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Eradicating Haemoglobin Variants Disorder: A Reality in Nigeria

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ABSTRACT

Haemoglobinopathies are among the most monogenetic disorders worldwide, inherited as autosomal recessive disorders from healthy-carrier parents. The most common are the sickle cell disorders and the thalassaemias, occurring in people of African, Asian, South European, and Middle Eastern descent. Our study aimed to understand the prevalence of the normal haemoglobin A among Nigerians and the ratio of HbAA to other Hb variants in the country to strategize an eradication algorithm for the haemoglobin disorders. Two independent studies of Hb genotyping were carried out from a neonatal population (387) and from an adult population (298). Haemoglobin electrophoresis at alkaline pH with cellulose acetate was the method of choice. Data analysis was done using simple percentages and the chi-square test.The participants' (685) results were as follows: 544(79.4%) were HbAA, 127 (18.5%) had HbAS, 9 (1.3%) had HbAC, 3 (0.4%) had HbSS, and 2 (0.4%) had HbSC. The ratio of Hb AA to Hb AS (4:1), HbAA to HbSS (181:1), HbAA to HbAC (60:1), and HbAA to HbSC (272:1), respectively. The knowledge and awareness of the participants were tested using a structured questionnaire, and on both sides, there was a statistically significant decrease in knowledge of the adult subjects and that of the parents and guidance of the neonates ($p>0.05$). It was concluded that making use of the high frequency of HbAA across the nation, a strategic and steady awareness campaign, along with education at all levels of life and guided government policy, eradication is achievable

Keywords: Haemoglobinopathies, Algorithm, Education and Eradication.

Introduction

Haemoglobin is a tetrameric metalloprotein that consists of two alpha (α or ζ) globin chains and two beta (β, ε, γ, or δ) globin chains that transport oxygen around the body (Pandya, *et al.,* (2019). Alpha-globin gene cluster is found on chromosome 16, and the beta-globin gene cluster is found on chromosome 11 (Jacob, (2016). These genes can be mutated, resulting in haemoglobin variants, by deletion, insertion, abnormal crossing over, point mutation, and/or mutations that alter the amino acid sequences (Buseri., & Okonkwo, (2014). The result of these abnormalities is haemoglobin disorders, a set of genetic illnesses conventionally categorized as thalassaemias (quantitative defects) and haemoglobinopathies (qualitative defects) [Hays, *et*

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al.,(2019).

The most prevalent monogenic disorders worldwide are hemoglobinopathies due to structural abnormalities in the production of haemoglobin (Harteveld, *et al*., (2022) These mutations can transform biochemical and physical features of haemoglobin that can cause abnormal phenotypes that can be identified using laboratory methods [Greene, *et al*., (2019).The inheritance occurs in an autosomal co-dominant manner, in which individuals with homozygous inheritance develop the disease, but those with hetero-zygous inheritance do not develop the disease (Buseri., & Okonkwo, (2014). These diseases constitute significant public health concern in most low and middle-income countries because of insufficient diagnostic, therapeutic and preventive facilities.

Haemoglobin Disorders in Humans

Haemoglobin disorders have significant worldwide morbidity. The most common ones are sickle cell disease (SCD) and thalassaemia, although other variants of haemoglobin C, D, and E are also associated with morbidity and mortality (Hirschsbli,*et al.,* (2019). Sickle cell disease has been endemic in sub-Saharan Africa, haemoglobin C is predominant in West Africa, haemoglobin D is predominant in Asian and British people, and haemoglobin E is highly prevalent in Southeast Asia (Nagababu, *et al*., (2019). The range of these disorders and their dispersion highlights their highly variable epidemiology and the necessity of region-specific eradication measures.

Table 1. Molecular Characteristics of Human Globin Chains and Haemoglobin

Globin chain	Molecular mass (Da)	Number of amino acids	Haemoglobins
α	15,126.4	141	Hb A; Hb A2; Hb F
β	15,867.2	146	Hb A
γA and γG	16,009.3 and 15,995.3	146	Hb F Hb Portland*a*
δ	15,924.3	146	Hb A2
ε	16,071.6	146	Hb Gower 1*a* Hb Gower 2*a*
ζ	15,505.8	141	Hb Gower 1*a* Hb Portland*a*

Adopted from: Troxler, *et al*., (2012)

Laboratory studies that involve the biochemical, biophysical, and molecular analysis, including but not limited to high-performance liquid chromatography (HPLC), polymerase chain reaction (PCR), and the sequencing of the DNA, are very important in diagnosis [Greene, *et al*., (2019) & Koutsi, & Vervesou, (2018). Through this type of technology, the variants can be identified accurately, and the foundation of prevention-based premarital, prenatal, and newborn screening can be laid. Yet, these diagnostics are often inaccessible in low-resource areas, which postpones diagnosis and proper treatment.

There are massive clinical implications related to haemoglobin disorders. Common presentations among patients include anaemia, vaso-occlusive problems, splenomegaly and low quality of life (Nagababu, *et al*., (2019) & Mantadakis, *et al.,* (2020). Lifelong interventions, including red blood cell transfusions, iron chelation therapy, or hydroxyurea, are the standard of care in the view of many (Bain, (2011). Treatment alternatives such as haematopoietic stem cell transplant and gene therapy have potential and are not yet accessible to the majority of patients because of cost, infrastructure, and donor constraints (Bain, (2011), Henry, *et al.,* (2020)*;* Rai, & Malik, (2016)). Therefore, carrier identification, counseling, and community education is one of the pillars of

eradication.

Educational Contributions to Haemoglobin Disorder Awareness

The role that the community plays in the management of haemoglobinopathies cannot be underrated. Knowledge of haemoglobin disorders is poor even with the rise in the level of formal education. In a Cameroonian study 60.4% tertiary education had been achieved by unmarried youths, 20.5% knew about sickle cell disease, and 13.2% knew their genotype (Ngwengi, *et al.,* 2020). Likewise, a study in Bangladesh found that there were a lot of misunderstandings about thalassaemia among college students (Hossain, *et al.,* 2020). This indicates that awareness is not the same as formal education and highlights the necessity of specific interventions in community of adolescents and young adults before they get married. (Ahmed, & Molla, (2017).

Theoretical Review of Haemoglobinopathy Eradication

Theoretically, eradication approaches are designed to aim at prevention, early diagnosis, genetic counseling and new treatments (Koutsi, & Vervesou, (2018), Aliouche, 2022 & Henry, *et al.,*2020). Prenatal, newborn, or premarital screening offers important opportunities to make interventions early. Genetic counseling facilitates informed decision-making and minimization of risk among risky couples (Aliouche, 2022). Gene therapies may provide potential solutions to more curative therapy in future and transfusion, chelation and hydroxyurea are still relevant as disease-modifying therapy (Bain, (2011) &, Crighton, *et al.,* 2016). Global and national plans, including World Health Organization (WHO)-coordinated efforts, focus on awareness campaigns, policy, and measures, as well as the distribution of resources fairly to enhance the situation in low-income states (Okwe, & Ofili,. (2025)

Various models of health risk mitigation due to haemoglobinopathy have been suggested. They are as follows: comprehensive follow-up screenings of newborns; access to combined healthcare services (Iheanacho, *et al.*, 2021), patient education to facilitate self-management (Hazaa, *et al*., 2021); multisectoral interventions, which address anaemia prevention and treatment (Alhazmi, *et al*.,2022); and patient purchasing of transfusion-dependent products via safe blood banking at the community level (Chowdhury, & Anwar, (2020). Together, these measures are a multidimensional approach to limit the prevalence of haemoglobinopathies, as well as to improve the lives of affected people.

Haemoglobin variant diseases have been neglected in the low-income countries, as they do not rank as a priority in the health systems of these countries, and prevention, diagnosis, and treatment are not effective in addressing the disease. The burden remains in spite of molecular diagnostics improvements and experimental therapies as the awareness is not appropriate, the policy is poorly implemented and the hazards are not properly provided. This research paper hence aims at formulating an algorithm to eliminate haemoglobin variant disorders in poor countries, by combining molecular diagnosis, genetic counseling, treatment modalities, and preventive measures aimed at the common population.

The central research question is: Can a comprehensive algorithm integrating diagnostic, therapeutic, educational, and policy approaches significantly reduce haemoglobin variant disorders in resource-limited settings? In answering this question, this study proposes the new framework that can help address the gap between high-level biomedical knowledge and its use in low-resource settings. This is important since it provides a guide on how to reduce the global burden of haemoglobinopathy using sustainable and context-based measures.

Materials and Methods

Study Area

The first study was conducted between 2019 and 2020 in Plateau State, Nigeria, with analyses performed at the Haematology Laboratory of Jos University Teaching Hospital (JUTH), Jos. Plateau

State, located in the North Central geopolitical zone, is the twelfth largest state in Nigeria, covering 26,899 square kilometers with an estimated population of about three million. Geographically, it lies between latitude 08°24′N and longitude 008°32′ and 010°38′E, approximately at the country's center (National Population Commission (2006).

The second study was undertaken in 2024 at Joseph Ayo Babalola University (JABU), situated at Kilometer 36, Ilesa-Akure Road, Ikeji Arakeji, Osun State, Nigeria. JABU, founded in 2004 by the Christ Apostolic Church (CAC) and named after its first spiritual leader, Joseph Ayo Babalola (1904–1959), is recognized as Nigeria's first entrepreneurial university (JABU,2024). Osun State, in the South-West geopolitical zone, is the ninth smallest (14,875 sq km) and nineteenth most populous state, lying between latitude 7°30′0″N and longitude 4°30′0″E. It is a constituent of 30 Local Government Areas (National Population Commission (2006). JABU is a fully residential university consisting of seven faculties which include sciences, arts, commercial courses and built environment courses.

Study Design

The present study used cross-sectional experimental design to determine the prevalence of Hb genotypes among neonates in JUTH and measure parental knowledge and awareness both neonatal parents and JABU students. The cross-sectional design allowed the measurement of Hb distribution at one time, whereas the structured questionnaires determined the awareness. To determine the accuracy and reliability of the results, Hb genotypes were determined by using cellulose acetate electrophoresis.

Study Population

The research group comprised of 387neonates and 782 parents and 298 members of the university population. A structured questionnaire was used to stratify the demographic information of the participants after each of them had signed an informed consent form.

The different sample size was obtained with the use of Yamene,1965 as presented under(Muyembe & Anselemo (2023) procedure of sample size determination using population. Once the sample size was acquired, stratified random sampling was used to select the respondents in terms of their faculties (Arts, Sciences, and Commercial disciplines) to get a good proportion of all respondents.

Data Collection Tools

A structured questionnaire to collect demographic data, evaluate the level of awareness of the Hb genotype and knowledge of its clinical relevance was used as the primary means of collecting data.

Data Analysis

Descriptive statistics were used to evaluate the subjects demographic data and awareness levels, whereas Chi-square tests were conducted to evaluate awareness and knowledge differences between participants.

Results

Table 2. Socio-demographic characteristics of the study participants

Variable	Category	Students n (%)	Parents (Male) n (%)	Parents (Female) n (%)
Age (years)	< 18	51 (17.1)		
	18–25	180 (60.4)		
	26–35	67 (22.5)		
	20–30		81	227
	31–40		207	154

	41–50		113	10
Gender	Male	160 (53.7)	391	
	Female	138 (46.3)		391
Colleges	Arts	90 (30.2)		
	Sciences	208 (69.8)		
Level of Study	100 Level	35 (11.7)		
	200 Level	26 (8.7)		
	300 Level	73 (24.5)		
	400 Level	100 (33.6)		
	500 Level	64 (21.5)		
Total		298 (100.0)	391	391

Table 3. Knowledge of Haemoglobin (Hb) Genotype of the Study Participants.

Demography	**Knows n (%)**	**Don't Know n (%)**	**χ^2 and P-value**
Neonate Parents	330 (42.2)	452 (57.8)	$\chi^2 = 19.03$, $p < 0.001$
Male Students	70 (43.8)	90 (56.2)	$\chi^2 = 1.57$, $p > 0.05$
Female Students	72 (52.2)	66 (47.8)	
Arts Students	40 (44.4)	50 (55.6)	$\chi^2 = 0.04$, $p > 0.05$
Science Students	102 (49.0)	106 (51.0)	–
Educational Level of parents			$\chi^2 = 126.791$, $p < 0.001$
None Formal	2 (13.3)	13 (86.7)	
Primary	5 (10.0)	45 (90.0)	
Secondary	59 (21.7)	213 (78.3)	
Tertiary	264 (59.3)	181 (40.7)	

Table 4. Test of knowledge of haemoglobin genotype of the "known" students (n=142)

Genotype	**Reported**		**Actual**		χ^2	***p*-value**
	Arts n (%)	Science n (%)	Arts n (%)	Science n (%)		
Hb AA	33(82.5)	86(84.3)	27	71(73.5)	0.0696	0.791
Hb AS	07(17.5)	12(11.78)	13	27(22.8)		
Hb AC	-	2(1.96)	0	2(2.3)		
Hb SS	-	1(0.98)	0	1(0.7)		
Hb SC	-	1(0.98)	0	1(0.7)		
Total	40(100)	102(100)	40(100)	102(100)		

* χ^2: Pearson Chi-square, *p*-value <0.05, S: Significant

Table 5. Comparison of Students' Self-Reported and Laboratory-Confirmed Haemoglobin Genotypes (HbAA and HbAS) Using McNemar's Test (n = 142)

Reported vs Actual Genotype	Actual Genotype: Hb AA		Actual Genotype: Hb AS	
	Hb AA	Not Hb AA	Hb AS	Not Hb AS
Reported HbAA	93	26	–	–
Reported Not HbAA	5	18	–	–
Reported HbAS	–	–	17	2
Reported Not HbAS	–	–	23	100
McNemar's χ^2 (df = 1)	**14.23**	**p < 0.001**	**17.64**	**p < 0.001**

Table 6. Haemoglobin genotype distribution of the two study groups

Items	Neonatal Group	University Group	Total	Ratio Of Hb to Aa
AA(+F)	327 (84.4)	217(72.8)	544 (79.4)	
AS	54 (14.0)	73 (24.5)	127(18.6)	4:1
AC	3 (0.8)	6 (2.0)	09 (1.3)	121:1
SS	2 (0.5)	1(0.4)	03 (0.4)	272:1
SC	1 (0.3)	1(0.4)	02(0.3)	181:1
Total	387 (100.0)	298(100.0)	685(100.0)	

Discussion

The research was conducted with a population of 387 newborns between 0 and 9 months of age female (50.4%) and male (49.6%). As the subjects were neonates, structured questionnaires were distributed to parents/guardians to collect demographic data and genetic background, and health history information, and there were 782 questionnaires in total. The research was to assess the level of knowledge and awareness of haemoglobin genotype in adults at various stages of life. The male age range of highest percentage among parents was 31-40 years (52.9%), and females were 20-30 years (58.1%). For the student group, aged <18–35 years, the majority were 18–25 years (60.1%), with 160 males (53.7%) and 138 females (46.3%).

A chi-square goodness-of-fit test was conducted to assess whether the distribution of the neonates' parents' knowledge of their haemoglobin genotype differed significantly from an equal distribution. The results revealed a statistically significant difference between those who knew their genotype and those who did not, $\chi^2(1, N = 782) = 19.03$, $p < 0.001$ (Table 3). A greater proportion of the parents (57.8%) did not know their haemoglobin genotype, indicating a gap in genetic health awareness. (Table 2).

The effect of education was considered on the knowledge gap of the respondents, and a significant difference was observed on the parents' group (Table 3). The primary and non-formal educated respondents had the highest "don't know" status (90.0% and 86.7% respectively), and the tertiary education group had the least "don't know" status (40.7%). These findings were in agreement with the works of Faremi *et al*.,2018 and Babalola *et al*.,2019, and just like

age, can be explained by the mode of awareness. A definite effort should be made by the government & NGOs to encourage education and make it appealing to each region.

A chi-square test of independence was conducted to determine the association between demographic variables and knowledge of haemoglobin genotype among university participants (n = 298). Results showed no significant relationship between gender and genotype knowledge (χ^2 =1.57, $p > 0.05$), nor between academic discipline and genotype knowledge ($\chi^2 = 0.44$, $p > 0.05$). This indicates that awareness of genotype status did not significantly differ between male and female students or between Arts and Science students. Table 5. Our findings were similar to those of [34] whose subjects had 19.2% misreported or misidentified genotype and 45% don't know cases, while Faremi *et al*., (2018) had 36.2% don't know. These scholars worked in the North-central and South-central zones, respectively. This knowledge gap, misreported or misidentified Hb genotype, subsequently endangers the process of the targeted newborn screening and fight against SCD and /or HVD in Nigeria.

The chi-square test of independence in Table 4 was subjected to McNemar's test and the agreement between students' reported and actual genotypes for HbAA and HbAS was understood. For HbAA, 26 students incorrectly reported being HbAA, while 5 students failed to report their true HbAA status ($\chi^2 = 14.23$, $p < 0.001$). Similarly, for HbAS, 2 students over-reported, while 23 students under-reported their carrier status ($\chi^2 = 17.64$, $p < 0.001$). Table 5. These discrepancies were statistically significant, indicating poor genotype knowledge among students. These poor awareness levels corresponded with the findings of Ngwengi *et al*., (2020) and Hossain *et al*., (2020).

Lastly, the distribution of haemoglobin genotypes of the study subjects, as presented in Table 6 showed that out of the 685 participants in this study (neonates and students), 544(79.4%) of the subjects had HbAA, 127 (18.5%) had HbAS, 9 (1.3%) had HbAC, 3 (0.4%) had HbSS, and 2(0.4%) had HbSC. The result showed a ratio of Hb AA to Hb AS (4:1), HbAA to HbSS (181:1), HbAA to HbAC (60:1), and HbAA to HbSC (544:1), respectively. These were in close keeping with findings of Nubila *et al.,* (2013) and Dirisu *et al* ., (2024).

From the results of table 6 above and the findings, some recommendations can be made. It is obvious that HbAA prevalence nationwide and even globally is greater than 70%. With sustained awareness of the method of acquisition and prevention at all levels; primary, secondary and tertiary along with consistent and precision based diagnostic method (e.g HPLC) at every government health facility, control and possibly eradication is possible. Education at both rural and urban centers must be made mandatory, with a quality monitoring system in place. After adequate education and awareness have been ascertained nationally for about two years or more, then laws and policies can be put in place for choices of marriage partners and illegal childbirth of HVD children. Also, the consequences of underage mothers of HVD children must be considered while educating parents, youths, religious leaders, as well as traditional rulers. These education and awareness methods must be all inclusive; I.e., those with disabilities of learning, hearing and others, along with the apparently abled persons. As government and non-government assistance has been made available for free treatment and management of HIV disease, such programs can be put up for HVD monitoring and management. When all has been put in place, punitive measures can be mapped out with time for those who fail to align.

Conclusion

Given the fact that scientific research has made great strides in treating HVD, such as haemopoietic stem cell transplantation and gene therapy, they are currently impractical for the greater population of the sub-Saharan nations. These options are both costly and inaccessible to most Nigerians, bearing

in mind our country's low/ middle-income status. Thus, prevention and eradication still remain the most effective strategies for the reduction of the disease burden. This will require a comprehensive and consistent awareness campaign with all educational skills for all classes of the Nigerian citizens. Precision-based diagnostic methods for HVD with neonatal screening inclusiveness have been prioritized in our health care systems for early detection and management. These must be backed up with an improved culture of training and re-training of health care staff for proper result reporting and interpretation, along with maintenance and services of equipment.

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