA1c between 5.7% to 6.4%			Diabetes defined by OGTT between 7.8mmol/L and 11.1mmol/L		
	Number of cases(N)	Sample size(S)	N/S%	Number of cases(N)	Sample size(S)	N/S%
Arab study[44]	54	482	11.25	223	482	46.3%
SIGT combined study[51], Georgia	918	4706	19.5%	1694	4706	36%
New Hoorn study[55]	184	2668	6.9%	439	2668	16.5%
Beijing study[56]	4	903	0.4%	202	903	22.4%

Table 9.1 indicates the pooled prevalence of pre-diabetes, while Table 9.2 shows ORs. The pooled OR is reflected in Table 9.3.

Table 9.1 The prevalence of pre-diabetes defined by HbA1c and OGTT in community based populations

Measurement method	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	12.2%(11.5%-12.9%)	7.9%(1.6%-18.4%)	99.4%(99.2%-99.6%)	<0.0001	1160	8659
OGTT	29%(28.1%-30%)	30.3%(18.4%-43.7%)	99.3%(99%-99.5%)	<0.0001	2558	8659

There were only 4 studies included when comparing prevalence of pre-diabetes between HbA1c and OGTT in community-based populations (Table 3). The prevalence of pre-diabetes was defined by these two methods in the community based subgroup. Again, random effects models were preferred because of heterogeneity. A total of 8759 people were included. Using HbA1c as diagnostic method, 1279 people were diagnosed with pre-diabetes. Using the OGTT, 2549 people were diagnosed with pre-diabetes. Therefore, using the OGTT as a diagnostic method, resulted in 24.4% more positive cases. Obviously, such big difference in prevalence of defining pre-diabetes is clinically significant.

Table 9.2 The Odds Ratios of HbA1c compared with OGTT in community based population

Study name	OR	95% CI	%W (fixed)	%W(random)
Arab study	0.15	0.10-0.20	9.12	26.65
SIGT study	0.43	0.39-0.47	62.80	28.85
New Hoorn study	0.38	0.31-0.45	18.82	28.30
Beijing study	0.01	0-0.04	9.26	16.19

Table 9.3 The pooled OR of pre-diabetes comparing HbA1c with OGTT in community based population

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.18	0.1-0.33	<0.0001	96.4% (93.3%-98%)	<0.0001

Table 9.2 analyzed odds ratios of each study comparing HbA1c with OGTT. Table 9.3 showed the pooled OR of 0.06 which is statistically significant, as the p value shown.

The sensitivity analysis was conducted in screening pre-diabetes with OGTT and HbA1c. The Arab study and the Beijing study probably contribute to heterogeneity. When removed, the results are as followed:

Analysis without the Arab and the Beijing studies

Table 9.3.1 The prevalence of pre-diabetes defined by HbA1c and OGTT in community based populations without both Arab and Beijing studies

Measurement metho	Fixed effect model prevalence (95% CI)	Random effect model prevalence (95% CI)	I^2 for heterogeneity between studies (95% CI)	P value for heterogeneity test	Number of cases	Sample size
HbA1c	14.3%(13.5%-15.1%)	12.5%(3%-27.3%)	99.6%	<0.0001	1102	7374
OGTT	28.4%(27.4%-29.4%)	25.6%(9.3%-46.7%)	99.7%	<0.0001	2133	7374

Table 9.3.1 The pooled OR of pre-diabetes comparing HbA1c with OGTT in community based population without both Arab and Beijing studies

Test method	OR of random effect model	95% CI	P value of OR	I^2 value of heterogeneity	P value of heterogeneity test
OGTT	reference	-	-	-	-
HbA1c	0.41	0.36-0.47	<0.0001	41.4%	0.19

When the Arab study and Beijing study were removed from the analysis, the remaining studies became homogenous. The pooled OR is 0.41, which is much higher than the original analysis. But the pooled OR and the difference in prevalence are still statistically and clinically significant.

3.3 Meta-analysis of diagnostic accuracy (sensitivity and specificity)

Table 10 and Table 11 below, summarize the individual studies, which were selected for current study. Two by two tables were drawn for each individual study. True positive cases (TP), false negative cases (FN), false positive cases (FP), as well as true negative cases (TN) were calculated.

The SIGT, the Greenland and Danish, and the UK Non-pregnant studies were not included in this diagnostic accuracy analysis, because the TP, FN, FP and TN could not be calculated by the original data.

This diagnostic accuracy analysis was done in STATA 11.0 with “metandi” option.

The HbA1c was set as index test for indicating T2DM in community based populations, comparing with reference test, OGTT. Unfortunately, there was no enough data captured for comparing FPG and HbA1c, regarding pre-diabetes.

Table 10 The diagnostic accuracy of T2DM defined by HbA1c according to OGTT in community based populations

Studyname	TP	FN	FP	TN
Arab study	10	42	0	430
South Africa study	68	79	27	645
Shanghai study	152	149	87	4498
Filipino-American study	62	92	27	812
Qingdao study, China	69	209	187	1867
New Hoorn study	26	81	25	2536
Beijing study	54	46	4	799

TP: true positive cases FN: false negative cases FP: false positive cases TN: true negative cases

True positives, false positives, false negatives and true negatives were calculated by drawing a 2*2 table, according to the existing data from the single study.

The HSROC curve was drawn as well.

Table 10 shows the diagnostic accuracy, while Table 10.1 lists the summary results of meta-analysis, done using STATA 11.0.

In the summary values as shown in Table 10.1, the sensitivity of HbA1c was found to be 69% (95% CI 0.49-0.84), so, when using HbA1c as diagnostic test it is likely that only 71% patients with T2DM will be detected, while 29% of T2DM patients would be missed.

Table 10.1 Meta-analysis of diagnostic accuracy of HbA1c and OGTT (OGTT is the reference test) in community based populations

Variable	Coefficient	Standard Error	z	p>|z|	95% Conf. Interval	
Summary value						
Se	0.69	0.09			0.49	0.84
Sp	0.93	0.01			0.9	0.96
DOR	31.12	14.81			12.25	79.08
LR+	10.40	2.46			6.54	16.53
LR-	0.33	0.1			0.19	0.59
1/LR-	2.99	0.88			1.68	5.33

Se: sensitivity Sp: specificity DOR:Diagnostic Odds Ratio LR+: positive likelihood ratio LR-: negative likelihood ratio

The specificity of HbA1c was 93% (95%CI 0.9-0.96) and the DOR was 34.6 (95%CI 12.42-96.42). This means that the odds for HbA1c>=6.5%, among subjects with T2DM, is 35 times higher than among subjects without T2DM. The positive likelihood ratio (LR+) showed that it was 10.75 (95%CI 6.76-17.11) times more likely to have HbA1c>=6.5% among T2DM compared to non-diabetic patients. The negative likelihood ratio (LR-) showed that it was 0.31 (95%CI 0.16-0.61) times as likely to have HbA1c<6.5% among T2DM compared to non-diabetic patients.

A value for the inverse of the negative likelihood ratio (1/LR-) was also given in Table 10.1, because larger value of the inverse of the negative likelihood ratio indicates a more accurate test. Comparing this with the positive likelihood ratio can indicate whether a positive or negative test result has greater impact on the odds of disease.[33,34] As shown in Table 10.1, 1/LR- (3.22) was less than LR+, which means that HbA1c >=6.5% has greater impact on the odds of T2DM than a negative test.

Figure 2 below, summarizes the HSROC curve of HbA1c, which is from the hierarchical summary ROC-model.

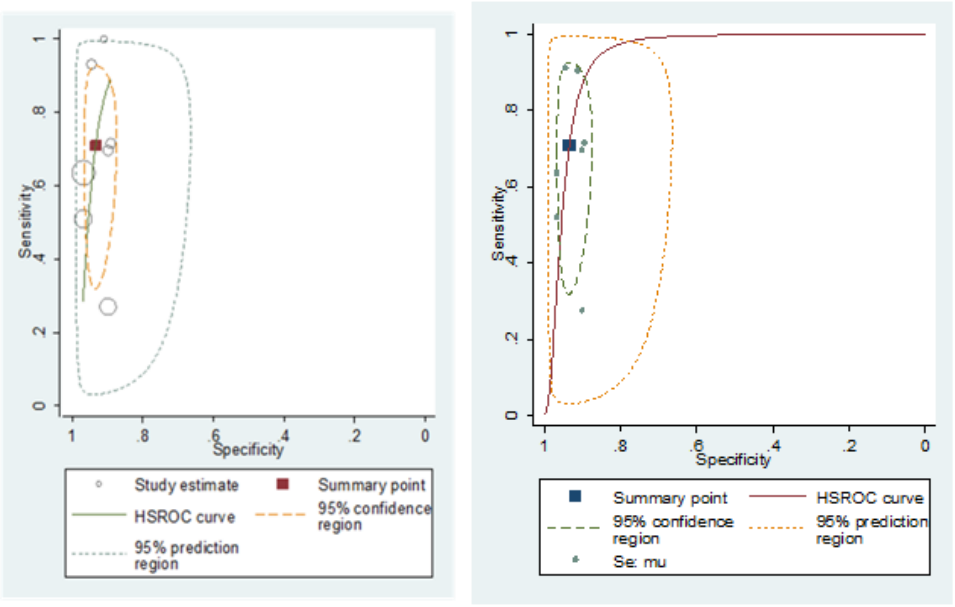

Figure 2. Left: The HSROC curve of HbA1c (from the hierarchical Summary ROC model) in community based populations. Right: Empirical Bayes estimates of HSROC model of HbA1c

In Figure 2 left plot, the sensitivity of a certain test is plotted against 1-specificity, allowing comparison of both parameters at the same time for multiple tests.

The round circles represent individual studies and the size of the circles is proportional to the number of patients included in the study. The filled red square is the summary estimates of sensitivity and specificity and the yellow dotted ellipses around the square represent the 95% confidence intervals around the summary estimate. The reference test used to determine the plotted accuracies in this figure is OGTT.

The blue dotted ellipses represent the 95% prediction region for a forecast of the true sensitivity and specificity in a future study.

The empirical Bayes estimates of the HSROC model of HbA1c, in community based populations, is shown in Figure 2 right plot.

Figure 2 right plot shows summary points, lines, and regions in conventional ROC space. The filled square is a summary point, solid line, a SROC curve, dotted line, the boundary of the confidence region for the summary point, dashed line, the boundary of the prediction region.

Comparing these two plots in Figure 2, showed that the shrinkage was generally greater for sensitivity than for specificity in this study (on the logit scale). This reflects the fact that most studies have fewer participants with T2DM, than without it, leading to more precise estimates of specificity than of sensitivity. In another words, in community-based populations, HbA1c can diagnose more non-diabetic cases than OGTT.

The sensitivity analysis was also conducted in this diagnostic accuracy analysis as some of the studies had low OR (Arab study) and high OR (Shanghai study and Filipino-American study).

Analysis without the Arab study

Table 10.2.1 Meta-analysis of diagnostic accuracy of HbA1c and OGTT (OGTT is the reference test) in community based populations

Variable	Coefficient	Standard Error	z	p>|z|	95% Conf. Interval	
Summary value						
Se	0.65	0.10			0.45	0.81
Sp	0.94	0.01			0.90	0.96
DOR	27.70	14.11			10.21	75.18
LR+	10.34	2.89			5.98	17.87
LR-	0.37	0.10			0.22	0.65
1/LR-	2.68	0.75			1.55	4.64

When the Arab study was removed from the analysis, the value of DOR are still within the 95% CI of original analysis.

Analysis without the Shanghai study and the Filipino-American study

Table 10.3.2 Meta-analysis of diagnostic accuracy of HbA1c and OGTT (OGTT is the reference test) in community based populations

Variable	Coefficient	Standard Error	z	p>|z|	95% Conf. Interval	
Summary value						
Se	0.71	0.13			0.41	0.89
Sp	0.93	0.01			0.89	0.95
DOR	32.15	21.85			8.49	121.78
LR+	10.09	2.91			5.74	17.76
LR-	0.31	0.14			0.13	0.75
1/LR-	3.19	1.42			1.33	7.63

Comparing with the original analysis, it could be said that there is no difference between the new results and original results.

So in the community based populations, using HbA1c as a screening method only can define around 70% of true positive cases of T2DM.

Table 11 summarizes the diagnostic accuracy of T2DM defined by HbA1c, according to OGTT in high-risk populations.

Table 11 The diagnostic accuracy of T2DM defined by HbA1c according to OGTT in high risk populations

Study name	TP	FN	FP	TN
Italian Caucasians study	61	70	54	834
Edmonton study	215	353	166	2429
Marshfield study	31	47	12	181
Spokane Washington study	183	59	247	869
Silent diabetes study	23	120	17	855

TP: true positive cases FN: false negative cases FP: false positive cases TN: true negative cases

The summary values of the meta-analysis of diagnostic accuracy of HbA1c in high-risk populations can be seen in Table 11.1, where OGTT is the reference test.

Table 11.1 Meta-analysis of diagnostic accuracy of HbA1c in high risk populations (OGTT is the reference test)

Variable	Coefficient	Standard Error	z	p>|z|	95% Conf. Interval	
Summary value						
Se	0.56	0.40			0.48	0.63
Sp	0.89	0.02			0.85	0.92
DOR	10.23	0.93			8.56	12.23
LR+	5.08	0.54			4.12	6.25
LR-	0.50	0.04			0.43	0.57
1/LR-	2.01	0.15			1.75	2.32

Se: sensitivity Sp: specificity DOR:Diagnostic Odds Ratio LR+: positive likelihood ratio LR-: negative likelihood ratio

In high risk populations, using OGTT as the reference test, HbA1c only could detect 56% (95%CI 0.50-0.63) of T2DM patients with respective specificity of 89% (95%CI 0.85-0.92). The DOR and values of comparing 1/LR- with LR+ also showed that HbA1c>=6.5% had greater impact on odds of T2DM than negative test of HbA1c<6.5%. The reasons of the differences between OR and diagnostic accuracy test could be explained as different analysis software and different selected single studies. The OR test was done in R software and diagnostic accuracy test was done in STATA 11.0. And besides the same selected studies, LEADER study, older adults study and high-risk south east Asian study were not included into diagnostic accuracy analysis because we could not calculate TP, FN, FP and TN.

Figure 3 summarizes graphically the HSROC curve of HbA1c in high-risk populations.

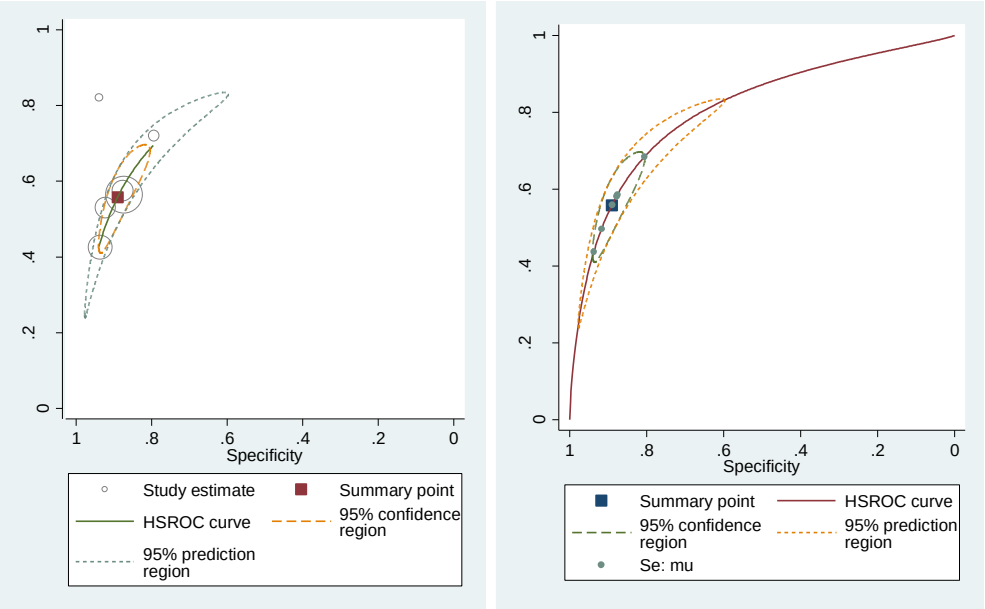

Figure 3 Left : The HSROC curve of HbA1c from the hierarchical summary ROC model in high risk populations. Right: Empirical Bayes estimates of HSROC model of HbA1c

In Figure 3, the sensitivity of a certain test is plotted against 1-specificity, allowing comparison of both parameters at the same time for multiple tests.

The round circles represent individual studies and size of the circles is proportional to the number of patients included in the study. The filled red square is the summary estimates of sensitivity and specificity and the yellow dotted ellipses around the square represent the 95% confidence intervals around the summary estimate.

The reference test used to determine the plotted accuracies in this figure is OGTT.

The blue dotted ellipses represent the 95% prediction region for a forecast of the true sensitivity and specificity in a future study.

The right plot of Figure 3 shows the empirical Bayes estimates of the HSROC model of HbA1c. It is summary of points, lines, and regions in conventional ROC space.

Comparing these two plots of Figure 4, it was shown that the shrinkage was greater for sensitivity than for specificity in this study (on the logit scale), leading to the

same precise estimates of specificity as sensitivity. In another words, in high-risk populations, HbA1c could also specify more non-diabetics than OGTT.

In the high-risk populations, sensitivity analysis was only conducted without Multicenter Spokane study as the Table 7.2 shown. And besides the same selected studies, LEADER study and high-risk Southeast Asian study were not included into diagnostic accuracy analysis because we could not calculate TP, FN, FP and TN.

Analysis without Spokane study

Table 11.2.1 Meta-analysis of diagnostic accuracy of HbA1c in high risk populations (OGTT is the reference test)

Variable	Coefficient	Standard Error	z	p>|z|	95% Conf. Interval	
Summary value						
Se	0.58	0.04			0.51	0.65
Sp	0.87	0.02			0.83	0.91
DOR	9.51	1.22			7.39	12.22
LR+	4.58	0.59			3.55	5.90
LR-	0.48	0.03			0.42	0.55
1/LR-	2.08	0.14			1.81	2.38

Also there is almost no difference with the original results when comparing the new analysis with the original analysis.

So it could be concluded that in the high risk populations, using HbA1c as the diagnostic method only can detect around 60% of T2DM which defined by OGTT.

3.4 Publication bias analysis (funnel plots)

When the standard error is used, straight lines may be drawn to define a region within which 95% of points might lie in the absence of both heterogeneity and publication bias.[64]

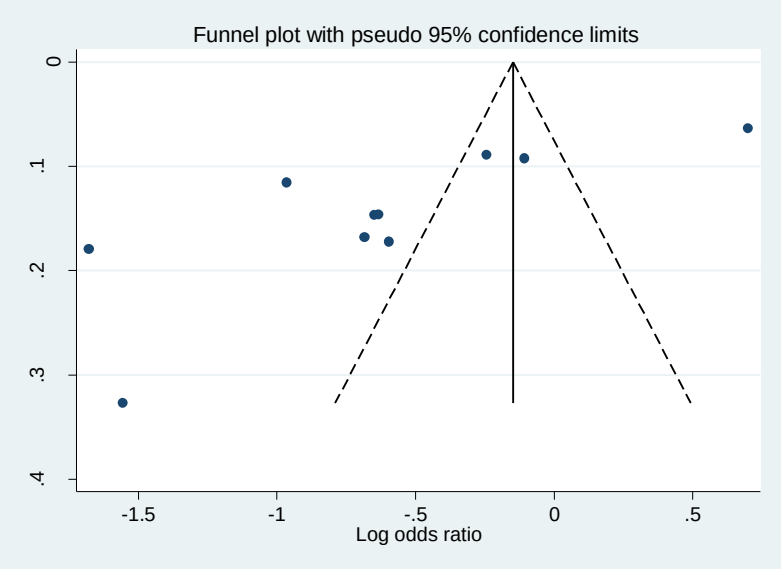

Figure 4 Funnel plot of OR comparing HbA1c with OGTT in community-based populations

In Figure 4, the heterogeneity and possible publication bias of the current analysis were confirmed, as it was shown that most of the studies lied outside of the region.

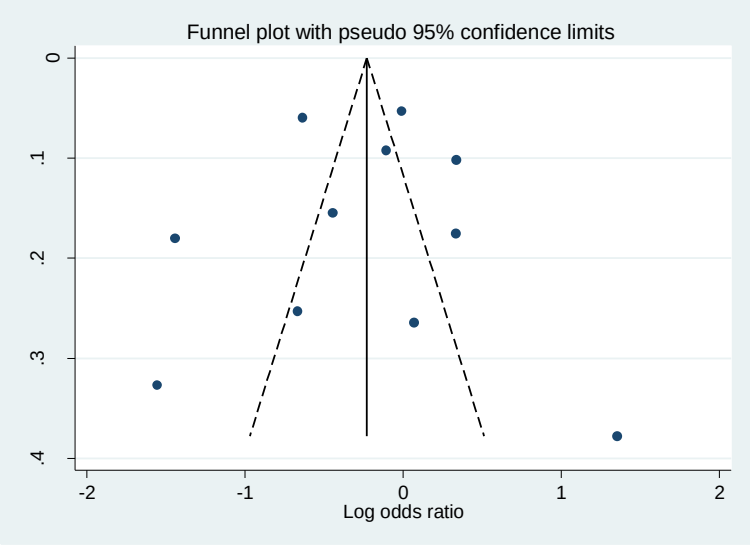

Figure 5 Funnel plots of ORs comparing HbA1c with FPG in community based populations

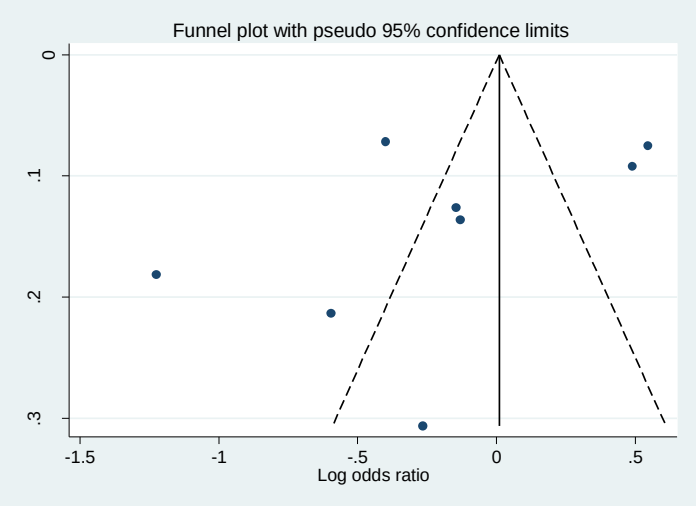

Figure 6 Funnel plot of OR comparing HbA1c with OGTT in high risk populations

Same as above, Figure 5 and Figure 6 did show the existing of publication bias and heterogeneity exist in current analysis.

The funnel plot was not drawn because of few studies were selected for comparing HbA1c with FPG in high risk populations.

For pre-diabetes detecting in community based populations, only four studies were included for analysis in community base populations, funnel plot was not drawn according to the lower validity and specify.

Chapter 4

Discussion

This systematic review and meta-analysis study was conducted on selected diagnostic accuracy tests studies that determined efficacy of the HbA1c test for detecting the T2DM and pre-diabetes. The results of sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, diagnostic odds ratios and receiver operating characteristic curves (ROC), which were defined by HbA1c, were then compared to the results of the OGTT test. The OGTT test was assumed to be the best available method to determine whether or not the patient has T2DM or pre-diabetes. A systematic and comprehensive search of the literature was done to review the eligible primary studies.

All the primary studies had to be cross-sectional studies, because the index test of HbA1c had to be compared with the reference tests of OGTT or FPG. Most of the selected studies were done around 2011, as laboratory equipment, blood collecting and testing skills had to be standardised.

Our analyses demonstrated that HbA1c diagnostic criteria have limitations for using in screening T2DM and pre-diabetes, which includes 1) lower pooled prevalence and lower pooled OR for screening T2DM and pre-diabetes comparing to OGTT in community based populations; 2) high specificity but low sensitivity compared to OGTT in both community based populations and high risk populations; 3) there is no difference of pooled prevalence and pooled OR to detect T2DM in high risk populations compared to OGTT and FPG; 4) equal ability to detect T2DM

compared to FPG in community based populations, as there are not statistically significant with pooled OR.

As the results show, in the community-based subgroup, the prevalence of T2DM detected by HbA1c and OGTT are 6.3% (95%CI 3.8%-9.3%) and 11.7% (95%CI 8.3%-15.6%). The difference is 5.4% which is statistically significant, confirmed by the pooled OR. In absolute terms, the difference is that 5.4% more cases are detected by OGTT. So for every 19 people screened by OGTT, 1 more case of T2DM will be detected compared to HbA1c (NNS). The NNS confirmed the clinical significant of this difference in prevalence.

The prevalence of T2DM detected by HbA1c and FPG, in high risk populations, are 18% (1.5%-46.8%) and 13.2% (1.5%-33.8%). The difference is 4.8% which is statistically significant, as the p value of pooled OR is 0.01. The NNS is 21, which is also clinically significant.

The prevalence of pre-diabetes detected by HbA1c and OGTT are 7.6% (95%CI 0-30.4%) and 32% (95%CI16.9%-49.4%). The difference is 24.4% which is also statistically significant, confirmed by the pooled OR. According to the absolute terms, for every 4 people screened by OGTT, 1 more case of pre-diabetes will be detected compared to HbA1c. Such a small number of NNS confirmed the clinical significance of this difference in prevalence.

In the meta-analysis of odds ratio, it was assumed that OGTT could pick up 100% T2DM and pre-diabetes in community-based populations. When using HbA1c as the diagnostic test, the odds were 0.50 (95% CI 0.31-0.82) times that of the OGTT to define T2DM, and 0.19 (95% CI 0.06-0.63) that of OGTT to define pre-diabetes.

Other analyses were done as well, for comparing HbA1c with OGTT in high risk populations and comparing HbA1c with FPG in community based populations. Differences were found but they were not statistically significant.

Because of the consistent heterogeneity found during the statistical analysis, sensitivity analysis was applied when comparing the HbA1c and the OGTT test for screening T2DM in both community based populations and high risk populations, comparing the HbA1c and FPG test for detecting T2DM in community based populations, and comparing the HbA1c and OGTT in detecting pre-diabetes in community based populations.

In the community based subgroup comparing HbA1c with OGTT for screening T2DM, the high OR study (Greenland and Danish combined study) and the low OR studies (The Arab study and the UK Non-pregnant study) were removed from the original dataset. It was found that individuals in the Greenland study had higher mean values of BMI, systolic and diastolic blood pressure and worse cardiovascular risk profiles, in comparison to populations in the rest of the selected studies. The Inuit migrants (256 people) in Denmark, one of the participant groups, had significantly higher levels of HbA1c than the Danish participants at any given level of FPG and OGTT. The Arab study demonstrated that heterogeneity of the current meta-analysis could be partly explained by ethnicity. Genetic variance was found to be related to different haemoglobin, which could affect the accuracy of HbA1c measurements. The authors of the Arab study had previously demonstrated that diabetes (both diagnosed and undiagnosed) and pre-diabetes were common in this culturally unique, largely immigrant, relatively young community.[65] A multivariate regression analysis was conducted, and it was also found that the decline in B-cell function was a stronger determinant of plasma glucose in Arabs

than the decline in insulin sensitivity, whereas the opposite was true for Japanese- and Mexican-Americans.[66,67] Besides the Arab study, other studies [50,53,56,61]also found that genetic variance was related to different haemoglobin, which could affect the accuracy of HbA1c measurements. Another reason for heterogeneity which could be explained by the Arab study was the age factor. In the Arab study, the proportion of the population who were older than 65 years old was only 6% (30/482). More (68%) younger people (<45yrs) were selected in the analysis. That is why in this ethnic group, HbA1c detected fewer diabetic cases.

When comparing HbA1c and OGTT for detecting T2DM in the high risk subgroup, the Spokane and the LEADER studies were dropped from the original dataset for sensitivity analysis. Although the results did not change much when compared to the original analysis, the authors of the Spokane study still confirmed the ethnicity issue, in regard to glucose concentration and haemoglobin. The LEADER study also found that the ethnicity factor had an influence on the level of HbA1c. A larger proportion of south Asians were detected by use of HbA1c >=6.5% compared with white Europeans. So it was assumed that lower socio-economics and rural populations were related to the higher prevalence of HbA1c. However further investigation of this relationship needs to be done.

Besides the Arab study and UK Non-pregnant study, the Peru study, Shanghai study and the Filipino-American study were removed from the original dataset for sensitivity analysis, when comparing HbA1c with FPG tests in community based populations. It was found that the difference in prevalence defined by FPG and HbA1c, became statistically and clinically significant, when the high OR studies, (Peru study, Shanghai study and Filipino-American study) were removed for sensitivity analysis. The possible explanation for this change could be study

population selection. In the Peru study, the T2DM defined by HbA1c were older, poorer, thinner and more likely to come from rural areas. However, further investigation needs to be done on this specific socio-economic factor, because there was no other study found to prove this assumption. The Shanghai study and the Filipino-American study did not have any proper explanations of heterogeneity, the only possible variable could be ethnicity.

In the pre-diabetes analysis, besides the ethnicity, the Beijing study also did not have any other proper explanation for heterogeneity in the analysis.

In the HSROC curve analysis, the OGTT was assumed to be the “gold standard”, which could screen 100% true positive cases of T2DM, and the HbA1c was the index test. In the community based populations, some studies[51,54,57] were not included in the analysis because of shortage of data for calculating TP, FN, FP and TN. Sensitivity analysis was also applied in HSROC curve analysis. The results of both HSROC curve analysis and sensitivity analysis confirmed that using HbA1c as the diagnostic screening test only, could successfully pick up around 70% of true positives. This result is the same as the prevalence and pooled OR analysis.

In high risk populations, HSROC curve analysis and sensitivity analysis were both conducted. Comparing to the prevalence and pooled OR analysis, OGTT was preferable to HbA1c as a screening test. The only explanation of this change is that OGTT was set up as the reference test, which was considered to be 100% correct. In reality, there is no perfect test to identify cases, so it is worth considering the choice of test methods in different participants. For example the Pochon CHA study, suggested combining FPG and HbA1c to detect T2DM at an early stage.[68] It may be also worth considering the cut-off points of HbA1c for detecting T2DM and pre-diabetes. During the selection process, it was found that quite a few studies

suggested HbA1c, with a cut-off point of 6.1%[68,69,70] to detect T2DM, rather than 6.5%. Thus, in high risk populations, OGTT and HbA1c were considered to have the same ability to screen for T2DM.

Unfortunately, there was not enough data to analyze the diagnostic accuracy – HSROC curve analysis between HbA1c and FPG in both T2DM and pre-diabetes in both subgroups. Also there was not enough data to compare HbA1c with OGTT in screening pre-diabetes in high risk populations, the HSROC curve analysis could not have done either in both subgroups for comparing HbA1c with OGTT.

The funnel plots (Figure 4, Figure 5 and Figure 6) were done to investigate the existence of publication bias and selection bias, as well as the heterogeneity of the study. Selection bias resulted because only published papers (a selected sample) were analysed, although unpublished articles might have shown different results.

As above discussed, HbA1c is not superior to OGTT in both community based and high risk populations; and HbA1c is a better choice comparing with FPG in high risk populations. But a further study is suggested to investigate the comparison of HbA1c with FPG in community based populations because both methods are convenient, but HbA1c costs more.

Secondly, although HbA1c was proposed as the gold standard to detect T2DM internationally, the precision of laboratory measurement in some countries had not yet matched international standards.[56] The precision of laboratory measurements, which were not indicated in most of the studies and were suggested to be improved in lower-or middle- income countries to meet international standards,[28] could also have affected the precision of the results.

Thirdly, some of the studies, such as the Arab study and SIGT combined study, used ADA criteria, while others, such as the Shanghai study, used the WHO criteria. Still others did not specify which was used.

Finally, the lower p values also resulted that most of the variability across studies was due to heterogeneity rather than chance in both subgroups. In most of individual studies, it was also found that A1c criteria had relatively higher sensitivity for detecting both diabetes and pre-diabetes in elder individuals.[45,47] And there was no relative evidence to show that HbA1c could be affected by smoking, anemia, blood pressure and lipid profiles factors.[47,56] But BMI factor contributed to higher level of HbA1c.[44,45,56]

Christensen D and Witte D, etc.[71] did an analysis in different ethnic groups and six studies were included in their study. It was found that diabetes prevalence was lower with the A1C-based diagnostic criteria in four of six studies, when compared with OGTT. Same results were also specified by the current study, and the ethnic issue has to be considered when choosing the diagnostic criteria for detect T2DM.In the Mayko study in Japan,[72] it was found that combining FPG and HbA1c would increase diagnosis of new cases of diabetes and it was suggested these two tests be combined for Japanese patients. The current study referred to two populations. In these, FPG and HbA1c each had their own benefits.

Tanaka Y and Atsumi Y, etc[73]. did another study in Japan, which compared HbA1c with OGTT and supported the current study in regard to DOR. In this Japanese study, HbA1c>=6.5% could define 49% of diabetic subjects who were defined by OGTT, while only 2% of NGT and IGT subjects had an HbA1c>6.5%, and 98% have a value less than 6.5%. The current study shows that the odds of

HbA1c>=6.5% among subjects with T2DM is 35 times higher than the odds for HbA1c>=6.5% among subjects without T2DM.

As George J[29] discussed, there are many factors that could affect the precision and validity of HbA1c measurement. So for new T2DM screening in general populations, HbA1c would not be the first choice.

The limitation of this study is that no sensitivity analysis of residence setting (rural or urban area),[50,53,54] or income variance (low- and middle- income)[47] was conducted. As the primary studies implied, these factors could be related to the haemoglobin, which also could affect HbA1c measurement. So a further sensitivity analysis needs to be considered.

Chapter 5

Conclusions

Compared to OGTT test, HbA1c is not a better choice in community based populations, because of the lower prevalence. When the OGTT test is applied as the “gold standard” test, using HbA1c as a diagnostic screening method can only detect about 70% of the true positive cases of T2DM.

In high risk populations, both the OGTT test and HbA1c test have the same ability to diagnose true T2DM cases. However, the HSROC curve analysis only showed 60% sensitivity for HbA1c, when used for screening T2DM cases.

Comparing HbA1c with FPG, it is found that HbA1c is not superior to FPG in community based populations, but tests in some of the population, such as poorer, older people or hose from rural areas, showed that the FPG test was more sensitive than HbA1c. In high risk populations, HbA1c is definitely superior to FPG.

For screening pre-diabetes, the OGTT test is much more sensitive than HbA1c.

Consistent heterogeneity was found during analysis, and sensitivity analysis was conducted for these heterogeneous studies. The heterogeneity in this study could possibly be explained by age, BMI, ethnicity and the cardiovascular profile.

Chapter 6

Appendix

Appendix 1

The diagnostic accuracy of T2DM defined by HbA1c according to OGTT in community-based study

studyname	sens	spec	sample size	TP	FN	FP	TN	Control positive	reference test
south africa study	46%	96%	819	68	79	27	645	147	OGTT
Qingdao study,china			2332	69	209	187	1867		
Arab study	19%	100%	482	10	42	0	430	52	OGTT
Beijing study	54%	99.50%	903	54	46	4	799	100	OGTT
Filipino-American study	40%	96.80%	933	62	92	27	812	154	OGTT
Shanghai study	50.50%	98.10%	4886	152	149	87	4498	301	OGTT
The new hoorn study	24%	99.00%	2668	26	81	25	2536	107	OGTT

Appendix 2

The diagnostic accuracy of T2DM defined by HbA1c according to OGTT in high-risk study

study name	sens	spec	sample size	TP	FN	FP	TN	Control positive	reference test
Shanghai study	54%	97%	3639	162	139	87	3251	301	OGTT
Hypertensive subjects study	76.70 %	96%	144	23	7	5	109	30	OGTT
Italian Caucasians study	46.60 %	93.90%	1019	61	70	54	834	131	OGTT
Edmonton study	37.90 %	93.60%	3163	215	353	166	2429	568	OGTT
Marshfield study	39.70 %	93.80%	271	31	47	12	181	78	OGTT
Spokane Washington study	75.50 %	77.90%	1358	183	59	247	869	242	OGTT
non-pregnant study,UK	29.20 %	100.00 %	401	52	126	0	223	178	OGTT
silent diabetes study	16.00 %	98.00%	1015	23	120	17	855	143	OGTT

Appendix 3 Meta-analysis of diagnostic accuracy of HbA1c and OGTT in community based populations (OGTT is the reference test)

Variable	Coefficient	Standard Error	z	p>|z|	95% Con. Interval	
Bivariate model						
E(logitSe)	0.89	0.49			-0.06	1.85
E(logitSp)	2.65	0.21			2.24	3.06
Var(logitSe)	1.41	0.97			0.37	5.46
Var(logitSp)	0.29	0.16			0.1	0.86
Corr(logits)	0.036	0.4			-0.68	0.63
HSROC						
Lambda	4.54	0.91			2.76	6.31
Theta	-1.67	0.53			-2.7	-0.63
Beta	-0.79	0.44	-1.79	0.07	-1.66	0.08
S2alpha	1.23	0.71			0.4	3.83
S2theta	0.33	0.2			0.1	1.1

Appendix 4 Meta-analysis of diagnostic accuracy of HbA1c and OGTT in high-risk populations (OGTT is the reference test)

Variable	Coefficient	Standard Error	z	p>|z|	95% Con. Interval	
Bivariate model						
E(logitSe)	0.90	0.49			-0.07	1.87
E(logitSp)	1.90	0.26			139	2.40
Var(logitSe)	1.48	1.1			0.34	6.35
Var(logitSp)	0.44	0.25			0.15	1.32
Corr(logits)	-0.89	0.12			-0.99	-0.30
HSROC						
Lambda	3.23	0.45			2.36	4.11
Theta	-0.95	0.40			-1.73	-0181
Beta	-0.61	0.31	-1.93	0.05	-1.22	0.08
S2alpha	0.17	0.19			0.02	1.57
S2theta	0.76	0.45			0.24	2.45

Chapter 7

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