academicJournals

Vol. 8(1), pp. 1-8, January 2017
DOI: 10.5897/JDE2016.0102
Article Number: 5F1633562394
ISSN 2141-2685

http://www.academicjournals.org/JDE

Journal of Diabetes and Endocrinology

Full Length Research Paper

The utilization of HbA_{1c} test in the screening and diagnosis of type 2 diabetes mellitus: An outpatient clinics survey in Botswana

Bernard Omech[1*], Jose-Gaby Tshikuka[2], Kennedy Amone-P'Olak[3], Julius Mwita[1], Billy Tsima[2] and Oathokwa Nkomazana[4]

[1]Department of Internal Medicine, University of Botswana, Private Bag UB 00713, Gaborone, Botswana.
[2]Department of Public Health and Family Medicine, University of Botswana, Private Bag UB 00713, Gaborone, Botswana.
[3]Department of Psychology, University of Botswana, Private Bag UB 00713, Gaborone, Botswana.
[4]Department of Ophthalmology, University of Botswana, Private Bag UB 00713, Gaborone, Botswana.

Received 23 November, 2016; Accepted 5 January, 2017

This study aimed to assess HbA1c performance against single fasting blood glucose (FBG) for diagnosis of undiagnosed type 2 diabetes (T2D) and impaired fasting glycaemia (IFG) among general medical outpatients in Botswana. Participants aged, ≥20 years were cross-sectionally surveyed from August to October, 2014. All the participants underwent testing for HbA1c and FBG. The HbA1c sensitivity, specificity and predictive values in the diagnosis of T2D and IFG were computed and their Pearson's correlation and scatter diagrams determined. A total of 291 participants (74.2% women) with a mean age of 50.1±11.0 years provided data for the current analysis. HbA1c at cut-off of ≥ 6.5% (48 mmol/mol) had a sensitivity and specificity for T2D of 100 (15.81 to 100.00) and 86.3% (86.16 to 89.92), respectively. Similarly, for IFG, the sensitivity and specificity was 100 (2.5 to 100) and 36.3% (30.3 to 42.6), respectively. The positive predictive value (PPV) was 4.8 (0.58 to 16.16) and 0.6% (0.02 to 3.45) for T2D and IFG screening, respectively. The negative predictive value (NPV) was 100% in both cases of T2D and IFG screening. HbA1c had a modest, positive correlation (r) with FBG for the overall population (r = 0.536, p < 0.001); for women, (r = 0 .578, p < 0.001) and men (r =0 .336, p =0.003). HbA1c had high sensitivity but widely varying specificity, high proportion of discordant results and poor prediction of T2D and IFG in this setting. Although, HbA1c correlation with fasting glucose was modest, both tests are required to improve diagnostic reliability in asymptomatic T2D screening program.

Key words: Diabetes screening, HbA1c test, Botswana.

INTRODUCTION

The growing incidence of type 2 diabetes mellitus (T2D) is a public health concern in sub-Sahara Africa (SSA) (Tuei et al., 2010). About 20 million people aged 20 to 79 years are estimated to live with diabetes in Africa and this

*Corresponding author E-mail: bgomech@gmail.com.

number is projected to double by 2035 (Guariguata et al., 2014). Owing to the increasing prevalence of overweight individuals, obesity and sedentary lifestyles (Ziraba et al., 2009), T2D accounts for over 90% of cases of diabetes globally (Chen et al., 2011). Botswana has a population of about 2.2million people and has been the fastest growing economy in SSA since 2000 (Botswana Report, 2016). The country is currently experiencing high rates of overweight, obesity and other non-communicable diseases risk factors (MOH, 2007). The national prevalence of diabetes was estimated to between 3.6 and 8.25% in 2012 (Unwin et al., 2010; Diabetes Association of Botswana (DAB), 2012). It is estimated that about 50 to 70% of people with diabetes may be unaware or undiagnosed in Africa due to under-resourced healthcare systems resulting in late diagnosis and poor outcomes (Beagley et al., 2014). An effective diagnostic test is therefore important to facilitate early diagnosis and management in order to avoid costly complications.

The current diagnostic criteria for T2D are based on fasting blood glucose (FBG) and a 75 g load of oral glucose tolerance test (OGTT) (Consultation, 1999; WHO, 2006). However, both methods have considerable intra-individual variability, require an overnight fasting of at least 10 h which is inconveniencing to patients, and require a strict quality assurance measures (Libman et al., 2008; Hyltoft Petersen et al., 2001). Although, OGTT is the recognized gold standard diagnostic test for asymptomatic diabetes, FBG is mostly used in clinical practice to screen/diagnose diabetes since it is more accessible. In 2009, an expert committee of American Diabetes Association (ADA) recommended the use of glycated haemoglobin (HbA1c) as an additional test to diagnose T2D (Committee, 2009). T2D is diagnosed when HbA1c threshold of ≥6.5% (48 mmol/mol) is measured and the level between 5.7 and 6.4% (39 to 46 mmol/mol) is considered impaired glycaemia, for individuals at high risk of progression to diabetes (Olson et al., 2010). HbA1c is formed by non-enzymatic glycosylation of erythrocytes, reflecting an average glucose concentration over the lifespan of the erythrocytes (Bunn et al., 1978).

HbA1c testing is highly standardized, requiring no fasting or preparation and has less intra-individual variability (Weykamp et al., 2013). Even though, the HbA1c test was adopted by World Health Organisation (WHO) in 2011 (WHO, 2011), its utility in the screening and diagnosis of T2D remains controversial in many parts of the world. The current diagnostic threshold of ≥6.5% was based on a similar relationship with prevalent retinopathy as that of both fasting plasma glucose and 2-h OGTT (Committee, 2009; Colagiuri et al., 2011). However, apart from several conditions that are known to preclude an accurate measurement of HbA1c, age and ethnic variability have been shown to affect diagnostic cut-offs values (Sharma and Signal, 2015). Hence, the recommendation by WHO that long-term prospective validation studies be done in various ethnic groups/ countries to establish the precise glucose and HbA1c levels that are predictive of microvascular complications (WHO, 2011). No such study has yet been done in SSA, raising concerns about its applicability for screening and diagnosis of T2D in this population.

The main aim of this study was to assess the HbA1c performance against a single fasting blood glucose (FBG) for diagnosis of undiagnosed diabetes and impaired fasting glycaemia among general medical outpatients in Botswana; specifically, to assess the correlation between the HbA1c and FBG among Batswana adults of African descent with no previous diagnosis of diabetes and determine the sensitivity, specificity and predictive values of HbA1c against undiagnosed diabetes and impaired fasting glucose.

MATERIALS AND METHODS

Study setting and design

Data was extracted from a cross-sectional study originally designed for evaluation of a Finnish diabetes risk Score (FINRISC) between August and October 2014 at two general medical outpatients' clinics in Gaborone and Maun (Omech et al., 2016) Gaborone and Maun are two cities located in the south and north of Botswana, respectively. The study was conducted in compliance with the ethical standards of relevant national and institutional committees on human experimentation and with the Helsinki declaration of 1975. All participants signed written informed consent after all the procedures were clearly explained to them. Permission was obtained from Botswana Ministry of Health and relevant institutional review board of both hospitals.

Participants and procedures

Recruitment and evaluation was done by research nurses with prior training on the study protocol, including anthropometric measurements and good clinical practice certification. The study was conducted simultaneously at both clinics. After obtaining informed consent, all the participants aged ≥20years were selected through a systematic random sampling from each of the two clinics. A sampling frame was made from a list of patients on scheduled visit each study day. On average, 30 patients were scheduled in each clinic. The first patient was picked randomly and evaluated for study eligibility. If eligible, then every sixth patient on the list was subsequently evaluated for study eligibility and enrollment. About five participants were enrolled per day until the sample sized was attained. Patients with conditions that could interfere with HbA1c diagnosis test such as acute illness less than two weeks prior to clinic attendance, established diagnoses of diabetes, anaemia, pregnancy and chronic kidney disease were excluded. Data collected from enrolled participants, included demographics, relevant history, anthropometric measurements and blood pressure (BP) measurements.

BP was measured by the research nurse after the subject had rested for at least 5 min by using the standard mercury sphygmomanometer. With the participant sitting in an upright position, BP was recorded to the nearest 2 mmHg. For each patient, two readings were recorded within a 2-min interval and averaged out. Weight was measured to the nearest 0.1 kg, and standing height was measured to the nearest 0.1 cm using a stadiometer attached to the same medical balance weighing scale

(HiCare International, Kerala, India). Body mass index was calculated as weight per square meters (kg/m^2). For each patient, venous blood was taken for HbA1c test. Blood samples were transported daily in an ice pack box for processing in one central accredited laboratory NGSP-certified using a high–performance liquid chromatography (HPLC) assay method (Abbot Architect, 2007, Germany). This method is aligned with the Diabetes Control and Complications Trial (DCCT) as recommended by WHO (Diabetes Control and Complications Trial Research Group, 1993). Plasma glucose was analyzed by the research nurse at point of care using oxidase method (Betachek glucometer; National Diagnostic Products Pty Ltd, Sydney, NSW, Australia). The HbA1c results were categorized according to American Diabetes Association (ADA) criteria into normal glycaemia (<5.6%), impaired glycaemia; 5.7 to 6.4% (39 to 46 mmol/mol) and diabetes; ≥6.5% (48 mmol/mol) (Olson et al., 2010). Plasma glucose was categorized according to WHO criteria, into normal glycaemia, <6.1mmol/l, impaired fasting glycaemia, 6.1-6.9mmol/l and diabetes, ≥7.0mmol/l (WHO, 2006)

Statistical analysis

Demographic data, anthropometric and laboratory measurements for individual participants were entered into a data base using IBM SPSS statistics version 24. Participants were grouped into three categories based on HbA1c status. Proportions and means (SD) were computed for categorical variables and continuous variables, respectively. Differences between the groups were ascertained using cross-tabulation. With MedCalc® software (BVBA, 2016), using HbA1c as screening test and FBG as the reference test, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were computed. Pearson's correlations coefficients with Scatter diagrams between HbA1c and FBG in the overall population and for each gender were computed and results tabulated. A linear regression line with 95% confidence interval and r^2 were provided. Level of statistical significance was taken to be $p < 0.05$.

RESULTS

Participant's characteristics

A total of 704 participants were screened; 304 (43.2%) were not eligible because of various reasons such as being acutely ill, already known diabetic or anaemic. Of the 400 patients enrolled, 109 (15.5%) did not turn-up for fasting blood test. Data from the remaining 291 (41.3%) were analyzed (Figure 1). This comprised of 216 (74.2%) women, 75 (25.8%) men and all were Batswana of black African descent residing in the urban centres of Gaborone and Maun. Their mean age was 50.1±11 years; 50.5±11.8 years for male and 49.9 ±11.0 years for female.

The majority (71%) of the participants had either completed secondary education level or primary education level, with only <7% having no formal schooling. Regarding employment status, 52% were formally employed and 23% were retired, while only 7% considered themselves unemployed. The most common reason for the visit was regular appointment for either one or more chronic disorders 197 (67.7%), followed by new referrals (26.5%). A majority of the participants: 215 (73.9%) had no symptom at presentation, and the common symptoms were headache, body pains, and shortness of breath. The most common chronic conditions were systemic hypertension; 135 (46.4%) and heart diseases, 37 (12.7%). However, 88 participants (26.9%) had no chronic disease. A total of 114 (39.2%) participants were HIV seropositive, 156 (53.6%) were HIV seronegative and 21 (7.2%) had unknown HIV status. Of those who were seropositive, 87 (76.3%) were on first-line antiretroviral therapy (ART; nucleoside/nucleotide analogues and non-nucleoside combinations). Only nine (7.9%) were on second-line ART (protease-based ART) and 18 (15.7%) were not on ART.

Using ADA diagnostic criteria, the overall population was stratified based on HbA1c diagnoses (Table 1); diabetes (n=42, 14.4%), impaired glycaemia (n=159, 54.3%) and normal glycaemia (n=90, 30.9%). The mean body mass index (BMI) and FBG were significantly higher in those diagnosed with diabetes as compared to those with impaired or normal glycaemia. Although, there were a trend towards higher mean age and elevated cholesterols in the diabetes groups as compared to the other groups, they were not statistically significant.

The outcomes of FBG testing in the various HbA1c glycaemic categories for the overall population were analyzed (Figure 1). Of those classified as diabetes, 37 (88.1%) had normal FBG, 3 (7.1%) had impaired fasting glycaemia and only two (4.8%) had diabetes. In the Impaired HbA1c glycaemia group, 158 (99.4%) had normal fasting glycaemia and only one participant was confirmed to have impaired fasting glucose. All the participants with normal HbA1c status had normal FBG.

Diagnostic accuracy of HbA1c

Table 2 summarizes the sensitivity, specificity, positive and negative predictive values of HbA1c as screening test as compared to FBG. HbA1c had a sensitivity and specificity of 100 and 86.2% for screening of T2D, respectively. Similarly, it had a sensitivity and specificity of 100 and 36.3% for screening of IFG, respectively. The positive predictive value (PPV) for T2D and IFG was 4.8 and 0.6%, respectively. The negative predictive value (NPV) for T2D and IFG was 100% in both cases.

Correlation between HbA1c and FBG

Figure 2 depicts the scatter plots diagram between HbA1c and FBG in the overall population. The Pearson's correlation coefficient, r was 0.546, p=0.001 for the overall population, the fitted regression line showed a slope of 0.43 and r^2 of 0.287. The coefficient r for women was 0.578, p= 0.001 and that for men was 0.336, p=0.003 and both were significant at $p< 0.05$ (Figure 3A

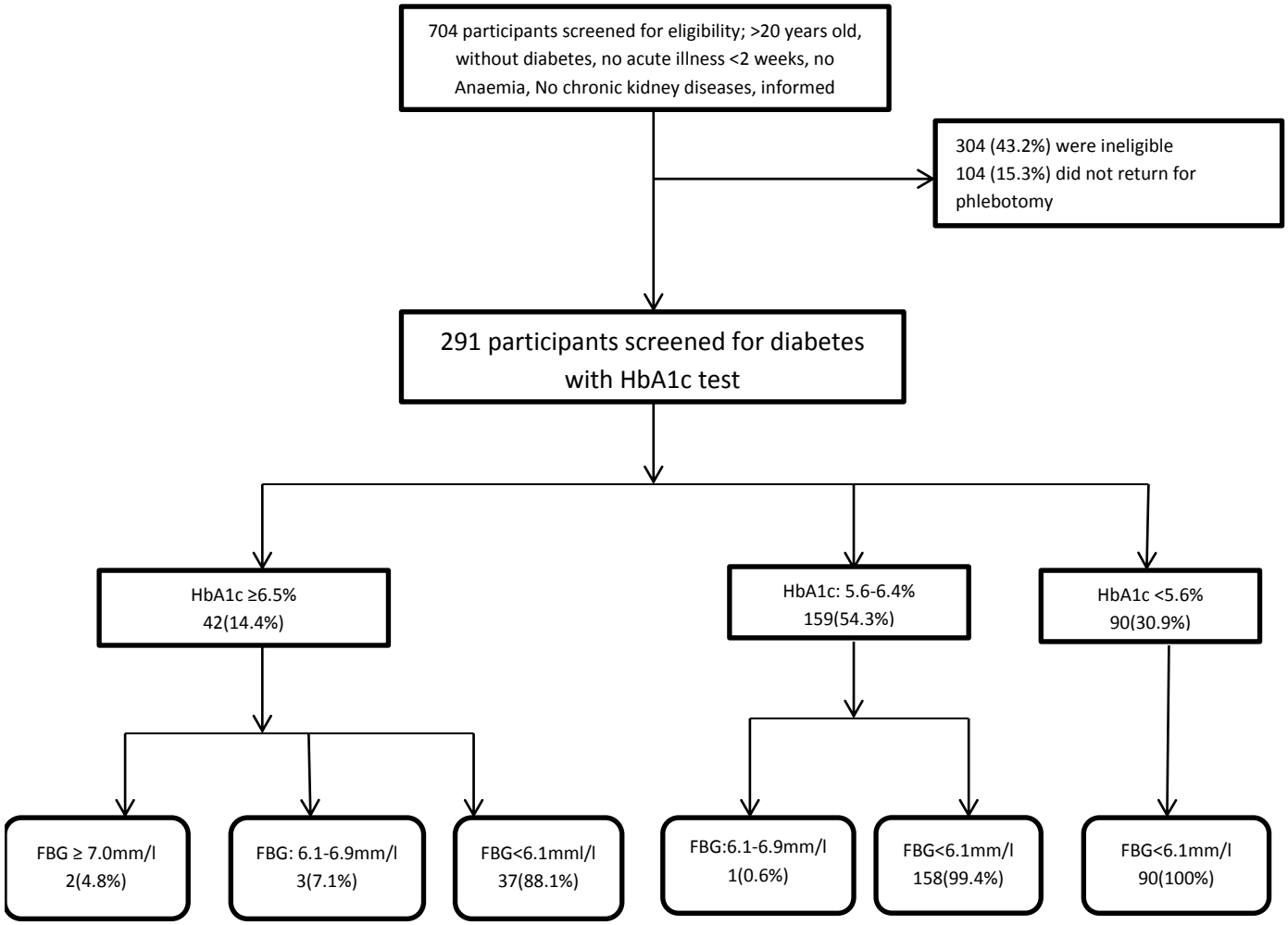

Figure 1. The flow diagram of participants' recruitment and outcomes of HbA1c tests, followed by FBG test under each glycaemic categories.

Table 1. General demographic and clinical characteristic of study participants stratified by HbA1c status (N=291).

Parameter	Total population (N=291)	Diabetes mellitus (≥6.5%) (N=42)	Impaired glycaemia (5.7-6.4%) (N=159)	Normal glycaemia (<5.7%) (N=90)
Gender (%)				
Female	216(74)	27(12.5)	122(56.5)	67(31.0)
Male	75(26)	15(20.0)	37(49.3)	23(30.7)
Age, years (±SD)	50.1(11.0)	56.0(10.0)	50.7(11.2)	46.2(9.4)
Body mass index, Kg/m^2(±SD)	28.7(6.5)	29.8(6.4)	27.9(6.2)	27.5(7.2)†
Fasting blood glucose, mmol/l (±SD)	3.5(0.9)	4.3(1.8)	3.4(0.6)	3.4(0.6)†
Triglycerides, mmol/l (±SD)	1.8(6.9)	3.7(14.3)	1.7(5.6)	1.1(0.6)
High density lipoprotein(SD)	1.2(0.4)	1.1(0.3)	1.2(0.4)	1.3(0.38)
Low density lipoprotein(±SD)	3.5(5.4)	5.6(14.0)	3.3(1.0)	2.9(1.0)
Total cholesterol(±SD)	4.7(1.2)	4.8(1.8)	4.8(1.2)	4.5(1.2)
BP-systolic (±SD)	128.6(19.3)	127.7(17.3)	130.6(19.6)	125.5(19.3)
BP-diastolic (±SD)	78.5(12.1)	78.3(12.5)	79.8(11.9)	76.3(12.0)

Values provided as means ±standard deviations; except for gender which are proportions;† p=<0.05.

Table 2. The sensitivity, specificity, positive and negative predictive values of HbA1c as a screening test against FBG.

Index	Diabetes(95% CI)	Impaired Glycaemia (95% CI)
Sensitivity	100%(15.81-100.00)	100%(2.5-100)
Specificity	86.2%(86.16-89.92)	36.3(30.3-42.6)
PPV	4.8%(0.58-16.16)	0.6(0.02-3.45)
NPV	100%(98.53-100.00)	100%(95.98-100.00)

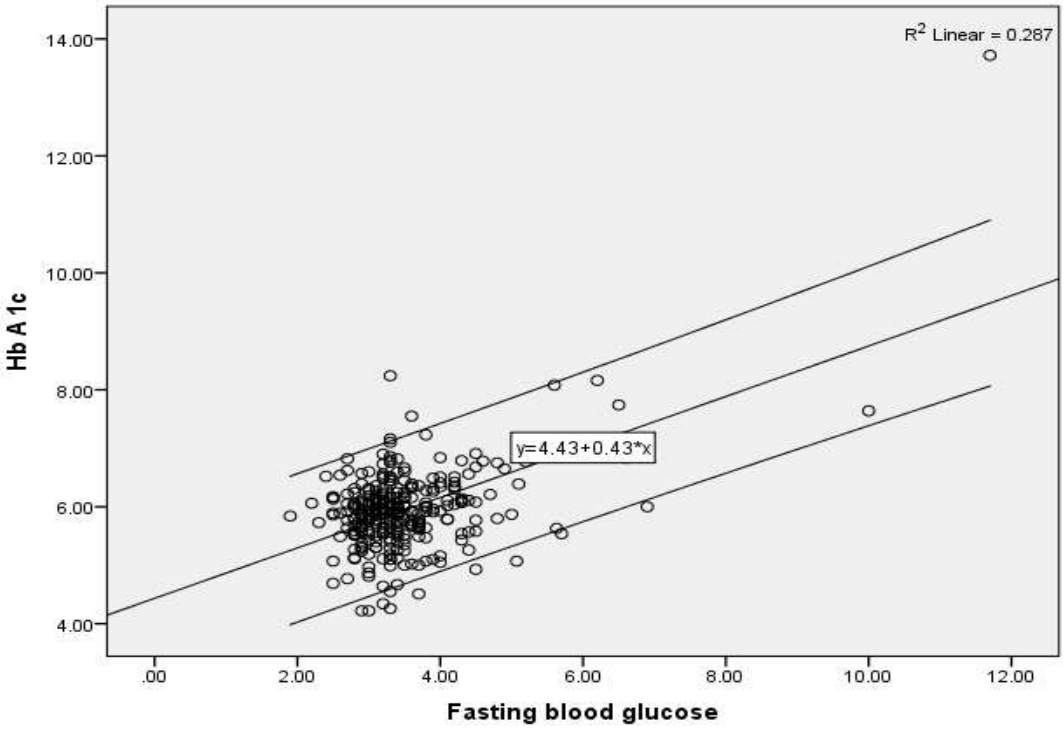

Figure 2. The scatter plots diagram depicting the linear correlation between HbA1c and FBG for the overall population.

and B).

DISCUSSION

The sensitivity of HbA1c for diagnosis of undiagnosed diabetes and impaired fasting glycaemia were high but its specificity varied widely. HbA1c specificity for impaired fasting glycaemia was very low (36.3%) as compared to undiagnosed diabetes (86.2%). These findings suggest a high proportion of discordancy between the HbA1c and fasting blood glucose based criterion for diagnosis of prevalent undiagnosed diabetes in this population. In addition, the current American Diabetes Associated recommended HbA1c cut-off values poorly predicted both prevalent undiagnosed diabetes and impaired fasting glycaemic status, but performed excellently in predicting normal glycaemic status (Table 2) and hence it can be considered an effective exclusion tool for a new case of undiagnosed diabetes during screening program.

The accuracy and predictive values of HbA1c is highly dependent on assays precisions, population's reference values, disease prevalence and the gold standard test. In this study, the HbA1c test was standardized to the currently recommended assay methods certified by NGSP and samples analysis was pooled in one central laboratory which is deemed to have minimized inconsistent findings. The HbA1c optimum cut-off values are known to vary in different races/ethnicities and in between studies which affect its sensitivity and specificity (WHO, 2011). For example, two previous studies in the U.S which included both black and white population

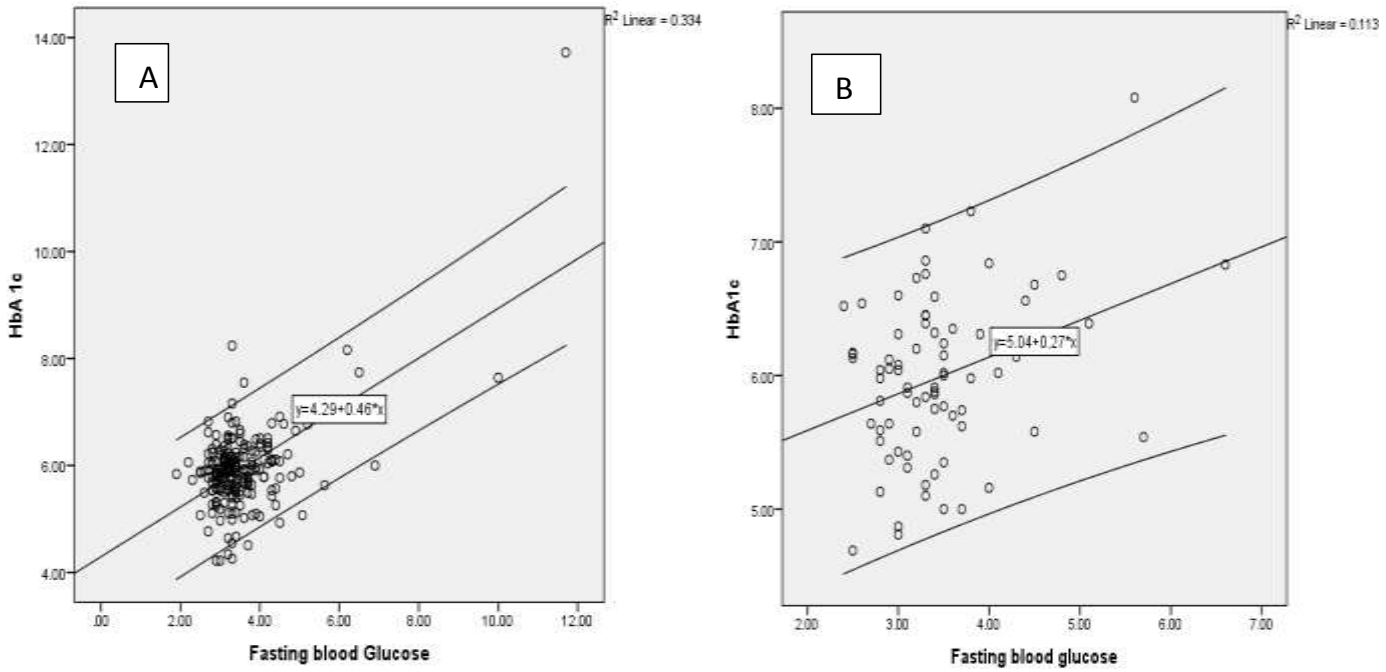

Figure 3. Scatter plot diagrams depicting the linear correlations between HbA1c and FBG in female (A) and male (B).

showed that blacks had significantly higher mean HbA1c values (5.8%) than whites (5.4%) (p<0.001) (Selvin et al., 2010; Herman et al., 2007). In the SSA context, a cross sectional study in KwaZulu-Natal estimated HbA1c optimal cut-off was 6.0%, with a sensitivity of 89.2% [95% CI :78.6-99.8] and a specificity of 92% [95% CI:90.3-93.7] (Hird et al., 2016). Previously, a similar study in Cape Town found a comparable HbA1c optimal value of 6.1% with a sensitivity and a specificity of 80 and 77%, respectively (Zemlin et al., 2011). These findings from South Africa support the need for population derived HbA1c references values in the African context.

A modest, but significant positive correlation was also observed between HbA1c and fasting blood glucose levels (Figure 2). However, the correlation was weaker in men as compared to women possibly due to smaller sample size of men in this study (Figure 3A and B). HbA1c is recognized as a reliable indicator of chronic glycaemic status, as compared to glucose based markers. In this study, the correlation were similar to other studies among non-diabetic Turkish outpatients study (r=0.47, p=0.001) (Giniş et al., 2012), as well as among the non-diabetic Dutch community study (r=0.46, p=<0.001) (van't Riet et al., 2010). Although, patients with known diabetes were not included in the sample, the correlation between HbA1c and FBG were stronger among known diabetics in the Dutch study (r=0.71), which is consistent with well-known positive association between HbA1c with glucose levels in established diabetes mellitus (Koenig et al., 1976). HbA1c and FBG discordance in this study may be attributed to intra-individual variability especially in the dysglycaemic phase as well as analytical and pre-analytical factors involved in the measurements of FBG (Chen et al., 2011; Sacks, 2011)

Despite the current limitations of HbA1c especially in SSA populations, HbA1c is a relatively convenient test, requiring no fasting and a single blood sample, though more costly. Based on the results, HbA1c and fasting blood glucose may still play a dual role in the diagnosis of undiagnosed diabetes in the clinic settings. The high proportions of discordancy, an elevated HbA1c test requires a confirmatory blood glucose test to define true diabetes cases for clinical interventions. This is consistent with most guidelines for screening and diagnosis of diabetes among high risk groups (Committee, 2009; d'Emden et al., 2012). It is an important consideration when planning improved uptake of testing and detection of diabetes, where majority of cases are undiagnosed especially in low and middle income countries (Beagley et al., 2014).

The results of this study should be considered in light of several limitations associated with HbA1c as a screening and diagnostic test that has been discussed in various literature (Sharma and Singal, 2015). For instance, HIV-sero positive individuals are considered to have ongoing low chronic haemolysis from viral infections or anti-retroviral drugs, affecting the reliability of HbA1c test resulting in under estimation of case diagnosis (Polygreen et al, 2003). Indeed, in this setting, HIV infection was highly prevalent (39.2%) and over 80% were on non-nucleoside reverse transcriptase inhibitors-, particularly efavirenz, which may have affected the HbA1c

levels and thus the need for further investigation to elucidate the influence of these factors on HbA1c test in this population. The haemoglobin, iron status, renal functions, liver functions were all not determined, all of which are known to affect the red cell survival time and may thus lead to misclassification of HbA1c levels (Sharma and Singal, 2015). Though, in this setting, haemoglobinopathies are known to be rare and all effort was made to exclude acutely ill patients including chronic renal failure patients and those with anaemia. Furthermore, the sample size was relatively small, especially the men, which could have affected the overall correlations findings. Lastly, FBG was used, instead of OGTT as the recommended gold standard test, which may have led to under diagnosis of diabetes.

In conclusion, the sensitivity of HbA1c at current ADA recommended cut off-value is high but with widely varied specificity for undiagnosed diabetes and IFG. HBA1c was poorly predictive of incident T2D and IFG, and had high proportion of discordant results but had an excellent negative prediction for normal glycaemia. However, HbA1c had a modest, but significant positive correlation with fasting glucose levels. The results support a dual role for HbA1c and fasting blood glucose in defining prevalent undiagnosed diabetes during screening program to improve the test reliability.

Conflict of interests

The authors declared that there is no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank all the patients who accepted to participate in the study and the following research nurses who collected the data: Irene N. Kebalefetse and Veronica O. Makopo. They wish to thank Dr Philip Opondo, Department of Psychiatry, University of Botswana for diligently proof reading the manuscript. The study was funded by the office of Research and Development (ORD) of the University of Botswana.

REFERENCES

Beagley J, Guariguata L, Weil C, Motala AA (2014). Global estimates of undiagnosed diabetes in adults. Diabetes Res. Clin. Pract. 103(2):150-160.

Bunn HF, Gabbay KH, Gallop PM (1978). The glycosylation of hemoglobin: relevance to diabetes mellitus. Science 200(4337):21-27.

BVBA M-MS (2016). Version 16.8 Easy to use statistical software. Available at: https://www.medcalc.org/history.php/ Accessed August 4, 2016.

Chen L, Magliano DJ, Zimmet PZ (2011). The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. Nat. Rev. Endocrinol. 8(4):228-236.

Colagiuri S, Lee CM, Wong TY, Balkau B, Shaw JE, Borch-Johnsen K (2011). Glycemic thresholds for diabetes-specific retinopathy implications for diagnostic criteria for diabetes. Diabetes Care 34(1):145-150.

Committee IE (2009). International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 32(7):1327-1334.

Consultation W (1999). Definition, diagnosis and classification of diabetes mellitus and its complications, vol. 1: Part; 1999. http://apps.who.int/iris/bitstream/10665/66040/1/WHO_NCD_NCS_99.2.pdf

d'Emden MC, Shaw JE, Colman PG, Colagiuri S, Twigg SM, Jones G, Goodall I, Schneider HG, Cheung NW (2012). The role of HbA1c in the diagnosis of diabetes mellitus in Australia. Med. J. Aust. 197(4):220-221.

Diabetes Association of Botswana (DAB) (2012). The Epidemiology of Diabetes Mellitus in Botswana. http://www.bankgaborone.co.bw/fileadmin/user_upload/downloads/Diabetes_Association_of_Botswana.pdf

Giniş Z, Öztürk G, Sirmali R, Yalçindağ A, Dülgeroğlu Y, Delibaşi T, Delibaş N (2012). The role of HbA1c as a screening and diagnostic test for diabetes mellitus in Ankara. Turk. J. Med. Sci. 42(Sup. 2):1430-1436.

Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, Shaw J (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res. Clin. Pract. 103(2):137-149.

Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, Lachin JM, Montez MG, Brenneman T, Barrett-Connor E (2007). Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. Diabetes Care 30(10):2453-2457.

Hird TR, Pirie FJ, Esterhuizen TM, O'Leary B, McCarthy MI, Young EH, Sandhu MS, Motala AA (2016). Burden of Diabetes and First Evidence for the Utility of HbA1c for Diagnosis and Detection of Diabetes in Urban Black South Africans: The Durban Diabetes Study. PloSOne 11(8):e0161966.

Hyltoft Petersen P, Brandslund I, Jørgensen L, Stahl M, de Fine Olivarius N, Borch-Johnsen K (2001). Evaluation of systematic and random factors in measurements of fasting plasma glucose as the basis for analytical quality specifications in the diagnosis of diabetes. 3. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. Scand. J. Clin. Lab. Invest. 61(3):191-204.

Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A (1976). Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. N. Engl. J. Med. 295(8):417-420.

Libman I, Barinas-Mitchell E, Bartucci A, Robertson R, Arslanian S (2008). Reproducibility of the oral glucose tolerance test in overweight children. J. Clin. Endocrinol. Metab. 93(11):4231-4237.

Ministry of Health Botswana (MOH) (2007). Botswana STEPS survey: Chronic Disease Risk Factor Surveillance 2007. http://www.who.int/chp/steps/2007_STEPS_Report_Botswana.pdf

Olson DE, Rhee MK, Herrick K, Ziemer DC, Twombly JG, Phillips LS (2010). Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria. Diabetes Care 33(10):2184-2189.

Omech B, Mwita JC, Tshikuka J-G, Tsima B, Nkomazna O, Amone-P'Olak K (2016). Validity of the Finnish Diabetes Risk Score for Detecting Undiagnosed Type 2 Diabetes among General Medical Outpatients in Botswana. J Diabetes Res. 2016:4968350. Diabetes Control and Complications Trial Research Group (DCCT) (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N. Engl. J. Med. 329:977-986.

Polgreen PM, Putz D, Stapleton JT (2003). Inaccurate glycosylated hemoglobin A1C measurements in human immunodeficiency virus-positive patients with diabetes mellitus. Clin. Infect. Dis. 37:e53-56.

Sacks DB (2011). A1C versus glucose testing: a comparison. Diabetes care 34(2):518-523.

Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J, Brancati FL (2010). Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N. Engl. J. Med. 362(9):800-811.

Sharma B, Singal P (2015). The utility and pitfalls of the currently used measures of glycaemia in the diagnosis and management of diabetes mellitus. J. Indian Acad. Clin. Med. 16(3-4):227-35.

Tuei VC, Maiyoh GK, Ha CE (2010). Type 2 diabetes mellitus and obesity in sub-Saharan Africa. Diabetes Metab. Res. Rev. 26(6):433-445.

Unwin N, Gan D, Whiting D (2010). The IDF Diabetes Atlas: providing evidence, raising awareness and promoting action. Diabetes Res. Clin. Pract. 87(1):2-3.

van't Riet E, Alssema M, Rijkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM (2010). Relationship Between A1C and Glucose Levels in the General Dutch Population The New Hoorn Study. Diabetes Care 33(1):61-66.

Weykamp C (2013). HbA1c: a review of analytical and clinical aspects. Ann. Lab Med. 33(6):393-400.

World Health Organization (WHO) (2006). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO. IDF consultation 2006. https://www.idf.org/webdata/docs/WHO_IDF_definition_diagnosis_of_diabetes.pdf

World Health Organization (WHO) (2011). Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. http://apps.who.int/iris/handle/10665/70523

Zemlin AE, Matsha TE, Hassan MS, Erasmus RT (2011). HbA1c of 6.5% to diagnose diabetes mellitus-does it work for us?-The Bellville South Africa study. PloS one 6(8):e22558.

Ziraba AK, Fotso JC, Ochako R (2009). Overweight and obesity in urban Africa: A problem of the rich or the poor? BMC Public Health 9(1):1.