UNIVERSITY OF THE WITWATERSRAND

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Title: The HbA1c threshold for diagnosis of type 2 diabetes mellitus in an adult Black South African population: A cross-sectional study from 2016 to 2020

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Declaration

I, Dr Wa Safi Guy Roger Safi, student number 2449294, declare that this research report is my work, unassisted except where otherwise stated. The report is being submitted for the fulfilment of a Degree of Master of Science in Epidemiology and Biostatistics at the University of Witwatersrand, Johannesburg. This is the first time it has been submitted.

Signature: Dr Wa Safi G.R Safi

Date: 02 October 2025

Dedication

I dedicate this work to the memory of my beloved mother, **Leonie Kipili,** whose love and wisdom continue to guide me. Her spirit has been a constant source of strength throughout this journey.

To my dearest children, **Ronald** and **Grace Safi**, and their mother, **Ketsee,** your love, patience, and understanding have motivated me to persevere. This accomplishment is as much yours as it is mine.

To my dearest friend **Thato**, for your support through my cancer's fight.

Thank you all for being my pillars of strength.

Abstract

Background

Diabetes mellitus is a growing public health concern in Africa. The HbA1c threshold of 6.5% used for diagnosing diabetes is based on populations of European ancestry and may not be optimal for African populations.

This study aimed to identify an optimal HbA1c threshold for the diagnosis of type 2 diabetes mellitus in a Black South African population and to compare its diagnostic performance using fasting plasma glucose (FPG) and two hour postprandial glucose (2hrPG) criteria.

Methods

A cross-sectional study (2016–2020) in Agincourt, Mpumalanga, was conducted among Black adults aged ≥40 years. HbA1c diagnostic performance was assessed using FPG, 2hrPG, and diabetic retinopathy as the reference standards. A receiver operating characteristic (ROC) curve analysis was used to determine optimal thresholds, sensitivity, specificity, and area under the curve (AUC). We used logistic regression to explore how HbA1c levels are associated with FPG, 2hrPG, hypertension, age, gender, and body mass index (BMI).

Results

Using FPG (standard diagnostic threshold ≥7.0 mmol/L) as the reference, an HbA1c threshold of 6.0% achieved a sensitivity of 100%, specificity of 83%, and AUC of 0.97.The optimal HbA1c threshold was 6.1% when compared to 2hrPG (standard diagnostic threshold ≥11.1 mmol/L), with a sensitivity of 85.0%, specificity of 85.9%, and AUC of 0.89. Diabetic retinopathy could not be meaningfully analysed as only one of 300 participants with interpretable images had diabetic retinopathy, with 157 images uninterpretable or missing.

Conclusion

Our findings indicate that using an HbA1c cut-off of 6.1% presents the best balance between sensitivity and specificity when compared with the results of the oral glucose tolerance test. A cut-off of 6.0% also performed very well against fasting glucose, making it useful in settings where fasting tests are more practical. This highlights the need to adapt HbA1c thresholds to local populations.

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Abbreviations and acronyms

2hrPG	Two hour plasma glucose
aOR	Adjusted odds ratio
ADA	American Diabetes Association
AIC	Akaike information criterion
AUC	Area under the curve
BMI	Body mass index
FPG	Fasting plasma glucose
HAALSI	Health and Ageing in Africa- A longitudinal Study in South Africa
HbA1c	Hemoglobin A1c
HDSS	Health and socio-demographic surveillance system
HIV	Human immunodeficiency virus
IQR	Interquartile range
OGTT	Oral glucose tolerance test
ROC	Receiver operator characteristic
RPG	Random plasma glucose
VIF	Variance inflation factor

1 Introduction

1.1 Background

Diabetes mellitus is a metabolic disease characterised by ongoing hyperglycemia, disruptions in glucose metabolism, fat metabolism and protein metabolism triggered by a lack of insulin secretion, lack of insulin action, or both[1]. Common symptoms of diabetes mellitus include polyuria, thirst, weight loss, and blurred vision[1].

Diabetes mellitus is a chronic disease that significantly impacts individuals, families, and societies worldwide. It affects both men and women, with similar global trends in rising prevalence observed. However, in Africa, sex differences show that age-standardised prevalence rates increased from 3.4% to 8.5% in men and from 4.1% to 8.9% in women between 1980 and 2014[2]. In 2019, the global diabetes prevalence was estimated to be 9.3% (463 million people), projected to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045[2]. Prevalence is generally higher in urban areas (10.8%) compared to rural areas (7.2%) and in developed countries (10.4%) compared to developing countries (4.0%)[2]. Diabetes mellitus is also one of the top ten causes of death globally, accounting for an estimated four million deaths in 2017[2].

In Africa, the burden of diabetes is growing rapidly. In 2010, approximately 12.1 million people were living with diabetes, a number expected to reach 23.9 million by 2030[3]. The prevalence of Type 2 diabetes in sub-Saharan Africa varies significantly, with recorded prevalence ranging from 0.6% in rural Uganda to 12% in urban Kenya[3]. Diabetes prevalence is notably higher in South Africa compared to the rest of sub-Saharan Africa, with rates nearly doubling from 5.5% in 2000 to 9% in 2009[4]. This is significantly greater than the overall regional trend, highlighting the unique challenges faced by South Africa in managing this growing public health concern.

The increasing prevalence of diabetes mellitus has significant consequences for both patients and the general public. On an individual level, diabetes mellitus can lead to various complications and contribute to the progression of other serious diseases, such as cardiovascular and renal diseases, as well as communicable diseases like pneumonia, bacteremia, and tuberculosis[3]. These complications have a substantial impact on morbidity and mortality in the region[3]. Additionally, diabetes mellitus and its related issues place a strain on healthcare systems and can hinder progress made in other health outcomes in sub-Saharan

Africa[5]. In 2015, diabetes mellitus cost around US$19.45 billion (1.2% of sub-Saharan Africa's GDP), and this cost is projected to reach $35.33-$59.32 billion (1.1%-1.8% of GDP) by 2030 if it is not addressed[5]. The lack of data on the prevalence of diabetes mellitus, its complications, and management in the region further complicates decision-making and provision of care when faced with limited resources and competing priorities[5].

1.2 Diagnosis of diabetes mellitus

Accurately diagnosing diabetes is crucial for both clinical care and research, but there is still ongoing debate about the most appropriate biomarker to use. One type of diagnostic test is based on measuring glucose levels. These tests assess fasting plasma glucose (FPG) and/or plasma glucose two hours after a 75 g glucose challenge (known as an oral glucose tolerance test) (2hrPG). FPG ≥7.0 mmol/L or 2hrPG ≥11.1 mmol/L are considered diagnostic of diabetes[6].

Glucose-based biomarkers have their advantages and disadvantages. On the positive side, these tests are generally widely available and relatively inexpensive. They are not affected by hemoglobin glycation, genetic factors or personal attributes like age or ethnicity[7]. However, they do require fasting and only reflect blood sugar at the time of sampling. Glucose levels may also be influenced by comorbidities and pre-analytic factors such as sample handling and transportation[8]. The two hour post-prandial glucose test is the most sensitive, serving as an early indicator of glucose dysregulation.

As an alternative to glucose-based biomarkers, HbA1c has gained attention for the diagnosis of diabetes. HbA1c was initially discovered as a glycated form of hemoglobin that was found to be elevated in people with diabetes mellitus in the late 1960s[1]. While HbA1c was initially used to monitor glycemic control, recent guidelines from the American Diabetes Association (ADA) Standards of Medical Care in Diabetes recommend using an HbA1c threshold of ≥ 6.5% as a diagnostic criterion for diabetes, based on evidence that this level is associated with an increased risk of diabetic retinopathy[6].

HbA1c has its own set of advantages and disadvantages. It reflects glycemic control over the preceding three months and is less influenced by acute illness. It shows less pre-analytic variation and is more consistent than glucose-based tests, while eliminating the need for fasting or extended time commitments[7]. HbA1c, however, can be influenced by non-glycemic factors,

such as anemias, hemoglobinopathies, nutritional deficiencies, kidney failure or variations in red blood cell lifespan[7].

In South Africa, the Society for Endocrinology, Metabolism, and Diabetes of South Africa (SEMDSA) guidelines align with international standards by recommending the same thresholds for FPG, 2hrPG, and HbA1c, along with a random plasma glucose (RPG ≥11.1 mmol/L) measurement in the presence of classic symptoms of diabetes[9].Substitution of HbA1c for glucose-based tests for diagnosing diabetes mellitus, as suggested by some international organisations, may present challenges in African-ancestry populations[10]. Existing evidence indicates individuals of African-ancestry may have higher HbA1c levels for a given blood sugar level compared to individuals of European-ancestry[11]. A cross-sectional quantitative study was conducted in Bellville , South Africa between January 2008 and March 2009, with a population that self-identified as Coloured. The study reported that the optimal threshold for HbA1c for the diagnosis of diabetes mellitus using ROC curves was 6.1%, using FPG ≥7mmol/L as gold standard and with a sensitivity of 80%. When the HbA1c value of 6.5% was used as the threshold, it had a sensitivity of 50% when using FPG[12].

Another study explored the association between laboratory-based FPG and HbA1c in an urban African population. This study found that an HbA1c value of 6.5% in the young Black urban population had a sensitivity of 74.1% and a specificity of 98.1% in identifying diabetes mellitus based on FPG≥7 mmol/L[10.] Based on existing evidence, further research is necessary to determine the appropriate HbA1c threshold for diagnosing diabetes mellitus in African-ancestry populations.

2 Literature review

2.1 Introduction

The internationally-accepted threshold for diagnosing diabetes mellitus is a HbA1c level of 6.5%. However, it is increasingly apparent that a one-size-fits-all approach may not be entirely applicable. This is particularly evident when considering the Black African population, where unique genetic and environmental factors may influence the sensitivity and specificity of the HbA1c benchmark. This literature review examines the HbA1c threshold for the diagnosis of type 2 diabetes in adult Black African populations. It aims to contribute valuable insights into more tailored diagnostic guidelines for this population by synthesising data from various studies. Such nuanced guidelines have the potential to reduce the risk of misdiagnosis while improving the overall effectiveness of diabetes care in Black South Africans.

2.2 The use of HbA1c in the diagnosis of diabetes mellitus

Glycated hemoglobin has emerged as a crucial diagnostic tool for diabetes mellitus. According to the American Diabetes Association, individuals with an HbA1c level ≥ 6.5% are classified as having diabetes mellitus[8]. Unlike fasting blood glucose tests, HbA1c testing does not require fasting before taking a blood sample. Its ease of use makes it more patient-friendly, encouraging more widespread screening and monitoring.

Elevated HbA1c levels have been linked to an increased risk of diabetes-related complications such as retinopathy, nephropathy, and cardiovascular disease. This link emphasises the clinical importance of HbA1c in predicting diabetes outcomes. However, a review conducted by Kowall and Rathmann in 2013 explored the association between high HbA1c levels and complications such as kidney, cardiovascular, and nerve diseases[13]. Kowall and Rathmann's findings raise questions about the universal applicability of the 6.5% HbA1c threshold for predicting diabetes mellitus complications, suggesting potential variation in this threshold, with studies reporting values ranging from 6% to 6.9%[13]. The study also suggests that the threshold for retinopathy may vary, different studies reported different values. A study in China established a cutoff of 6.4%, for the entire group, but after excluding subjects receiving antihyperglycemic medication, the cutoff was 6.7%. In a South Korean study, the ROC-derived cut-off for retinopathy was 6.6% and 6.9% for moderate or severe retinopathy[13]. Furthermore, thresholds of 6.6% and 7.0 % were calculated from ROC in Malay people for mild and moderate retinopathy, respectively[13]. Literature generally recognises HbA1c as a marker for

glycemic control and diabetes complications, but the specific threshold for predicting these complications is nuanced. The variation is attributed to factors such as age, gender, and ethnicity, as highlighted by Kowall and Rathmann[13]. This variability highlights the need for a nuanced understanding of HbA1c thresholds, which may depend on the specific complications and the characteristics of the population under study.

2.3 The benefits and challenges of using an HbA1c of 6.5% in African ancestry populations

A crucial aspect of the literature review centres on the applicability of the HbA1c threshold of 6.5% in populations with African ancestry. Paterson's 2017 study revealed differences in HbA1c levels between Black and White populations for a given level of glycemia, suggesting that individuals of African ancestry tend to have higher HbA1c values compared to their White counterparts despite having similar blood glucose levels[14]. Paterson emphasises that differences in HbA1c levels between Black and White populations are significant, particularly due to the prevalence of certain genetic variants, such as the glucose-6-phosphate dehydrogenase (G6PD) variant, in individuals of African descent. This variant may result in elevated HbA1c levels that do not necessarily correlate with actual glucose levels, potentially leading to misclassification of diabetes status. The misclassification is concerning because it potentially prevents timely diagnosis and treatment for those who actually have diabetes based on glucose measurements.

Cavagnoli et al. conducted a systematic review and discovered that HbA1c levels differ significantly between ethnic groups[15]. In particular, South Asian and African-Caribbean individuals had statistically higher HbA1c levels compared to White Europeans. The meta-analysis included data from 49,238 people across multiple studies and highlighted that these differences were consistent across different populations, including Blacks, Asians and Latinos, compared to Whites. The authors emphasised that the variability in HbA1c levels was primarily due to ethnic differences and not to fluctuations in glucose levels, as the study included studies in adults without diabetes and ensured that the methods for HbA1c measurement were standardised.

A study by Wade et al. in a rural African population examined the use of HbA1c and FPG for diagnosing diabetes. The study found a noticeable difference between the two methods, with poor agreement between HbA1c and FPG[16]. This suggests that the relationship between them is not fully understood and may be influenced by various factors[16].

2.4 Factors other than blood glucose affecting HbA1c

The literature emphasises that factors other than blood glucose can affect HbA1c levels. Kowall and Rathmann identified several factors influencing HbA1c levels beyond glucose levels and highlighted the complexity of interpreting these values due to nonglycemic influences. A key factor is red blood cell turnover. Conditions that reduce red blood cell turnover, such as iron deficiency, kidney failure or vitamin B12 deficiency, lead to higher HbA1c levels. In contrast, diseases that shorten the lifespan of red blood cells, such as hemolytic anemia and chronic liver disease, result in lower HbA1c levels. Genetic factors also play an essential role, as evidenced by twin studies indicating that hereditary elements influence HbA1c levels. In addition, individual characteristics such as hemoglobinopathies (e.g. HbS, HbC, HbD), age and ethnicity significantly influence HbA1c levels. These results highlight the importance of considering a wide range of nonglycemic conditions when interpreting HbA1c values[13].

2.5 Conclusion

In conclusion, the literature review emphasises the critical role of HbA1c in diagnosing diabetes mellitus. The evidence suggests that a universal HbA1c threshold of 6.5% may not be suitable, especially in populations with African ancestry, due to genetic variability and other influencing factors. The review emphasises the need for population-specific guidelines to accurately diagnose diabetes, taking into account factors beyond blood glucose, such as age and ethnicity.

3 Problem statement

The current diagnostic threshold for diabetes mellitus of HbA1c of 6.5% may not accurately diagnose diabetes in African ancestry populations. It is necessary to assess its diagnostic accuracy in African ancestry populations in comparison to glucose-based tests, such as fasting plasma glucose and oral glucose tolerance tests, considering potential genetic and environmental factors that can affect HbA1c levels.

4 Justification

Using HbA1c for diabetes mellitus diagnosis is widely recommended. However, in African ancestry individuals, an inappropriate HbA1c threshold may result in under-diagnosis or over-diagnosis of diabetes mellitus, which has implications for individuals and the healthcare system. Determining a population-specific threshold would ensure an accurate classification of glycemic status.

5 Research question

What is the appropriate HbA1c threshold for diagnosing diabetes mellitus in individuals of African ancestry?

6 Objectives

The objectives of this study are as follows:

1. To identify the optimal threshold of HbA1c for identifying type 2 diabetes mellitus and compare it to the current recommended threshold in individuals of African ancestry.
2. To compare risk factors for type 2 diabetes mellitus identified using FPG, 2hrPG and study-derived HbA1c diagnostic criteria.
3. To investigate the association between HbA1c≥6.5% and diabetic retinopathy.
4. To measure the agreement between diabetes classification using HbA1c and the presence of diabetic retinopathy using retinal photography.

7 Aims and methodology

7.1 Introduction

This study aimed to establish an optimal HbA1c threshold for diagnosing type 2 diabetes mellitus in an adult population of Black African ancestry and to compare its diagnostic accuracy with FPG and 2hrPG criteria.

During household interviews and clinic visits, socio-demographic information, health status, and metabolic information were collected. Blood samples were analysed for FPG, 2hrPG and HbA1c, while retinal photography assessed diabetic retinopathy. Rigorous data management practices ensured accuracy and confidentiality, while statistical analysis, including descriptive statistics, logistic regression and ROC curve analysis were used to identify the optimal HbA1c threshold and evaluate its diagnostic performance compared to glucose-based criteria.

7.2 Study design and primary study

A primary cross-sectional study was conducted from 2016 to 2020 among Black adults aged ≥ 40 years residing in Agincourt, Bushbuckridge, Mpumalanga. Each participant was assessed only once over five years without repeated measurements. In 2014, the population under study had a prevalence of diabetes mellitus of about 11%[17].

The Agincourt Health and Socio-Demographic Surveillance System (HDSS) covers an area of 420 square kilometres and includes 120,000 people living in 21,000 households across 31 villages. This location is in South Africa's semi-arid rural northeast, 40 kilometres west of the Mozambican border.

A convenience primary study sample of 457 individuals aged 40 years or older from the Agincourt Health and Socio-Demographic Surveillance System (HDSS) was recruited by Health and Ageing in Africa-a Longitudinal Study in an INDEPTH community (HAALSI) and the Africa Wits- INDEPTH partnership for Genomic Studies (AWI- Gen). To be eligible, individuals had to be 40 years of age or older, residents in the study area, self-identify as Black, and be able to fast. Those with a known history of diabetes mellitus, taking glucose-lowering medication, and pregnant individuals were excluded.

7.3 Data collection

Primary data were collected through household and clinic visits. During household visits, participants from the sample provided socio-demographic and health status information. During clinic visits, venous blood samples were collected for FPG analysis in potassium oxalate/sodium fluoride tubes and in EDTA tubes for HbA1c analysis.

HbA1c was measured once for each patient during the study duration. Following blood collection, fasting participants were given a 75 g oral glucose solution. Two hours after finishing the solution, a second venous sample was taken for the glucose assay. Using colorimetric techniques, plasma glucose levels were measured on the Randox Plus clinical chemistry analyser, with a coefficient of variation (CV) of less than 2-3%. The Vironostika Uniform II screening assay (Biomerieux, France) was used to determine HIV serostatus from dried blood spots. Positive results were verified with Roche Elecsys (Roche, USA).

During visits, a qualified fieldworker used a non-mydriatic fundal camera to take retinal photographs, which an ophthalmologist later evaluated and classified each participant as either having diabetic retinopathy or not.

7.4 Data management

Data were kept on servers at the Sydney Brenner Institute for Molecular Bioscience, where strict enforcement of access controls ensured that sensitive data could only be accessed by authorised individuals.

To maintain confidentiality, de-identified data were received for analysis. As part of the data cleaning protocol, systematic methods were developed to locate and resolve errors or outliers. Transparency and reproducibility were ensured through careful documentation of all cleaning process steps. This comprehensive data management approach adhered to best practices, promoting the safe, ethical, and efficient handling of information throughout the research process. Data were analysed to examine relevant variables, including age, gender, and data types, ensuring consistency and accuracy across these parameters. Checks were performed to ensure the appropriate format of the variable, internal consistency, length, and identification of outliers within the variable. Various techniques, including data visualisation and graphical analysis, were used to remove missing data proxies, coding errors, and misspellings. The data cleaning process was performed using R software.

7.5 Data analysis

Categorical variables, such as gender and the presence of diabetic retinopathy, were described using frequencies and percentages to represent the distribution across the sample group. The distribution of continuous variables such as age and body mass index was examined using visual representations to summarise the data accurately. Additionally, the number of individuals with retinal changes compatible with diabetes mellitus was described, highlighting the prevalence of a diabetes-related complication in the study population. The proportion of individuals meeting diabetes criteria was determined based on FPG, 2hrPG, and study-derived HbA1c diagnostic thresholds. This detailed descriptive analysis laid the foundation for subsequent inferential analyses.

An evaluation of the performance of HbA1c as a diagnostic tool was made to determine its ability to identify individuals meeting the criteria for diabetes based on FPG and 2hrPG levels. The Youden index was used to identify the HbA1c threshold that optimised sensitivity and specificity, balancing accurate positive detections and minimising false negatives. Further analysis of the performance of HbA1c, using the ROC, was undertaken to assess its sensitivity and specificity at a threshold of 6.5% to identify individuals meeting glucose-based criteria for diabetes and its accuracy in differentiating between people with or without diabetic retinopathy. We also calculated the AUC to measure how accurately HbA1c could distinguish between individuals with and without diabetes mellitus.

Logistic regression was used to identify risk factors for diabetes across different diagnostic criteria; FPG $\geq$ 7.0 mmol/L, 2hrPG $\geq$ 11.1 mmol/L and standard and study-derived HbA1c. To select the model, an assessment was made of how accurate and valid the selected regression model was, using various goodness-of-fit tests for this study's purposes. To assess the model's calibration and alignment with real results, the Hosmer-Lemeshow test was used to compare predicted results with observed data. A calibrated model means that its predictions are mainly consistent with results within the target population. To maintain the credibility of study estimates, an examination of variance inflation factors (VIF) was conducted to detect any multicollinearity issues among the predictors and to ensure that correlated variables did not bias the results. Checks were conducted to identify any data points and key observations that could significantly affect the results. By addressing these concerns, the model's strength and accuracy were improved. Variables included in the multivariate logistic regression model were

selected based on their established clinical and biological relevance to diabetes mellitus, as they are well-documented risk factors influencing glycemic control. The backward and forward selection techniques were also used. Additionally, age and gender were retained in the model as they are essential demographic factors. This approach ensured that the final model captured both statistically significant associations and clinically meaningful predictors, enhancing its interpretability and applicability in the real world.

The relationship between HbA1c levels and diabetic retinopathy was examined using the Mann-Whitney test to determine if there was a statistically significant difference in HbA1c levels between the two retinopathy groups. The Cohen's Kappa statistic was used to evaluate the level of agreement between the diagnosis of diabetes mellitus based on HbA1c ≥6.5% and the detection of diabetic retinopathy. This approach allowed for quantifying the consistency between these two diagnostic methods while accounting for the possibility of agreement occurring by chance. A high Kappa value indicated a strong level of agreement, suggesting a close relationship between HbA1c ≥ 6.5% and the presence of diabetic retinopathy in identifying individuals with diabetes.

In summary, a comprehensive approach was taken to evaluate the diagnostic performance of HbA1c. Through descriptive analyses, the population characteristics were clearly understood, while ROC curve analysis quantified the diagnostic accuracy of HbA1c. Univariate and multivariate analyses identified significant predictors of diabetes mellitus classified based on different glycemic and HbA1c thresholds, and goodness-of-fit assessments confirmed the robustness of the predicted models. Kappa statistics provided a key measure of agreement between HbA1c cut-off values and diabetic retinopathy. R-version & Rstudio version 2024.12.1+563 were used to conduct the statistical analysis. P values < 0.05 were considered statistically significant.

8 Ethical considerations

Each study participant gave written informed consent, and the concepts of anonymity, confidentiality, and voluntary participation were upheld. The ethics approval numbers were M141157 and M1911199 (Wits HREC) and MP 2015RP12-260 (Mpumalanga Research Ethics Committee).

9 Results

9.1 Introduction

This chapter reports the outcomes of a cross-sectional investigation carried out between 2016 and 2020, which aimed to establish a suitable HbA1c level for diagnosing type 2 diabetes mellitus in an adult South African population of Black descent. The ROC was used to compare various HbA1c thresholds to the gold standard thresholds of FPG ≥ 7 mmol/L and 2hrPG ≥11.1 mmol/L. Logistic regression models were used to examine the associations between diabetes mellitus defined by different criteria and different variables such as gender, age, BMI, and hypertension.

9.2 Descriptive statistics

Descriptive characteristics of the study population

Table 1. Clinical and demographic characteristics of the study population by 2 hour plasma glucose-defined diabetes status

Characteristic	**No diabetes** N = 411[1]	**Diabetes** N = 21[1]
Age (years)	54 (9)	53 (7)
Gender		
Female	239 (58%)	12 (57%)
Male	172 (42%)	9 (43%)
HIV status		
Negative	235 (60%)	12 (60%)
Positive	159 (40%)	8 (40%)
Unknow	0 (0%)	0 (0%)
No answer	17	1
Family history of diabetes		
No	350 (85%)	14 (70%)
Yes	52 (13%)	5 (25%)
Unknow	9 (2.2%)	1 (5.0%)
No answer	0	1
Diabetic retinopathy		

Characteristic	No diabetes N = 411[1]	Diabetes N = 21[1]
Image missing or uninterpretable	127 (31%)	9 (43%)
No	284 (69%)	11 (52%)
Yes	0 (0%)	1 (4.8%)
Alcohol consumption	133 (32%)	6 (29%)
Body mass index(kg/m^2)	27 (7)	30 (6)
Unknown	1	0
Hypertension(mmHg)		
No	204 (50%)	6 (29%)
Yes	207 (50%)	15 (71%)

Categorical data are presented as n(%); continuous data are presented as mean (standard deviation)

Table 1 presents the clinical and demographic characteristics of participants grouped by 2hrPG-defined diabetes classification, with 21(4.9%) individuals classified as having diabetes.

Of the 457 individuals enrolled, 432 had complete data for the variables included, and are therefore presented in the table. Participants with missing information were not included in this analysis. The mean age of participants with diabetes was 53 years (SD 7), which was slightly lower but not meaningfully different from those without diabetes who had a mean age of 54 years (SD 9). The gender distribution was also very similar between the two groups, with women representing 57% of the diabetes group and 58% of the group without diabetes.

Clearer differences emerged in body composition and cardiovascular risk factors. Participants with diabetes had a higher average BMI (30 kg/m², SD 6) compared to those without diabetes (27 kg/m², SD 7). Hypertension was also more common among those with diabetes, affecting 71% compared to 50% of the group without diabetes . Family history of diabetes showed a similar pattern: 25% of those with diabetes reported a positive family history, compared to 13% of those without. Diabetic retinopathy was very rare in the study population overall, but one participant (4.8%) in the diabetes group was identified with retinopathy, whereas none were observed in the group without diabetes. A higher proportion of participants in the diabetes group also had missing or uninterpretable retinal images (43% vs 31%). Due to the high number

of uninterpretable or missing retinal images and the low prevalence of retinopathy, no further analyses relating to retinopathy are presented in this report.

For HIV status, the distribution was identical in relative terms: 40% of participants in both groups were HIV positive and 60% were HIV negative. Alcohol consumption did not differ substantially, with 29% of the diabetes group and 32% of the non-diabetic group reporting use.

9.3 Inferential and modelling analysis

An evaluation of the diagnostic performance of HbA1c using FPG ≥ 7.0 mmol/L and 2hrPG ≥ 11.1 mmol/L diagnostic thresholds as reference standards was made. These standards are widely acknowledged for their precision and are regarded as the gold standard for diagnosing diabetes.

9.3.1 ROC analysis using fasting plasma glucose as a response and HbA1c as a predictor

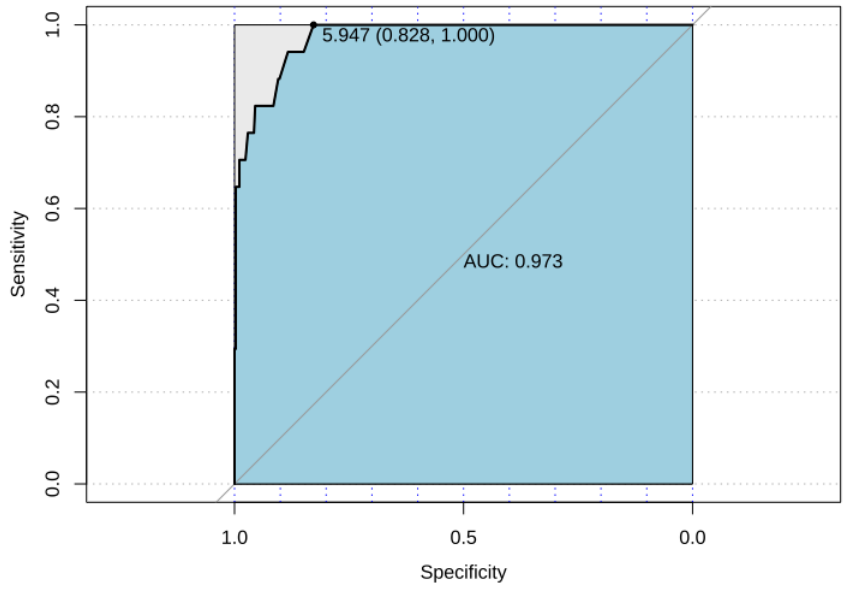

Figure 1:ROC curve analysis for optimal HbA1c cut-off values for diagnosis of diabetes mellitus using fasting plasma glucose in the population of Agincourt, Bushbuckridge, Mpumalanga from 2016 to 2020

The ROC curve (Figure 1) from the study illustrates the performance of HbA1c in diagnosing diabetes mellitus using FPG ≥ 7 mmol/L as the reference standard. The AUC was 0.97, indicating excellent discriminatory power and a highly reliable ability to differentiate between individuals with and without diabetes. The optimal threshold of 6.0% achieved a sensitivity of 1.00 and a specificity of 0.83, reflecting a high capacity to correctly identify all true positive

cases while maintaining strong specificity to exclude negative cases. The curve's proximity to the top-left corner of the plot underscores the accuracy of this diagnostic model, confirming that HbA1c at this threshold was a robust predictor of diabetes within the context of this study's population.

9.3.2 ROC analysis using two-hour plasma glucose as a response and HbA1c as a predictor.

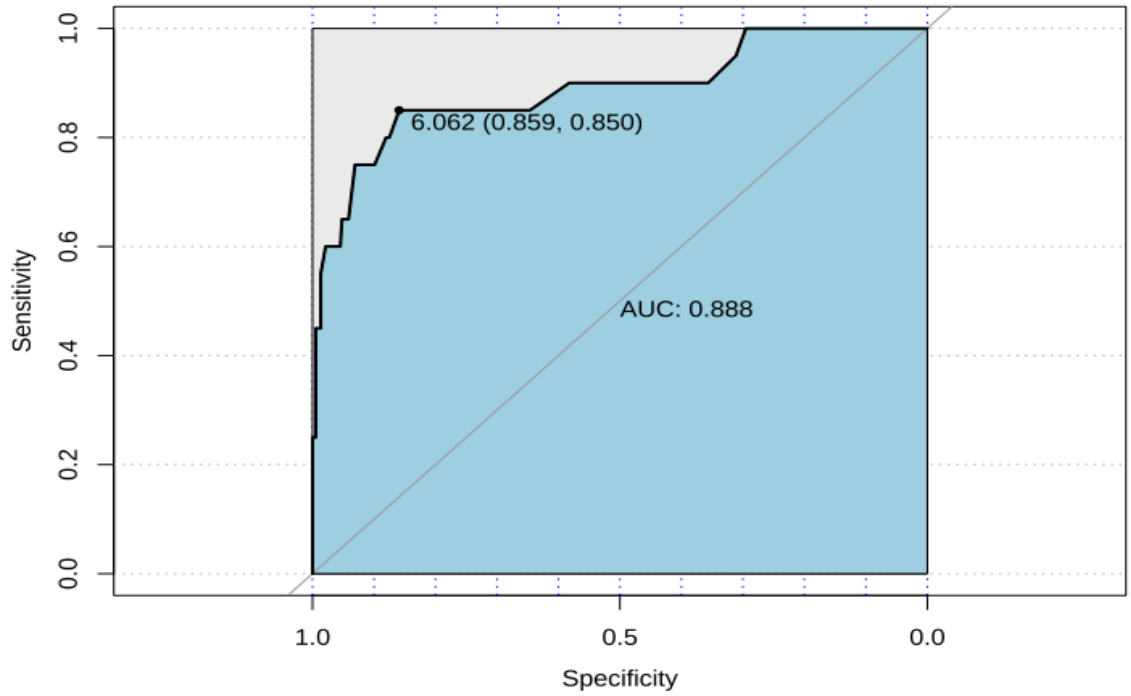

Figure 1: ROC curve analysis for optimal HbA1c cut-off values for the diagnosis of diabetes mellitus using two hour plasma glucose as a reference in the population of Agincourt, Bushbuckridge, Mpumalanga from 2016 to 2020

The ROC curve (Figure 2) from this study demonstrates the diagnostic performance of HbA1c in identifying diabetes mellitus using 2hrPG≥11.1 mmol/L as a reference standard. With an AUC of 0.89, the model showed strong discriminatory power, indicating its effectiveness in distinguishing between individuals with and without diabetes mellitus. A threshold of 6.1% was identified as optimal, achieving a sensitivity of 0.86 and a specificity of 0.85. These metrics reflected a balanced performance, where 86% of true positive cases and 85% of true negative cases were correctly identified. The curve's deviation from the diagonal line further supported its robustness, highlighting the suitability of HbA1c as a diagnostic tool in the study population.

9.3.3 Comparison of diagnostic performance: standard HbA1c threshold vs. study-derived thresholds

Table 2: Diagnostic performance of different HbA1c threshold using different reference standards

HbA1c threshold	N	Sensitivity	Specificity	AUC
HbA1c ≥ 6.0%				
Fasting plasma glucose	457	1.0	0.83	0.97
HbA1c ≥ 6 .1 %				
Two-hour plasma glucose	457	0.85	0.86	0.89
HbA1c ≥ 6.5%				
Fasting plasma glucose	457	0.83	0.93	
Two hour plasma glucose	457	0.75	0.93	

Table 2 shows the diagnostic performance of the HbA1c threshold using different reference standards.

The study-derived HbA1c thresholds of 6.0% (using FPG) and 6.1% (using 2hrPG) exhibited slightly higher sensitivity but lower specificity compared to the standard 6.5% threshold. Specifically, the 6.1% threshold achieved a sensitivity of 85% and a specificity of 86% (AUC: 0.89), whereas the 6.0% threshold achieved a sensitivity of 100% but a specificity of 83% (AUC: 0.97). The higher sensitivity of the lower HbA1c thresholds (6.0% and 6.1%) suggested a greater ability to detect true diabetes cases. However, their lower specificity implied an increased risk of false positives, which might lead to overdiagnosis.

In contrast, the diagnostic performance of the standard HbA1c threshold of 6.5% was evaluated using two reference standards: FPG and 2hrPG. For FPG ≥7.0 mmol/L, the sensitivity was 83%, with a specificity of 93%. Similarly, when using 2hrPG ≥11.1 mmol/L as the reference, the sensitivity was 75%, and the specificity was 93%.

9.3.4 Prevalence of diabetes mellitus by diagnostic criteria

Table 3:Diabetes mellitus prevalence across biomarkers

Diagnostic threshold	Individuals with diabetes (n)	Total tested (n)	%	95% confidence intervals
FPG ≥ 7.0 mmol/L	17	416	4.1	(2.5-6.6)
2hrPG ≥ 11.1 mmol/L	21	432	4.9	(3.1-7.5)
HbA1c ≥ 6.0%	74	401	18.5	(14.8-22.7)
HbA1c ≥ 6.1%	63	401	15.7	(12.4-19.7)
HbA1c ≥ 6.5%	41	401	10.2	(7.5-13.7)

Table 3 outlines how diabetes prevalence changed depending on the diagnostic method used, specifically FPG, 2hrPG, and three different HbA1c thresholds. People with missing data were excluded. Across a sample of 401 to 432 individuals, the percentage identified as having diabetes varied notably. Using FPG ≥ 7.0 mmol/L, 4.1% were classified as having diabetes, while 2hrPG ≥ 11.1 mmol/L identified 4.9%. The HbA1c criteria showed a wider spread: 18.5% at a threshold of ≥ 6.0%, 15.7% at ≥ 6.1%, and 10.2% at ≥ 6.5%. These results highlighted the significant impact of the choice of diagnostic tool and cutoff point on estimates of diabetes prevalence, with the lowest HbA1c threshold capturing the highest proportion of cases.

The variation in sample sizes across Tables 1–3 reflects differences in data completeness for specific variables. Table 1 (N = 432) presents descriptive characteristics and therefore includes only participants with complete demographic and clinical data, excluding those with missing data. Table 2 (N = 457) presents the diagnostic performance of HbA1c against FPG and 2hrPG, and thus includes all participants with valid HbA1c and glucose measurements (the full analytic sample). Table 3 (N = 401–432) presents diabetes prevalence across biomarkers, with the range reflecting exclusion of individuals with missing data for the biomarker under analysis—for example, 432 participants had valid 2hrPG results, while only 401 had complete HbA1c data.

9.3.5 Logistic regression analysis

Univariate logistic regression table

Table 4 presents the findings of a univariate logistic regression analysis of factors associated with different HbA1c thresholds.

For HbA1c≥6.0%, the univariate logistic regression analysis also identified significant associations with key metabolic factors. FPG had an OR of 2.52 (95% CI: 1.87–3.54, $p < 0.001$), while 2hrPG showed an OR of 1.47 (95% CI: 1.32–1.65, $p < 0.001$). BMI was significantly associated with HbA1c ≥ 6.0%, with an OR of 1.06 (95% CI: 1.03–1.10, $p < 0.001$). Family history of diabetes had an OR of 1.68 (95% CI: 0.90–3.07, $p = 0.095$), which was not statistically significant. Similar to HbA1c≥6.1%, age, gender, hypertension, HIV status, smoking, and alcohol consumption did not show significant associations with HbA1c ≥ 6.0% ($p > 0.05$).

The univariate logistic regression analysis for HbA1c≥ 6.1% revealed several significant associations. FPG showed a strong positive association, with an odds ratio (OR) of 2.92 (95% CI: 2.11–4.28, $p < 0.001$). Similarly, 2hrPG was significantly associated with HbA1c≥6.1%, with an OR of 1.62 (95% CI: 1.43–1.87, $p < 0.001$). BMI also demonstrated a significant association, with an OR of 1.08 (95% CI: 1.04–1.12, $p < 0.001$).

1 *Table 4: Univariate logistic regression analysis for factors associated with different threshold of HbA1c*

	HbA1c ≥ 6.0%				HbA1c ≥ 6.1%				HbA1c ≥ 6.5%			
Characteristic	**N**	**OR[1]**	**95% CI[1]**	**p-value**	**N**	**OR[1]**	**95% CI[1]**	**p-value**	**N**	**OR[1]**	**95% CI[1]**	**p-value**
Gender	401				401				401			
Female		—	—			—	—			—	—	
Male		0.95	0.60, 1.50	0.8		0.88	0.50, 1.51	0.6		0.76	0.38, 1.47	0.4
Age(years)	401	1.00	0.98, 1.03	0>0.9	401	0.98	0.95, 1.01	0.2	401	0.99	0.95, 1.02	0.4
Fasting plasma glucose (mmol/L)	394	2.52	1.87, 3.54	<0.001	394	2.92	2.11, 4.28	<0.001	394	2.95	2.12, 4.39	<0.001
Two hour plasma glucose (mmol/L)	396	1.47	1.32, 1.65	<0.001	396	1.62	1.43, 1.87	<0.001	396	1.68	1.47, 1.96	<0.001
Body mass index(kg/m^2)	400	1.06	1.03, 1.10	0.001	400	1.08	1.04, 1.12	<0.001	400	1.08	1.03, 1.13	<0.001
Hypertension	401				401				401			
No		—	—			—	—			—	—	
Yes		1.23	0.78, 1.95	0.4		1.12	0.65, 1.93	0.7		1.71	0.89, 3.42	0.11
HIV status	383				383				383			
Negative		—	—			—	—			—	—	
2												
Positive		0.86	0.53, 1.4	0.6		0.72	0.39, 1.29	0.3		0.95	0.47, 1.88	0.9
Family history of diabetes	400				400				400			
No		—	—			—	—			—	—	

Yes		1.68	0.90, 3.07	0.095		2.24	1.12, 4.31	0.018		2.54	1.15, 5.31	0.016
Don’t know		0.00		>0.9		0.00		>0.9		0.00		>0.9
Smoking	401				401				401			
No		—	—			—	—			—	—	
Yes		0.69	0.32, 1.42	0.3		0.30	0.09, 0.79	0.029		0.42	0.10, 1.26	0.2
Don’t know		0.72	0.20, 2.12	0.6		0.21	0.01, 1.06	0.13		0.39	0.02, 2.05	0.4
No answer		0.70	0.42, 1.16	0.2		0.49	0.26, 0.87	0.018		0.66	0.32, 1.31	0.2
Alcohol consumption	401				401				401			
No		—	—			—	—			—	—	
Yes		0.80	0.48, 1.30	0.4		0.62	0.32, 1.13	0.14		0.57	0.25, 1.19	0.2

OR[1] = Odds Ratio, CI = Confidence Interval

3

Family history of diabetes was another significant factor, with an OR of 2.24 (95% CI: 1.12–4.31, p = 0.018). However, age, gender, hypertension, HIV status, smoking, and alcohol consumption did not show significant associations with HbA1c ≥6.1% (p > 0.05).

There was no significant association between gender and an HbA1c threshold of 6.5% (OR 0.76; 95% CI 0.38 -1.47, p=0.4). Age had an OR of 0.99 (95% CI: 0.95-1.02) with a p-value of 0.4, indicating no significant association between age and HbA1c≥ 6.5%. FPG level was significantly associated with HbA1c≥6.5%, with an OR of 2.95 (95% CI: 2.12 -4.39) and a p-value < 0.001. Similarly, 2hrPG had an OR of 1.68 (95% CI: 1.47 -1.96) with a p-value < 0.001, indicating a strong association with HbA1c≥6.5%. There was also a significant association between BMI and HbA1c≥ 6.5% with a p-value < 0.001 and an OR of 1.08 (95% CI: 1.03-1.13). Hypertension had an OR of 1.71 (95% CI: 0.89-3.42) with a p-value of 0.11, indicating it was not significantly associated with HbA1c ≥ 6.5%.

HIV status, family history of diabetes, smoking, alcohol consumption and age were also analysed, with mixed results. Family history of diabetes showed a significant association, with an OR of 2.54 (95% CI: 1.15 to 5.31) and a p-value of 0.016; however, other variables like alcohol consumption, smoking, and HIV status were not associated with HbA1c≥ 6.5% with p-values greater than 0.05.

Table 5 presents the findings of a multivariate logistic regression analysis. An analysis of various factors, including gender, FPG, 2hrPG,BMI, age, and hypertension, and their associations with different HbA1c thresholds was performed.

The multivariate logistic regression analysis identified FPG and 2hrPG as significant predictors of HbA1c ≥6.0%. Fasting plasma glucose had an OR of 2.17 (95% CI:1.47–3.41, p < 0.001), indicating a strong association between higher FPG levels and HbA1c ≥ 6.0%. 2hrPG had an OR of 1.39 (95% CI: 1.20–1.62, p < 0.001), further supporting the relationship between postprandial glucose levels and HbA1c. BMI was not associated with HbA1c ≥ 6.0%, with an OR of 1.04 (95% CI: 0.99–1.09, p = 0.101). Age was also not associated to HbA1c≥6.0. The multivariate logistic regression analysis for HbA1c≥6.1% identified FPG, 2hrPG, and BMI as significant predictors of diabetes mellitus. Fasting plasma glucose had an odds ratio of 1.93 (95% CI: 1.3–3.0, p < 0.001), indicating that higher FPG levels were significantly associated with HbA1c ≥ 6.1%. 2hrPG also showed a strong positive association with an OR

of 1.43 (95% CI: 1.2–1.7, p < 0.001). BMI was another significant predictor, with an OR of 1.08 (95% CI: 1.0–1.1, p = 0.005), suggesting that higher BMI increased the likelihood of HbA1c ≥6.1%. Age was not associated with HbA1c ≥6.1% (OR = 0.98, 95% CI: 0.94–1.02, p = 0.449), indicating that after adjusting for other variables, age did not independently influence HbA1c levels.

The multivariate model retained FPG, and 2hrPG with p-values < 0.001 as factors associated with HA1c ≥ 6.5%. The variable gender was added a priori. The odds ratio for men was 0.96, with a 95% confidence interval of 0.3 - 2.5 and a p-value of 0.938, indicating that after adjusting for the other variables in the study model, there was no statistically significant association between gender and HbA1c ≥6.5%. FPG showed a strong positive association with HbA1c ≥6.5% with an OR of 1.86 (95% CI: 1.3-2.9, p <0.001). The 2hrPG also showed a significant positive association with HbA1c ≥6.5%, with an OR of 1.46 (95% CI: 1.2-1.8) and a p-value<0.001. The result indicated that higher 2hrPG levels were significantly associated with an increased likelihood of HbA1c≥6.5%. BMI had an OR of 1.06 (95% CI: 0.98 -1.13) with a p-value of 0.450, suggesting BMI was not associated with HbA1c≥ 6.5%.

Overall, the multivariate logistic regression analysis identified fasting plasma glucose, 2hrPG and BMI as significant predictors of different HbA1c thresholds, while gender, hypertension, and age did not appear to have a significant influence when these other variables were considered.

Table 5: Multivariate logistic regression analysis for factors associated with different HbA1c thresholds.

	HbA1c ≥ 6.0%			HbA1c ≥ 6.1%			HbA1c ≥ 6.5%		
Variable	**OR[1]**	**95% CI[1]**	**p-value**	**OR[1]**	**95% CI[1]**	**p-value**	**OR[1]**	**95% CI[1]**	**p-value**
Gender			0.452			0.405			0.938
Female	—	—		—	—		—	—	
Male	1.27	0.68, 2.38		1.45	0.60, 3.47		0.96.	0.36, 2.46	
Fasting plasma glucose (mmol/L)	2.18	1.47, 3.41	<0.001	1.93	1.32, 3.00	<0.001	1.86	1.27, 2.89	0.001
Two hour plasma glucose (mmol/L)	1.39	1.20, 1.62	<0.001	1.43	1.23, 1.68	<0.001	1.46	1.23, 1.76	<0.001
Body mass index (kg/m^2)	1.04	0.99, 1.10	0.101	1.08	1.02, 1.14	0.005	1.06	0.98, 1.13	0.125
Age	0.97	0.94,1.01	0.101	0.98	0.94,1.03	0.449	0.98	0.93,1.13	0.450
Hypertension			0.644			0.122			0.892
No hypertension	-	-		-	-		-	-	
Hypertension	0.85	0.43,1.69		0.55	0.25,1.17		1.07	0.40,2.90	

OR[1] = Odds Ratio, CI = Confidence Interval,

Table 6 presents the associations between selected risk factors and diabetes defined by FPG and 2hrPG. Gender was not significantly associated with diabetes, regardless of the definition used. Compared with females, males had slightly lower odds of diabetes when defined by FPG (OR = 0.80, 95% CI 0.2–2.1, p=0.683) and higher odds when defined by 2hrPG (OR = 1.46, 95% CI 0.55–3.78), but in both cases the confidence intervals were wide and included unity. Similarly, age did not demonstrate any significant relationship with diabetes, with odds ratios close to one under both diagnostic definitions (FPG: OR = 1.00, 95% CI 0.9–1.1; 2hrPG: OR = 0.97, 95% CI 0.92–1.02, p=0.332).

Hypertension was also not significantly associated with an increased risk of diabetes. While no relationship was observed with FPG-defined diabetes (OR = 0.94, 95% CI 0.3–2.8, p=0.905), a positive but borderline non-significant trend was noted for 2hrPG-defined diabetes (OR = 2.31, 95% CI 0.9–7.1, p = 0.089). By contrast, BMI was a strong predictor for FPG-defined diabetes (OR=1.09,95%CI1.0-1.2, p=0.018. Each one-unit increase in BMI was associated with a 9% increase in the odds of diabetes defined by FPG. There was no association between BMI and 2hrPG-defined diabetes (OR = 1.05, 95% CI 1.0–1.1, p = 0.127).

Table 6. Multivariate logistic regression analysis for factor associated with fasting plasma glucose and 2 hour plasma glucose

	Fasting Plasma Glucose			2 Hour Plasma Glucose		
Variable	**OR[1]**	**95% CI[1]**	**p-value**	**OR[1]**	**95% CI[1]**	**p-value**
Gender			0.683			0.434
Female	—	—		—	—	
Male	0.80	0.24, 2.31		1.46	0.55, 3.78	
Age	0.99	0.94, 1.06	0.801	0.97	0.92,1.03	0.332
Hypertension			0.905			0.089
No hypertension	-	-	-	-	-	
Hypertension	0.94	0.32,2.81		2.31	0.85,7.05	
Body mass index (kg/m^2)	1.09	1.02, 1.17	0.018	1.05	0.99, 1.12	0.127

OR[1] = Odds Ratio, CI = Confidence Interval,

	Fasting Plasma Glucose			2 Hour Plasma Glucose		
Variable	**OR[1]**	**95% CI[1]**	**p-value**	**OR[1]**	**95% CI[1]**	**p-value**

10 Discussion

10.1 Introduction

The primary objective of this study was to identify the optimal threshold of HbA1c for identifying type 2 diabetes mellitus in individuals of African ancestry and compare it to the current recommended threshold.

10.2 Principal findings

The main finding of this study was that in a rural Black South African population, the optimal HbA1c threshold for diagnosing type 2 diabetes mellitus was ≥ 6.1%, when using the internationally recognised gold standard of a 2hrPG ≥11.1 mmol/L. At this cut-off, HbA1c showed good diagnostic accuracy, with a sensitivity of 85% and a specificity of 86%. This indicates that an HbA1c threshold of 6.1% is both reliable and practical for identifying true cases of diabetes while limiting the risk of misclassification.

A secondary but still noteworthy finding was that an HbA1c threshold of ≥6.0% performed extremely well when compared with fasting plasma glucose (FPG ≥7.0 mmol/L), identifying all true cases (100% sensitivity), although at the expense of slightly lower specificity (83%).

Taken together, these findings suggest that HbA1c ≥6.1% may be considered the most appropriate diagnostic threshold for this population, as it performs well against the recognised gold standard of the oral glucose tolerance test (2hrPG). At the same time, the strong performance of HbA1c ≥6.0% against fasting glucose highlights its potential relevance in settings where that measure is used.

Table 3 illustrated the variability in diabetes prevalence depending on the diagnostic criterion applied. Notably, the proportion of individuals classified as having diabetes mellitus ranged from just over 4% using FPG or 2hrPG to nearly 19% when using the lowest HbA1c threshold

(≥ 6.0%). This wide disparity underscores the impact of diagnostic thresholds on disease classification. In our study, the proportion of participants classified as having diabetes varied significantly depending on the diagnostic test used. When glucose-based criteria were applied, the prevalence was low and fairly consistent, at approximately 4% using FPG and just under 5% using the 2hrPG test. In contrast, using HbA1c resulted in much higher estimates. Almost one in five participants was classified as diabetic at the 6.0% threshold, and even at the standard 6.5% cut-off, the prevalence was still around 10%. This shows that HbA1c may identify a far larger group of individuals as having diabetes compared with glucose-based tests. Such differences are significant because they influence how many people in a community are diagnosed as having diabetes, with direct implications for healthcare planning, resource use, and individual treatment decisions.

10.2.1 Strengths and weaknesses of the study

This study used both fasting and two-hour glucose-based measures as the gold standards against which to compare HbA1c performance, which is critical as these measures can contribute differentially to HbA1c. Both FPG and postprandial glucose play a key role in determining a person's average blood sugar levels, as reflected by their HbA1c. However, FPG seems to have more impact[18]. For every 1 mmol/L increase in FPG, HbA1c goes up about 0.25%, while the same increase in PPG raises the HbA1c by 0.16%[18].

Another strength of this study is its focus on Black adults aged 40 years or older residing in a rural area of South Africa, a population that is underrepresented in diabetes research. By excluding individuals with a known history of diabetes or those taking glucose-lowering medications, the study minimised these potential confounding effects on HbA1c, FPG, and 2hrPG levels. This approach ensured that the findings reflect the diagnostic accuracy of HbA1c in a population without pre-existing diabetes or treatment-related influences, thereby providing more reliable insights into the optimal HbA1c threshold for diagnosing diabetes in this specific demographic. This cross-sectional study, conducted from 2016 to 2020, therefore provides important insights into the applicability of HbA1c as a diagnostic marker for type 2 diabetes mellitus.

However, the study had limitations. A key limitation was the use of convenience sampling, a non-probability sampling approach. This method may have limited the generalisability of the results as the sample may not fully represent the broader population. Another drawback was

the relatively small sample size, which reduced the study's statistical power and limited the ability to detect significant associations between HbA1c thresholds and other key metabolic and demographic variables. This might have decreased the estimates' accuracy and made finding subtler but meaningful relationships harder.

Another limitation of this study was the presence of missing or uninterpretable retinal images, which affected 157 subjects. Because of the low prevalence, diabetic retinopathy could not be meaningfully analysed.

Despite the study's strengths, these limitations related to the sampling strategy, sample size, data interpretability, and data distribution must be considered when interpreting the results.

10.2.2 Strengths and weaknesses in relation to other studies

The optimal HbA1c thresholds identified in this study and Hird et al.'s study show some differences, reflecting variations in population characteristics and reference standards. Hird et al. determined an optimal HbA1c threshold of 6.0% when using 2hrPG ≥ 11.1 mmol/L as the reference standard, with a sensitivity of 89.2% and specificity of 92.0%[10] . In comparison, we found a slightly higher optimal threshold of 6.1%, with a sensitivity of 85.9% and a specificity of 85.0% when using the same reference standard. When using FPG ≥ 7.0 mmol/L as the reference, Hird et al. reported a sensitivity of 96.3% and specificity of 91.4% at an HbA1c threshold of 6.0%, while we found an optimal threshold of 6.0%, which achieved a sensitivity of 100% and specificity of 83.0%.

Regarding the standard diagnostic threshold of HbA1c ≥ 6.5%, the two studies also revealed notable differences in diagnostic performance. Hird et al. reported that the 6.5% threshold detected diabetes with a sensitivity and specificity of 74.1% and 98.1%, respectively, when using FPG ≥ 7.0 mmol/L, with a sensitivity of 70.3% and specificity of 98.7% when using the 2hrPG ≥ 11.1 mmol/L as the reference.. In contrast, we found that HbA1c ≥ 6.5% had a sensitivity of 83.0% and specificity of 93.0% when using FPG as a reference and a sensitivity of 75.0% and specificity of 93.0% when using 2hrPG as a reference. These differences highlight the impact of population-specific factors, such as genetic and metabolic variations, on HbA1c-based diabetes diagnosis. The findings reinforce the need for population-specific thresholds to improve diagnostic accuracy in African populations, as the universal threshold of 6.5% may underperform in certain settings.

Unlike our study, Hird et al. used multistage cluster sampling to guarantee representativeness in their large, randomly chosen sample of 1,190 individuals aged at least 18 years old. On the other hand, we focused on an older demographic using a smaller, convenience sample of 435 individuals who were 40 years or older, and our sample was less representative. Hird et al's rigorous sampling technique lessened selection bias. However, our study still offers important insights into an older group, a demographic at increased risk for diabetes mellitus and in whom the relationship between HbA1c and glucose may differ from that in younger ages. In our study, age did not show a significant association with HbA1c level across different thresholds (6.0%,6.1% and 6.5%), as demonstrated by both univariate and multivariate logistic regression analyses. This contrasts with a study conducted by Hashimoto et al, which revealed that HbA1c levels were associated with age and tended to increase as age increased[19]. The study concluded that the increase in HbA1c with age may be a consequence of the ageing process itself, independent of other factors such as BMI, physical activity, or a family history of diabetes. The absence of a significant age effect in our study may be attributed to the narrow age range of the study population, which might have reduced variability in HbA1c levels associated with ageing.

Zemlin et al. identified in a Coloured community in Bellville South, Cape Town, South Africa, an HbA1c threshold of 6.1% with a sensitivity of 80% and specificity of 77% when using FPG ≥7.0 mmol/L as the reference standard[12]. When using 2hrPG≥11.1 mmol/L, the sensitivity was 75%, and specificity was 78%. In comparison, our study identified an HbA1c threshold of 6.0% with a sensitivity of 82.8% and specificity of 100% when using FPG and 6.1% with a sensitivity of 85.9% and specificity of 85.0% when using 2hrPG. In the study by Zemlin et al., an HbA1c threshold of 6.5% demonstrated a sensitivity of 50% and specificity of 95% when using FPG as the reference. When using 2hrPG as the reference, the sensitivity was 46%, while the specificity remained high at 96%[12].

Zemlin et al. included a larger, more representative sample of 946 individuals than we did, selected through multistage stratified random sampling from a mixed-ancestry community aged 16 to 95 years. Their population also differs from ours in both age range and ancestry, which may explain the differences in study findings.

A study by Bvumbi et al. found that an HbA1c level of 6.1% was the optimal threshold for diagnosing diabetes, with a sensitivity of 87.1% (95% CI: 78.5–94.5%) and a specificity of

93.2% (95% CI: 85.5–97%) when using FPG ≥ 7.0 mmol/L as the reference standard[20]. Their study, conducted between February and May 2023, involved 120 participants from Harare, Zimbabwe, all with a BMI of ≥ 25 kg/m². While their findings provide useful insights, the small sample size might limit the broader applicability of their results.

In comparison, our study, which included 457 adults aged 40 years and older, identified a slightly different HbA1c threshold depending on the reference standard used. When using 2hrPG ≥ 11.1 mmol/L as the reference, an HbA1c threshold of 6.1% was found, with a sensitivity of 85.0% and a specificity of 85.9%. Using FPG ≥ 7.0 mmol/L as the reference, the optimal threshold was 6.0%, achieving a perfect sensitivity of 100% and a specificity of 82%. Although both studies identified a threshold of 6.1%, the difference in the reference standards highlights the importance of considering population-specific factors and diagnostic criteria when determining optimal HbA1c levels.

In the multivariate model, FPG and 2hrPG maintained a positive association with the three different thresholds of HbA1c and BMI maintained a positive association with only HbA1c ≥ 6.1%. For instance, multivariate analysis showed that higher FPG and 2hrPG levels were significant predictors of having an HbA1c at 6.0% or 6.1%. This suggests that these alternative thresholds may improve sensitivity and help detect early diabetes mellitus, particularly in populations with distinct metabolic profiles. However, gender, hypertension, and age had no significant effect on HbA1c in the adjusted model, suggesting that the observed differences in HbA1c between men and women could have been due to differences in metabolic factors such as BMI and hypertension rather than gender. When diabetes is defined by FPG ≥ 7.0 mmol/L BMI stands out as the only significant risk factor. Other variables, including hypertension, age, and gender, did not show meaningful associations in these models. This finding suggests that excess body weight remains a key driver of glucose-based diabetes risk, while other commonly considered factors may have less predictive power depending on the diagnostic method used. It highlights the importance of focusing on BMI as a central target in screening and prevention efforts when relying on glucose criteria.

The findings contrast with those of Zemlin et al., who identified age and family history of diabetes as independent risk factors for diabetes mellitus [12]. using an HbA1c threshold of 6.5%. Our study failed to observe these associations, maybe due to the differences in the study population and sample size.

A previous systematic review and meta-analysis found that the thresholds of HbA1c at which diabetic retinopathy is evident range from 6.1% to 7.8% in participants from 11 countries, with age ranging from 18 to 79 years and retinopathy prevalence between 1.6% and 15.8%[7]. Kowall and Rathmann demonstrated significant variability in HbA1c thresholds for the diagnosis of diabetic retinopathy and other complications, with cut-off values ranging from 5.2% to 7.8%[13]. The low prevalence of diabetic retinopathy in our study precluded us from drawing any meaningful conclusions about its relationship with HbA1c.

We did not assess anemia as a confounding factor in this study but different types of anemia are known to affect HbA1c levels independent of glucose levels[13]. Assessing the effect of anemia on diagnostic HbA1c thresholds should be explored in future studies. A study conducted by Guo et al. found that the appropriate HbA1c cutoff for diagnosing diabetes mellitus in patients with iron deficiency is debated[21].

A study conducted by Doultrey et al. observed no meaningful effect of HIV status on HbA1c levels[22]. These results suggest that HIV may play only a limited role in shaping HbA1c values. HIV and its treatment are common in South Africa so it remains important for future research to explore how factors such as antiretroviral therapy or related conditions might influence diabetes diagnosis using HbA1c.

10.3 Recommendations

Based on the results of this study, several areas were identified for further research and consideration. The HbA1c threshold of 6.1% showed a balanced sensitivity and specificity with the use of 2hrPG ≥ 11.1 mmol/L as a reference, while a threshold of 6.0% had an optimal performance against FPG ≥7.0 mmol/L. These results suggest that the population-specific HbA1c threshold values can improve the sensitivity of diabetes diagnosis in Black South African adults. In view of the limitations of this study, including the small sample size, the convenience sampling and the low prevalence of diabetic retinopathy, further research is necessary to confirm these results in larger, randomly selected samples. Larger studies should investigate the association between potential demographic and clinical factors and HbA1c levels.

11 Conclusion

This study aimed to establish an optimal HbA1c threshold for diagnosing type 2 diabetes mellitus in Black South African adults using FPG and 2hrPG criteria as gold standards and to compare its diagnostic accuracy with existing recommended thresholds. The findings suggest that a threshold of 6.0% performs optimally against FPG, while an HbA1c threshold of 6.1% provides a balanced sensitivity and specificity when using 2hrPG as a reference. These results highlight the need for population-specific diagnostic thresholds to improve the accuracy of diabetes diagnosis in African populations.

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Appendix 1

Figure 1: Distribution of age (years) among the population

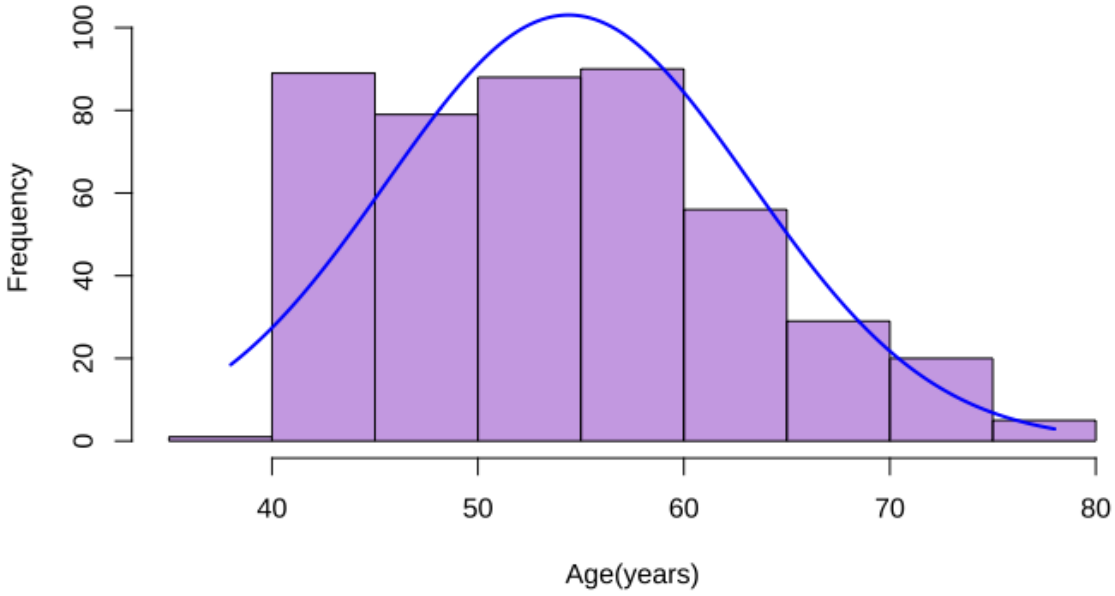
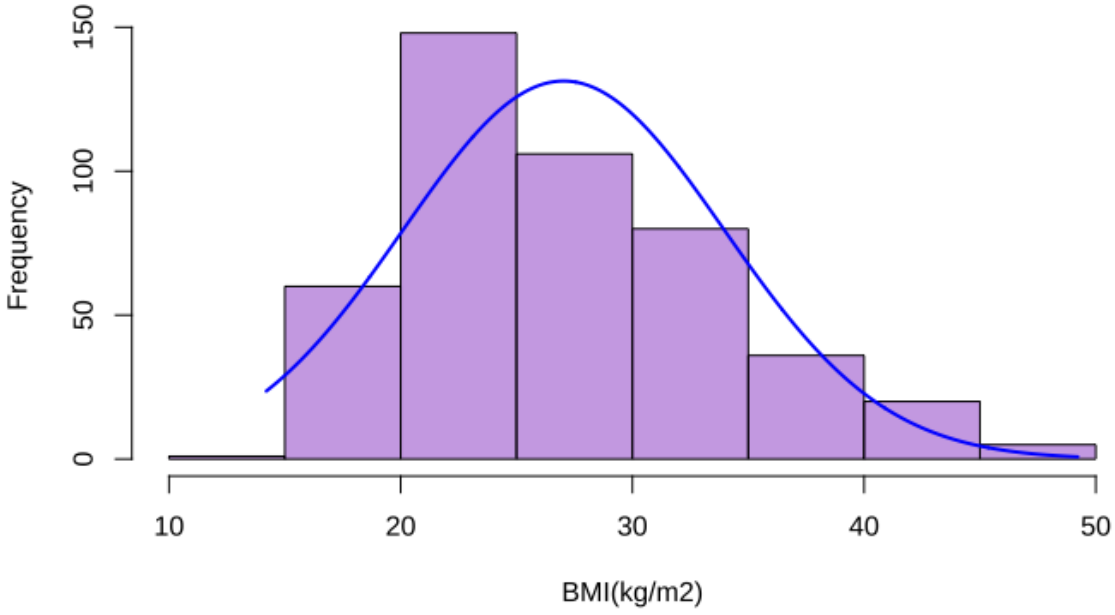


Figure 2: Distribution of BMI (kg/m2) among the population.



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FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

I, DR WA SAFI GUY ROGER SAFI (Student number: 2449294) am a student registered for the degree of MASTER IN BIOSTATISTICS AND EPIDEMIOLOGY in the academic year 2025.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
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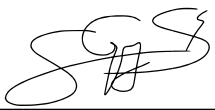
Signature:  Date: 02 OCTOBER 2025

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Ethics Certificates

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Wade, Dr A, et al
School of Public Health
Rural Public Health & Health Transitions Research Unit
Medical School
University

E-mail: Alisha.Wade@wits.ac.za

CC: Supervisor: Not applicable <>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2020/01/24

REF: *R14/49*

PROTOCOL NO: **M1911199** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Evaluating of glycosylated haemaglobin (HbA1c) for diagnosis of Type 2 Diabetes Mellitus in Black African people with and without HIV*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Wade, Dr A, et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M1911199

NAME: (Principal Investigator)	Wade, Dr A, et al
DEPARTMENT:	School of Public Health Rural Public Health & Health Transitions Research Unit Medical School University
PROJECT TITLE:	Evaluating of glycosylated haemoglobin (HbA1c) for diagnosis of Type 2 Diabetes Mellitus in Black African people with and without HIV
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Renewal of M141157
SUPERVISOR:	Not applicable
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/01/24

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **November** and will therefore reports and re-certification will be due early in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES