OFFICIAL JOURNAL

ORIGINAL ARTICLE

Association between G6PD G202A Polymorphism and HbA1c among Patients with Type II Diabetes in Nigeria

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Abstract

Background: Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. In hyperglycemic cases, haemoglobin binds irreversibly with glucose to form glycated haemoglobin (HbA1c) an accepted diagnostic test for type 2 diabetes mellitus (T2DM) used for monitoring glycaemic control in patients with diabetes. Some genetic variants influence the characteristics of HbA1c, via the erythrocytic pathway (erythrocytic variants), while some act through the glycemic pathway (glycemic variants). Glycemic variants increase the risk of developing diabetes, while erythrocytic variants have no association with the risk of diabetes. G202A, a variant of *G6PD,* is a single nucleotide polymorphism (SNP) associated with HbA1c in non-diabetic African Americans. G202A variant lowers HbA1c levels irrespective of an individual's blood glucose level. Alterations in HbA1c caused by a G202A SNP of the *G6PD* in normal African Americans leads to a massive under-diagnosis of T2DM. There is no previous study on the effect of this SNP on HbA1c in diabetic individuals hence the need for this study. **Methods:** Thirty-two non-diabetic and 54 diabetic male adult subjects were recruited after signing informed consent. Venous blood was collected from which fasting blood glucose and glycated haemoglobin were measured. DNA was extracted from whole blood. The samples were amplified, and amplicons digested using the PCR-RFLP technique and were visualised using a UV transilluminator. Data were analyzed using SPSS version 20 and SHEsis online software. **Results:** The genotypic distributions of G6PD G202A in the diabetic group were statistically significant (HWE $p<0.05$). The minor allele frequency 202A was 20% and 16% in diabetics and non-diabetics, respectively. *G6PD* G202A polymorphism was associated with HbA1c in diabetic subjects. *G6PD* G202A SNP showed association with diabetes (p-value=0.003) and HbA1c in diabetic subjects. **Conclusion:** There is an association between HbA1c and *G6PD* G202A SNP in diabetic subjects.

Keywords: T2D,*G6PD,* G202A, HbA1c.

Introduction

Diabetes mellitus is a metabolic disorder of carbohydrate, lipid, and protein metabolism characterized by high blood glucose levels, resulting from the inability of the body to either produce a sufficient amount of insulin or its failure to respond to insulin (Saltiel and Kahn, 2001). Diabetes is characterised by chronic hyperglycaemia, glycated haemoglobin (HbA1c), or C-peptide, a component of proinsulin used as a marker of endogenous insulin production (Singh, Clements and Singh, 2001; Jain et al., 2014).

HbA1c is an indicator of long-term blood glucose concentrations, measuring the amount of glycated haemoglobin, which shows the average ambient glycemia for the preceding 2–3 months (Mortensen and Christophersen, 1983). World Health Organisation (WHO) recommends the use

Received: 18 June 2021**; accepted revised manuscript:** 24 August 2021 **Published online:** 08 September 2021
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 | ISSN (Online): 2716-649X

of HbA1c to diagnose diabetes (WHO, 2011); it is therefore used for diagnosis and as well as prognosis of T2DM (ADA, 2012). HbA1c is a more convenient and stable marker of glycemic status and a good predictor of T2DM associated complications (Selvin et al., 2010). Previous studies reported genetic factors as vital players in determining HbA1c in individuals with T2DM (Snieder et al., 2001; Simonis-Bik et al., 2008; Mathias et al., 2009). GWAS and Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC) previously identified 61 loci influencing HbA1c either through glycemic or non-glycemic pathways (Chen et al., 2013a; Wheeler et al., 2017). Factors that affect erythrocyte biology are considered non-glycemic (erythrocytic) factors. Erythrocytic genetic variants reduce the erythrocyte lifespan, thereby giving false low HbA1c values (Wheeler et al., 2017). In diagnosing diabetes using HbA1C, these non-glycemic genetic variants falsely lower HbA1c levels, leading to missed T2DM cases (Bry, Chen and Sacks, 2001; Venkataraman et al., 2012).

A genetic study of HbA1c in African Americans showed the predominance of G202Avariant, which has been associated with HbA1c and reportedly mutates the protein produced by G6PD, thereby shortening the RBC lifespan and lower HbA1c levels in African Americans irrespective of the blood glucose level (Wheeler et al., 2017). This results in massive under-diagnosis of T2DM.

G6PD deficiency and its variants are common in areas with high occurrence of malaria, and both affect the diagnostic accuracy of HbA1c (Howes et al., 2012; Leong and Wheeler, 2018), screening for the G6PD genotype when diagnosing diabetes using HbA1c in populations where G6PD deficiency is highly prevalent was suggested (Paterson, 2017). Similarly, the American Diabetes Association recommends race/ethnicity be put into account when using HbA1c to diagnose diabetes in clinical practice guidelines (ADA, 2017). However, to the best of our knowledge, a study on the relationship between G202A and HbA1C in normal non-diabetic and diabetic patients in Nigeria has not been conducted.

Methods

Eighty-six subjects comprising of 54 diabetics and 32 non-diabetic individuals between the ages of 35 and 65 were recruited from Murtala Muhammad Specialist Hospital, Kano. Patients with any history of diagnosed metabolic disorders, cerebral vascular disease, coronary heart disease, known neuropsychiatric, diabetic complications such as cardiomyopathy, nephropathy, and retinopathy were excluded. All physically healthy subjects within the age group were included as normal subjects in the study. Subjects with substance abuse were also excluded. A signed informed consent approved by the Kano State Ministry of Health was obtained from each participant before commencing the study.

A total of 5 mL of blood was collected and poured in EDTA coated tubes for FBG, HbA1c measurements, and DNA extraction. FBG was measured using an Accucheck glucometer, while HbA1c was measured using ion-exchange chromatography. Following the world health organisation criteria for diagnosis of type II diabetes, individuals with fasting Blood glucose (FBG) ≥ 7 mmol/L were regarded as diabetics, while those with FBG < 7.0 were classified as normal. HbA1c ≥6.5% was also considered as diabetic,while values less than 6.5% were regarded as normal.

DNA was extracted from whole blood using a GF-1 blood DNA extraction kit following manufacturers' instructions. DNA samples were amplified by PCR, using primers as mentioned previously (Beutler et al., 1989):

Forward Primer 5'-GTCTTCTGGGTCAGGGAT-3'

Reverse Primer: 5'-GGAGAAAGCTCTCTCTCC-3'.

A total of 25mL of Polymerase chain reaction was formulated and performed. A total of 5 mL 5X PCR Master Mix, 1 mL of forward and reverse primers each, and 16 mL PCR grade water. Primers synthesized by integrated DNA technologies were used. Thermal cycling with an initial denaturation at 94 oC for 2 min., 35 cycles of denaturation at 94oC for 30 seconds, annealing at 60oC for 30 seconds, and elongation at 72oC for 1 min., then final extension at 72oC for 2 min.

For RFLP, a total of 10U of the restriction enzymes NlaIII (New England Biolabs, Ipswich, MA, USA) was incubated with 15 mL of PCR amplicons for 1 hour.

To the first well on the right side, a 2.5% agarose gel stained with ethidium bromide, 5µl of 50 bp DNA ladder were loaded, followed by 5 µl of the digested PCR products in the subsequent wells.

 | ISSN (Online): 2716-649X

The gel was run at a voltage was set to 100 volts 30 minutes, after which the gel was visualized and photographed using the UV benchtop transilluminator. Fragment sizes of GG: 109 bp; GA: 109, 63, 46 bp; AA: 63 and 46 were obtained.

Statistical analysis

Descriptive statistics were used to determine the percentage frequencies of diabetic and non-diabetic subjects using fasting blood glucose and the percentage of missed diagnoses of T2DM using the HbA1c values. Mann-Whitney U test was used to determine the differences in glycemic variables between diabetic and non-diabetic groups. Kruskal-Wallis test was used for differences in glycemic variables between the different genotypic groups. Results were presented as Mean ± SD and $P \leq 0.05$ was considered to be statistically significant. Shesis software was usedof genotypic and allelic frequencies as well as the Hardy-Weinberg p-value. $P \leq 0.05$ was considered statistically significant. Chi-square test of association was used for the association between G6PD G202A polymorphism and HbA1c. All statistical tests were conducted using SPSS software version 23, except for the genotypic and allelic frequencies.

Results

Subjects classified as non-diabetic using FBG were re-categorized using HbA1c. It was observed that there would have been about 42% missed diagnoses if HbA1c was used as a diagnostic test. This is depicted in Figures 1 and 2.

Fasting blood glucose (FBG) was statistically higher ($p = 0.001$) in the diabetic group compared to their non-diabetic counterparts. The FBG was within the normal physiologic range in the non-diabetic subjects (FBG = 5.4 mmol/L), while it was above the normal range in the diabetic subjects (FBG = 8.6 mmol/L). There was a significant difference in HbA1c between the diabetic and non-diabetic subjects ($p = 0.001$). HbA1c was higher in the diabetic group and exceeding the normal range (HbA1c ≥ 6.5%). In the normal non-diabetic subjects, however, HbA1c levels were lower and within the normal range, which is less than 6.5%, as shown in Table 1.

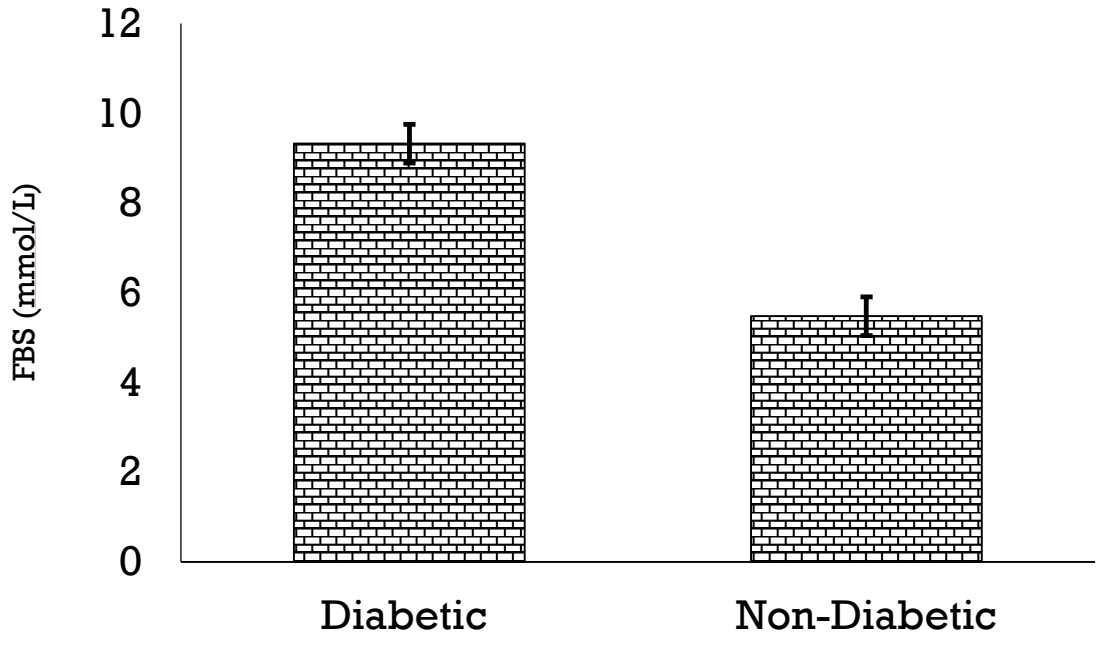

Figure 1. Fasting blood sugar among diabetic and non-diabetic subjects

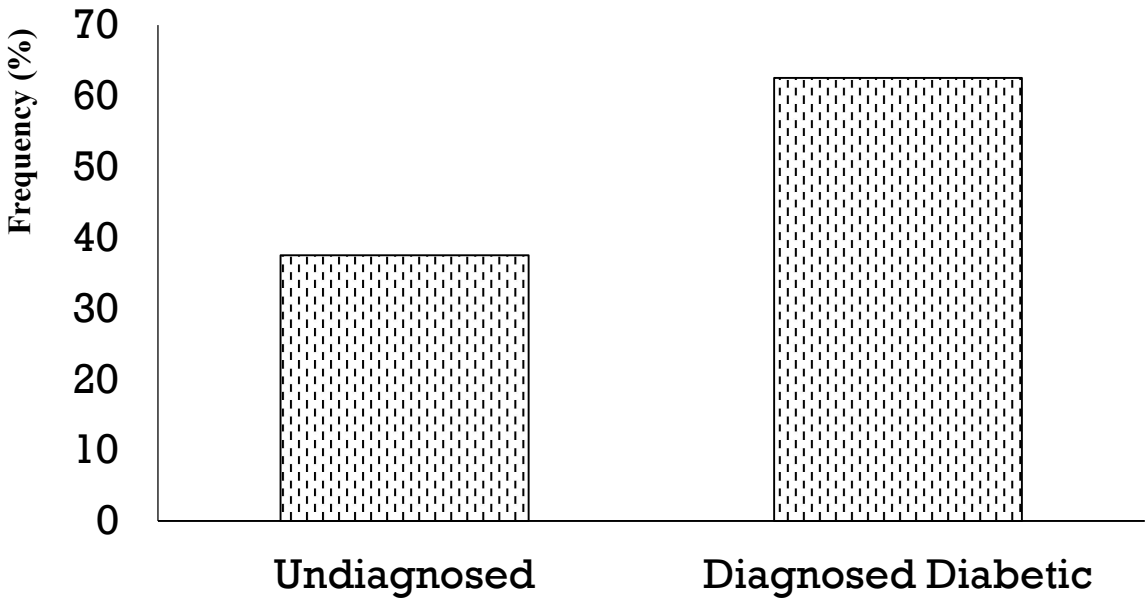

Figure 2. Percentage of missed diagnoses using HbA1c

Table 1.

	Non-Diabetic Mean± SD	Diabetic Mean± SD	t-statistic	p-value
FBG (mmol/l)	5.65 ± 0.18	9.10 ± 0.57	-4.747	0.001*
HbA1c (%)	5.04 ± 0.20	7.15 ± 0.26	-6.049	0.001*

The size of the PCR amplicon for G6PD G202A was 109 base pairs (bp). Following restriction digestion analysis, the fragment sizes were 109 bp for GG, 63 bp, and 46 bp for AA, while 109, 63, and 46 bp were for GA. Thus, about 53% of the normal individuals were normal for the G6PD G202A genotype (GG), and 16.7% were mutant (AA). In the diabetic subjects, however, 19% of them were found to be GG, 24% were heterozygous (GA), and 57% had an AA genotype.

The genotypic and allelic distributions of the G6PD G202A were determined, as shown in table 2. Among the diabetic group, the proportions of the G6PD G202A variant were as follows: GG had 0.148, GA = 0.222, and AA = 0.603. The Hardy-Weinberg equilibrium was not maintained as its p-value was significant ($p \leq 0.05$). In the non-diabetic group however, the proportions of the genotype were GG = 0.576, GA = 0.273 and AA = 0.152. Having a $p \leq 0.05$, Hardy–Weinberg equilibrium was therefore maintained.

 | ISSN (Online): 2716-649X

Table 2. Genotype and Allele distribution of G6PD G202A Variant between the Diabetic and Non-diabetic groups.

Genotype	Diabetic (Freq)	HWE of Diabetic p-value	Non-Diabetic (Freq)	HWE of Non-Diabetic p-value	Genotypic p-value	Chi2
GG	31(0.574)	0.0003*	16(0.533)	0.0929	0.718	
GA	12(0.222)		9(0.300)			0.663 df=2
AA	11(0.204)		5(0.167)			

Allele	Diabetic (Freq)	Non-Diabetic (Freq)	Allelic p-value	OR [95% CI]
G	74(0.685)	41(0.683)	0.980	1.008608 [0.51~1.99]
A	34(0.315)	19(0.317)		

Both FBG and HbA1c showed no significant difference in the three genotypic groups ($p > 0.05$) in diabetic subjects. FBG was higher in the AA and least in the GA groups in diabetic subjects. HbA1c had no significant difference between the genotypic group in diabetic ($p = 0.918$), as shown in Table 3. Nonetheless, the HbA1c levels were lower in the group with the mutant genotype compared to those with the wild-type genotype.

The current study results also show a significant association between G6PD G202A polymorphism and HbA1c in diabetic patients, as shown in Table 4.

Table 3. Genotypic distributions of FBG and HbA1c among diabetic subjects

	GG	GA	AA	Chi-Square	P-value
FBG (mmol/L)	9.3 (4.1 – 23.2)	7.8 (4.7 – 17.8)	8.2 (3.3 – 18.0)	0.381	0.827
HbA1c (%)	7.1 (4.5 – 10.6)	7.1 (4.9 – 13.7)	6.9 (4.9 – 10.6)	0.170	0.918

Table 4. Association between Fasting blood glucose, HbA1c and G6PD G202A Variant.

	Chi-square	p-value
FBG (mmol/l)	36.857	0.955
HbA1c (%)	96.872	0.001*

Discussion

This study reported about 37% missed diagnoses using HbA1c, thus questioning its sensitivity and diagnostic accuracy. Similar results were reported in T2DM subjects in Kano (Yarube et al., 2019) and East-Asian participants with diabetes (Leong et al., 2020). The association between G202A SNP of the G6PD and HbA1c among diabetic subjects of African origin was determined in the current study. The minor allele frequency (MAF) of the 202A allele to be 16% in the normal non-diabetic subjects. This is similar to the MAF in the Esan population of Nigeria (Auton et al., 2015). The MAF observed in this study falls within the frequencies in the sub-Saharan African populations, which ranges from 3.0 to 19% (Tishkoff et al., 2001). Previous evidence of significant variations in the 202A allele frequency based on ethnicity as reported in the Genome project Consortium (GPC) (Auton et al., 2015). They reported varying MAF in different populations, viz: African Caribbeans in Barbados; 13%; Americans of African Ancestry in the south-west USA; 17%, the Esan in Nigeria; 16%, the Mende in Sierra Leone; 7%; Gambian in Western Divisions in the Gambia; 4%; Luhya in Webuye,

Kenya; 18%; Yoruba in Ibadan, Nigeria; 21% (Auton et al., 2015). Other studies reported a MAF of approximately 0.6% and 7.7–11.9% were reported among Fulani and Dogon ethnic groups in Mali (Maiga et al., 2014; Dolo et al., 2014), 6.0–14.9% in Burkina Faso (Dolo et al., 2014), 20.0% in apparently healthy Tanzanian individuals (Manjurano et al., 2015). The 202A allele frequency of 15% was reported in healthy subjects from Lagos (Babalola et al., 2018). Furthermore, 2.8% for 202A was observed in the mixed ethnic groups of Gambians (Clark et al., 2009). The MAF for the 202A allele in Malian subjects was recorded at 7.7% (Battistuzzi et al., 1977). These heterogeneous allele frequencies may be due to the complexity of the G6PD haplotype signature in the tropical African region.

In the current study, HbA1c was associated with G202A SNP of the G6PD in diabetic subjects. There were, however no differences in the HbA1c distribution between genotypic groups in the diabetic subjects in the current study. This, to the best of our knowledge, is the first study on the association as well as the effect of G202A SNP on HbA1c in diabetic individuals. The diabetic subjects with the mutant genotype had HbA1c level 0.2% lower than the hemizygous diabetic subjects, contrary to the 0.7% lower HbA1c in hemizygous non-diabetic African American individuals reported by Wheeler and colleagues (Wheeler et al., 2017). This variant was first identified as a cause of G6PD deficiency, showing association with numerous red cell parameters such as lower hematocrit, haemoglobin, red cell count, and Red cell Distribution Width (RDW); higher mean corpuscular volume and mean corpuscular haemoglobin concentration (Chen et al., 2013b; Ding et al., 2013). Surprisingly, in a GWAS of HbA1c, the SNP was associated with lower HbA1c levels in an African American population (Wheeler et al., 2017).

In this study, we found no association between G202A and FBG in diabetic subjects. Similarly, a GWAS of glucose in African Americans showed no association between this SNP and FBG (Wheeler et al., 2017). This lack of association with FBG led researchers to classify the SNP as erythrocytic and not glycemic, concluding that the SNP is associated with HbA1c via erythrocytic mechanisms (Paterson, 2017). Therefore, being an erythrocytic variant, G202A of G6PD can be inferred to decrease HbA1c levels by reducing the life span of red blood cells, thereby causing hemolysis.

One of the limitations of this study is that the red cell indices of the individuals were not taken into consideration. Also, the G6PD status of the subjects was not determined.

Conclusion

We found an association between G6PD G202A polymorphism and HbA1c levels in diabetic individuals in an African population.

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 | ISSN (Online): 2716-649X