



## Effect of Haemoglobin Variants on Glycemic Indices

Victor Tuanwii Ideede<sup>1</sup>, Jonathan Nyebuchi<sup>2\*</sup>, Albert Lesere Nwibani<sup>2</sup>,  
Friday Ogidigba<sup>1</sup>, Felix Eedee Konne<sup>2</sup> and Fyneface Chikadibia Amadi<sup>2</sup>

<sup>1</sup>Department of Medical Laboratory Science, Bayelsa State College of Health Technology, Otuogidi-Ogbia, Bayelsa, Nigeria.

<sup>2</sup>Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/IBRR/2021/v13i1130161

Editor(s):

(1) Dr. Dharmesh Chandra Sharma, J. A. Groups of Hospital and G. R. Medical College, India.

Reviewers:

(1) Iraklis Avramopoulos, Hygeia Hospital, Greece.

(2) Matthew Adu, Edwin Clark University, Nigeria.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/74242>

Original Research Article

Received 01 August 2021

Accepted 05 October 2021

Published 12 October 2021

### ABSTRACT

Haemoglobin genotypes have been known to be linked with groups of diseases such as diabetes. The aim of this study is to assess the impact of haemoglobin variants on glycemic indices (fasting blood glucose and glycated haemoglobin) in subjects in Bayelsa State, Nigeria. A total of 150 subjects were enrolled for the study with AA group = 99 subjects and AS group = 51 subjects. 4mls of blood was collected into EDTA bottle for each subject and was assayed for Hb electrophoresis and glycated haemoglobin (HbA1C) using electrophoretic method and automated CLOVER A1c Analyser respectively. 2mls was collected into fluoride oxalate bottle for spectrophotometric analysis of fasting blood glucose (FBG). Results revealed that there were no significant differences in the FBG and HbA1C mean levels of the two studied groups (AA and AS). This study has shown that AA and AS blood genotypes may not have any impact on FBG and HbA1C glycemic parameters.

**Keywords:** Haemoglobin variant; glycated haemoglobin; fasting blood glucose.

## 1. INTRODUCTION

Haemoglobin variants are inherited abnormal or mutant forms of haemoglobin and their presence could cause certain disorders in the blood. The commonly found haemoglobin variants among Nigerians include; HbAA, HbAS and HbSS as reported by Umoh *et al.*, (2010). Glycated haemoglobin (HbA1c) is a type of haemoglobin estimated mainly for the identification of average plasma glucose level for three months. It is restricted to a three-month average since the lifecycle of erythrocyte is usually between three to four months. Nevertheless, glycated hemoglobin is usually assumed as an incomplete measure of three months. This is because erythrocytes do not all experience haemolysis at once. The mode of formation is through a glycation pathway that is non-enzymatic through haemoglobin's exposure to glucose in plasma. The degree of the beta-N-1-deoxy fructosyl part of hemoglobin is glycated haemoglobin (Miedema, 2005). The frequency of type 1 and type 2 diabetes mellitus, specifically type 2, has increased in the years past and continues to increase at a frightening degree worldwide as revealed by a wealth of epidemiological data. History revealed that diabetes became common first among wealthy western populaces, then spread rapidly to other populaces in due to better conditions of living and western way of life embracement (Coca-colonisation). Other factors that contributed majorly to the diabetes epidemic are changes in environment due to development, excessive fat accumulation and getting old. Differences in genetic composition among populations are possibly a contributor of diabetes mellitus. The prevalence of type differs among tribes, with a greater danger in those of European origin compared to African descent, and considerably reduced in Asian and Pacific Islanders although the prevalence of type 1 diabetes is relatively low (<1%) across the world [1]. Haemoglobin genotypes have been known to be linked with groups of diseases [2]. These variants change hemoglobin organization and chemical characteristics causing insignificant to severe physiological effects [3]. Knowing the relationship between haemoglobin variants and glycated haemoglobin could aid in appropriating vulnerability to diabetes mellitus amongst a genetically related population [4]. This study is intended to compare the glycemic indices between AA and AS haemoglobin variants in Bayelsa State.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted among subjects who reside in Yenagoa Local Government Area of Bayelsa State, Nigeria.

### 2.2 Study Population and Sample Size Description

The study population consisted of apparently healthy male and female subjects residing in Yenagoa Local Government Area, Bayelsa State of Nigeria. A total of 150 subjects were enrolled for the study. All subjects were aged between 16 and 48 years in the general population and included both males and females.

### 2.3 Subjects Eligibility Criteria

All the subjects utilized for the study were apparently healthy as portrayed by the research clinician. Subjects with history of diabetes mellitus and other chronic diseases were excluded from the study. More so, subjects that were not residing in Bayelsa were not included in this study.

### 2.4 Specimen Collection

Four milliliters (4 mls) of Blood samples was collected from the subjects utilizing standardized phlebotomy venepuncture method [5,6,7]. 2 mls of the blood was withdrawn into EDTA for HbA1c evaluation and blood genotype while the other 2mls was withdrawn into fluoride oxalate for fasting blood glucose.

### 2.5 Sample Analysis

#### 2.5.1 Haemoglobin Genotype determination

The Haemoglobin Genotype was determined by the alkaline Cellulose Acetate Electrophoresis technique as described by Cheesbrough, [8]. The first step in Haemoglobin electrophoresis is the preparation of hemolysate, which involves destroying the intact red cell membrane to free the haemoglobin. To achieve this, 0.5ml to 1ml of well mixed anticoagulated whole blood was placed into a well labeled test tube and filled normal saline, centrifuged at 2500 RPM for 5 minutes. Supernatant was removed and cell washing repeated for 2-3 times. The supernatant was removed with a Pasteur pipette. One drop of the washed cell was placed into an appropriately

labeled tube and 5 drops of distilled water added. This was mixed and allowed to stand for 5 minutes for complete hemolysis of red cells.

About 100 ml of the Tris buffer was poured into the outer section of the electrophoresis chamber. The two wicks were wet in buffer and one draped over each support bridge, ensuring that each makes contact with the buffer. 5µl of each hemolysate samples (test and control) was transferred into the well plate. The cellulose acetate membrane was placed in the zip- zone and sample applied using applicator. The cellulose acetate membrane was immediately placed in the electrophoresis chamber. To electrophorese, the power supply was turned on and electrophorese at 350V for 25 minutes. At the end of 25 minutes, the hemoglobin types present in the patient's sample was identified by comparing the migration distance with the known controls separated under identical conditions to the test sample.

Control samples; AA, AS, SS.

### 2.5.2 Estimation fasting blood glucose

Fasting blood glucose was determined using Glucose Oxidase method. The reaction is a coupled reaction with hydrogren peroxidase, an enzyme that catalyzes the hydrolysis of hydrogen peroxide. The overall effect is the production of a coloured solution that is read spectrophotometrically. The intensity of the colour is proportional to the concentration of glucose in the sample.

### 2.5.3 Determination of glycated haemoglobin

Glycated haemoglobin (HbA1c) was determined using the automated CLOVER A1c Analyser as described by Diabetes Management Technology [9]. It is a spectrophotometric self Analyser that consist of self-Test cartridge and provides a convenient method for measuring the percent concentration of haemoglobin A1c (HbA1 %) as specified by Diabetes Management Technology. The CLOVER A1c self-system is a fully automated boronate affinity assay for the determination of the percent of Hemoglobin A1c (HbA1c %) in whole blood.

The Test Cartridge is composed of a cartridge and a reagent pack containing the reagent necessary for the determination of HbA1c with a sample collecting area for blood sample collection. The reagent pack is pre- filled with reaction solution and washing solution. The reaction solution contains agents that lyse erythrocytes and bind hemoglobin specifically, as well as boronate resin that bind cis-diols of glycated hemoglobin. The blood sample (4µl) is collected at the sample collecting area of the reagent pack, then the reagent pack is inserted into the cartridge, where the blood is instantly lysed releasing the hemoglobin and the boronate resin binding the glycated hemoglobin. The reagent pack containing the blood sample is inserted in CLOVER A1c self Analyser (in which the cartridge has been placed). The cartridge is automatically rotated, placing the blood sample in the measuring zone. The total hemoglobin is photometrically measured by the diffused reflectance of the optical sensor composed of both a LED (Light Emiting Diode) and a PD (Photo Diode). Then, assembled cartridge is rotated and the rinsing solution washes out non-glycated hemoglobin from the blood sample, enabling photometrical measurement of glycated hemoglobin. The ratio of glycated hemoglobin and total hemoglobin is calculated.

### 2.6 Statistical Analysis

Data were analyzed with Statistical Package for Social Sciences (SPSS) version 20, and Microsoft excel. Student *t*-test was used for comparing glycemic indices between AA and AS haemoglobin variants and  $p \leq 0.05$  was considered significant.

## 3. RESULTS

Fasting Blood Sugar (FBS) and glycated haemoglobin (HbA1c) were evaluated between the two common haemoglobin variants (AA) and (AS). Subjects with genotype HbAA were 99 while subjects with HbAS genotype were 51. For FBS, a mean value of  $4.08 \pm 1.09$  was observed for Hb Phenotype AA and  $4.15 \pm 1.15$  observed for AS. A mean concentration of  $5.12 \pm 0.79$  for AA and  $5.01 \pm 0.88$  for AS was also observed when HbA1c was compared. No significant difference was observed between groups for all parameters,  $p > 0.05$  using independent t-test.

**Table 1. Evaluation of glyceic indices based on haemoglobin electrophoretic patterns**

Parameters	AA(n=99)	AS(n=51)	p-value
FBS(mmol/L)	4.08±1.09	4.15±1.15	0.75 NS
HbA1c(%)	5.12±0.79	5.01±0.88	0.46 NS

Key: FBS = Fasting Blood Glucose; AA = Haemoglobin AA phenotype; AS = Haemoglobin AS phenotype; HbA1c = Glycated Haemoglobin

#### 4. DISCUSSION

Few recent published data have been encountered in Nigeria on the influence of hemoglobin variants on glycated hemoglobin but none on the influence of Hemoglobin variants on glycated hemoglobin among residents in Yenagoa, Bayelsa state, Nigeria. The study reported two key haemoglobin variants (AA and AS). There was no sickle cell hemoglobin (HbSS) and haemoglobin C trait (HbAC) recorded in this study. It is thus consistent with a study carried out by Jeremiah, [10] on abnormal hemoglobin Variants, ABO and Rhesus Blood groups among students of Africa descents in Port Harcourt where the frequency of HbSS and HbAC were equally zero. Evaluation of the concentration of Fasting Blood Sugar (FBS) and Glycated Haemoglobin (HbA1c) between the two common haemoglobin variants (HbAA and HbAS) among the study subjects showed no statistical significant difference in FBS ( $p > 0.05$ ) between the subjects with Hb AA and those with Genotype HbAS. This is probably due to the fact that subjects for this study were apparently healthy and not diabetic. The mean concentration of glycated Hb of HbAA subjects was not statistically different from that of HbAS subjects. This is in consonance with a study by Bleyer and others in 2010 that showed that sickle cell trait does not have any impact on glycated haemoglobin as well as glucose concentration [11]. This may be true as there was no observed abnormal haemoglobin variants such as HbAF and HbSS encountered in this study and thus in agreement with Chanadrassen et al., [12] who reported that subjects with HbF and HbS trait may have abnormal HbA1c values. However, majority of persons having diabetes mellitus across the world also had haemoglobin disorder [12]. Moreso, a significantly higher level of glycated haemoglobin has been reported in sickle cell anaemia (HbSS) and sickle cell trait (HbAS) than in normal Haemoglobin (HbAA) [13].

#### 5. CONCLUSION

This study has revealed that haemoglobin variants (AA and AS) may not have any

significant impact on glyceic index in Bayelsa State. However, further studies are encouraged to consider other variants not considered in this study to really understand the impact of haemoglobin variants on glyceic status.

#### CONSENT AND ETHICAL APPROVAL

The experimental protocol was approved by the Ethics Committee of the Bayelsa State Ministry of Health. Informed consent was also obtained from each subject after been told of the nitigrities of the study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

- Serrano-Rios M, Goday A, Larad TM. Migrant Populations and the Incidence of Type 1 Diabetes Mellitus: An Over View of the Literature with Focus on the Spanish – Heritage Countries in Latin America. *Diabetes Metabolites Research Review*. 1999;15:113-132.
- Kassai T, Fesus L, Hendriks WM, Takats C, Fok E, Redl P, et al. Is there a relationship between haemoglobin genotype and the innate resistance to experimental haemonchus contortus infection in merino lambs? *Veterinary Parasitology*. 1990;37(1):61-77.
- Christopher ST, Claire FD, David AG, Mitchell JW. *Hemoglobin Variants: Biochemical Properties and Clinical Correlates*. Cold Spring Harbor Perspectives Medicine. 2013;3(3):231-239.
- Okon UA, Akpanyung EO. Relationship between haemoglobin genotype, incidence of diabetes mellitus and some clinical complications in Southern Nigeria Tertiary Health Institution. *Journal of Dental and Medical Sciences*. 2017;16(1):88-92.
- Fyeface CA, Joel BBK, Felix EK. Assessment of creatinine levels in blood and saliva of haemodialysed subjects.

- International Journal of Advances in Nephrology Research. 2020;3(1):21-25.
6. Fyeface CA, Ibama O, Davies TE. Evaluation of Saliva for Monitoring Renal Function in Haemodialysis Patients at University of Port Harcourt Teaching Hospital. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2018; 1(2):1-6.
  7. Onengiyeofori I, Fyeface CA. Assessment of serum levels of some heavy metals in carpenters residing port harcourt in relation to their lifestyle. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2018;4(4):1-7.
  8. Cheesbrough M. District laboratory practice in tropical countries Part 2. Cambridge University Press; 2002.
  9. Diabetes Management Technology. Clover A1c self Test Cartridge. Ref: INFHS01AS2. P 1-2. Infopia Co Ltd Publisher; 2017.
  10. Jeremiah ZA. Abnormal Haemoglobin Variants, ABO and Rh Blood Groups among Students of African Descents in Port Harcourt, Nigeria. African Journal of Health Science. 2006;6:177-181.
  11. Bleyer AJ, Vidya S, Suyat L, Russel GB, Akinifesi D, Ffredman BI. The impact of sickle cell trait on glycated hemoglobin in diabetes mellitus. Diabetic Medicine. 2010;27(9):1012–1016.
  12. Chanadrasena I, Hemantha P, Senani W, Siribadana SH. Hemoglobin variants in patients with type 2 diabetes mellitus. South East Journal of Tropical Medicine of Public Health. 2010;1(2):48-56.
  13. Reid HL, Famodu AA, Photiades DP, OSamo ON. Glycosylated Haemoglobin HbA1c and HbS1c in Non-Diabetic Nigerians. Tropical Geographical Medicine. 1992;44(1-2):126-130.

© 2021 Ideede et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:  
<https://www.sdiarticle4.com/review-history/74242>*