International Journal of Current Research and Review
DOI: https://doi.org/10.31782/IJCRR.2023.15301

Original Article

IJCRR
Section: Healthcare

Validation of Self-Reported Parental Sickle Cell Status

Eneh Chizoma I[1], Nwankwo Chinwoke A[2], Agunyenwa Chioma U[3]

[1]Department of Paediatrics, Enugu State University of Science and Technology Enugu, Enugu State Nigeria and Enugu State University Teaching Hospital (ESUT-TH) Parklane, P.M.B. 1030, Enugu, Enugu State, Nigeria; [2,3]Enugu State University Teaching Hospital (ESUT-TH) Parklane, P.M.B. 1030, Enugu, Enugu State, Nigeria.

ABSTRACT

Introduction: Parents with heterozygous sickle cell status [haemoglobin AS (HbAS) or sickle cell trait (SCT)] may produce sickle cell anaemia (SCA) patients. Proper diagnosis and correct reporting of sickle cell status is therefore necessary to guide genetic counselling of prospective partners/parents. However, misreporting of SCD status is common in adult population leading to increase disease incidence. Self-reported sickle cell status may be validated by comparing the reports with more reliable and more sensitive diagnostic methods such as high performance lipid chromatography (HPLC) if not genetic studies.

Aims/Objectives: To determine the accuracy of self-reported parental genotype status by comparing self-reported and HPLC determined genotype.

Methodology: This was a hospital based, cross-sectional analytical study. Participants were consecutively enrolled at clinic visits, socio-demographic as well as self reported SCD status were obtained and documented in a pre-tested questionnaire, HPLC analysis of SCD status was conducted and documented. Data was anlyzed using Statistical Package for the Social Sciences (SPSS Version 21).

Result: The post marital reports were more sensitive, more accurate and had higher positive predictive value (all 100%) than the premarital reports. The premarital reports were more specific than sensitive and correctly predicted the truly positive (AS/AS) more than the truly negative (AA/AS, AA/AA) couples. The premarital reports were less accurate than the post marital reports.

Conclusions: Post marital self-reports are more accurate than premarital reports. Knowledge of personal sickle cell status seems marred by poor access to proper diagnostic tools. HPLC is useful for ambiguous reports.

Key Words: HbAS, SCT, SCA, Misreporting, Validation, Self-reports, HPLC

INTRODUCTION

Background: Sickle cell disease (SCD) is an autosomal recessive disorder caused by mutations in the β-globin gene of the haemoglobin molecule and defined by the presence of at least one hemoglobin S (Hb S) allele.[1] Homozygous SCD or inheritance of 2 Hb S genes (SCA)[1] is the most common and most severe form of SCD. SCD can also result from co-inheritance of Hb S with a second abnormal β-globin chain variant, such as a hemoglobin C(HbSC disease) or β-thalassemia allele (HbSβ-thalassemia). Individuals heterozygous for the mutation [one hemoglobin S (Hb S)allele and a normal haemoglobin (Hb A) allele] are said to have the sickle cell trait (SCT)[1] otherwise called 'Carriers' do not have SCD themselves but may have affected offsprings.SCD is associated with serious adverse effects and so constitute a significant public health problem[2] but the distinction between disease and trait is not always clear to the general population[1] even amongundergraduates.[3] Misreporting of SCD is common in an older adult population,[1,4] this has potential for increasing disease incidence as 'carriers' (SCT) do not have SCD themselves but may have affected children. Wrong parental self-reports would endanger the ability of a targeted newborn screening to identify infants with SCD[5] as well as genetic counselling of prospective parents. Therefore it is imperative to establish a proper diagnosis of SCD. HPLC is the most validated[6,7,8] methods for screening and detection of various hemoglobinopathies providing rapid, reproducible, and precise results.[6,7,8] DNA analysis or amino acid sequencing remains the gold standard for the definitive diagnosis of hemoglobinopathies.[2]

Literature review: SCD is a widely prevalent hemoglobi-

Corresponding Author:
Eneh Chizoma I, Senior Lecturer and Consultant Paediatrician, Department of Paediatrics, Enugu State University of Science and Technology and Enugu State University Teaching Hospital (ESUT-TH) Parklane, P.M.B. 1030, Enugu State, Nigeria; Email: zomafrank@yahoo.com

ISSN: 2231-2196 (Print) **ISSN:** 0975-5241 (Online)

Received: 03.01.2023 **Revised:** 18.01.2023 **Accepted:** 28.01.2023 **Published:** 14.02.2023

nopathy in sub-Saharan Africa with 30% of the world's annual SCD and SCT births located in two sub-Saharan African countries: Nigeria and the Democratic Republic of the Congo.[5] Frequently deadly in early life, killing 70-80% of affected children before their fifth birthday.[9] SCA is one of the top five non-communicable diseases in Nigeria,(accounting for more than 50% of deaths among carriers).[10,11] About 10 - 40% of Nigerian adults are healthy carriers of the defective S gene, leaving the country with a ~2% prevalence rate of SCA.[10,11] Despite that Nigeria has six zonal centers for sickle cell control, the disease remains endemic with an annual infant death rate of over 150,000 in its wake and 29/1000 under 5 mortality rate.[10,11] Fifty to ninety percent of the children born with SCA in low-income developing countries of sub-Saharan Africa and India will die before the age of 5.[12]

SCA is normally diagnosed by measuring the HbS content (%HbS) in blood of patients with HPLC,[13] hemoglobin electrophoresis (HE)[14] or isoelectric focusing (IEF).[15,16] HPLC however has the limitation of not detecting alpha thalassemia, normal A2 beta thalassemia or other hemoglobinopathies that elute with a similar retention values on HPLC.[17] Cellulose acetate electrophoresis at alkaline pH (pH 8·2–8·6) identifies A, F, S/G/D, C/E/O-Arab, haemoglobin F levels>2%[2] while citrate agarose gel at acid pH 6·0–6·2 distinguishes haemoglobinC from E,C-HarlemandO-Arab, S from D/G, but donot distinguish D and G types. Beta-thalassaemia trait is diagnosed by quantification of haemoglobin A2 using micro column chromatography which also distinguishes between SS (haemoglobin A2<4%) and Sb-thalassaemia(haemoglobinA2>4%)[2]. IEF distinguishes haemoglobins A, F, S, C, D-Punjab, G-Philadelphia/Lepore, E/A2/O-Arab which cause false positive increase in haemoglobin A2 but IEF has not been validated for haemoglobin A2 quantification.[2,15] HPLC provides precise quantification of haemoglobin A2 (suitable for the diagnosis of b-thalassaemia trait), quantification of haemoglobin F and variant haemoglobin[2]. Currently immunoassays (HemoCard) detect as low as 5 to 10 % haemoglobins S, C, E and A except D-PunjaborO-Arab.[2] The Kleihauer test is used to differentiate sickle cell disease, thalassaemias and hereditary persistence of fetal haemoglobin (HPFH) in early infancy.[2,16]

In Africa management of SCA is still widely limited to supportive, symptomatic, preventive measures.[18] However, the cornerstone of such programs; adequate, reliable, parental and newborn diagnostic screening has not been widely implemented in most African countries.[2,9] This dilemma springs from the cost and logistic problems with accepted diagnostic methodologies such as HPLC,[13] IEF[15] and DNA analysis which require laboratory equipment, highly-trained operating personnel, uninterrupted electrical supply as well as sample transport away from the point-of-care (POC).[12] Thus conventional laboratory methods used for diagnosing SCA remain prohibitively expensive and impractical in our setting.

It has been estimated that wide-spread screening and follow-up care could result in >95% survival for affected infants.[19] This could save the lives of nearly 10 million African children by 2050.[20] In Nigeria there are few attempts at population-wide screen for carriers of sickle cell gene for purposes of genetic counseling. It is difficult to inform adults of their risk of having a child with sickle cell disease if they do not know their or their partner's sickle cell status, or if they believe that it is different from the true status. Inadequate knowledge of personal sickle cell status and community awareness of how sickle cell disease is inherited constitutes a significant barrier to better treatment and care.[1]

Older adults regardless of sex and level of education often incorrectly report their sickle cell status.[1] Most adults reporting that they had sickle cell disease[1] or HbAA were actually persons with sickle cell trait, based on their laboratory results. One in four adults claiming to have sickle cell disease had neither sickle cell disease nor sickle cell trait.[1] Individuals may misreport their status because they do not remember as in a survey of Ghanaian women in which only 47% tested for SCT could remember their results[21] and 29% in a Tanzanian study.[22] Eneh[4] in an earlier study documented 82.1% discordance between premarital and post marital self reported sickle cell status among parents of children with SCA. The present study seeks to validate parental self-reported genotype by determining their accuracy when compared with HPLC derived sickle cell status of these parents.

MATERIALS AND METHODS

Aim: To determine the accuracy of self-reported parental genotype status

Objectives:

(1) Compare premarital self-reported and HPLC determined genotype
(2) Compare post-marital self-reported and HPLC determined genotype.
(3) Evaluate the reliability of HPLC determined genotype.

Methods: This was a cross-sectional analytic study conducted at the Paediatric SCD clinic of Enugu State University Teaching Hospital, Enugu South-East, Nigeria. The study sample was drawn from a population of parents of 74 SCA children (aged 1 to 18 years) registered in the clinic. The sample being those parents who were paired and whose children had been regular (not missed 3 appointments in a year) with clinic visits in the one year proceeding the study. Sample size was determined by the convenience sampling technique at each clinic visit. The sample size was 40 paired parents who both had either premarital(3), post marital(11), discordant premarital and post marital (17), concordant

premarital and post marital (3) self reported genotype as well as those who could not remember (4) or in whom only one of the pairs had either a premarital or a post marital (2) self reported genotype. Only the 34 parent-pairs who both had self reports (pre maritally, post maritally or both) were eligible for validation by HPLC. Six of these 34 parent-pair had either the father or mother absent at the time of the investigation while 9 pairs of parents declined this step on the basis that they didn't see the need or the difference the result would make nor could afford even the much subsidized cost of HPLC analysis. So 19 pairs of parents none of whom had received blood transfusion in the previous three months had the HPLC estimation of their genotype. Ethical protocols involving human blood samples were as approved by the institutional review board (IEC number ESUTHP/C-MAC/RA/034/VOL.3/195. Written informed consent was obtained from each parent.

For validation, the accuracy of the self reported screen results was assessed by comparison with HPLC determined Hb variants which depend on separation and quantification.. The D-10 Dual Program is based on chromatographic separation of the analytes by ion-exchange HPLC. Whole blood were collected into vacutainer vials (K_2EDTA, BD Diagnostics, USA) using standard venipuncture technique. Blood specimens were stored up to 4 days at 2–8 °C or 1 day at room temperature (15–30 °C/?20–25°Cand used within 4–5 hours. The samples were automatically diluted (with wash diluent) on the D-10 (BIO-RAD D-10) and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength (Elution Buffer 1 and 2) to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. The equipment runs 10 samples in one hour; each sample takes about 6 minutes. The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration (HbA2/F/A1c Calibrator) is used for quantitation of the obtained electrophoresis values. A sample report is generated for each sample. Quality Control values (Lyphochek HbA2 Controls levels 1 and 2) are used to compare results.

Data analysis: Statistical Package for the Social Sciences (SPSS Version 21) was used for analysis of the descriptive statistics and frequencies. For evaluation of test performance we calculated the following: sensitivity=TP/(TP+FN); specificity=TN/(FP+TN); positive predictive value=TP/(TP+FP); negative predictive value=TN/(TN+FN); and accuracy=(TP+TN)/(TP+FP+TN+FN); where TP=true positive, FP=false positive, TN=true negative and FN=false negative.

RESULTS

Table 1 presents the demographic characteristics of the study participants. The patients' age range was 1-17 years, the mean and standard deviation, 7.70±4.30 and the modal age group, 1-5 years (40.0%). 52.5% of the patients were males and 47.5%, females. Their mothers' age range was 22-58 years, the mean and standard deviation, 35.92±8.01 and the modal age group, 31-40 years (45.0%). Their fathers' age ranged from 28-65 years with mean and standard deviation, 43.62±8.67 and modal age group, 41 years and above (60.0%). Majority of the parents were in SEC 2 (42.5%) and 3 (32.5%) respectively.

From Table 2, Out of the 40 parent pairs, 24 (60.0%) had pre-marital genotype, 31 (77.5%) had post-marital genotype and 19 (47.5%) HPLC genotype. Of the 19 parent pairs with HPLC genotype, 13 (68.4%) had pre-marital genotype with the HPLC and 18 (94.7%), post-marital genotype with the HPLC. Total females with HPLC genotype was 19 of which 14 (73.7%) had pre-marital genotype and 18 (94.7%), post-marital genotype. Total males with HPLC were 19 of which 13 (68.4%) had pre-marital genotype, and 18 (94.7%), post-marital genotype.

Table3: computed based on the number with paired result (that is, for pre marital self reports and HPLC, 13 (instead of 19) was used since 6 parents with HPLC didn't have pre-marital result; for post-marital and HPLC, 18 (instead of 19) was used since 1 didn't have post-marital result). Comparing premarital self-reported and HPLC determined SCD status, 76.9% discordance for self-reported ['AA/AS' and 'AA/AA'] and 23.1% concordance was observed with a 100% concordance and 0.0% discordance for post marital self-reported ['AS/AS'].

Table 4: Sensitivity is the probability that the pre-marital genotype is positive when it is actually positive based on HPLC. E.g. There were actually 12 positives (by HPLC), and pre-marital genotype only found 2. Probability of pre-marital genotype correctly detecting positive (AS/AS)

Sensitivity: 16.7%;(95% confidence interval, C.I: 2.09% to 48.41%)

Specificity is the probability that the pre-marital genotype is negative when it is actually negative based on HPLC. E.g. There were actually 1 negative (by HPLC), and pre-marital genotype found it. Probability of pre-marital genotype correctly detecting negative (AA/AS or AA/AA)

Specificity: 100.0%;(95% confidence interval, C.I: 2.50% to 100%).

Positive predictive value is the probability that it is actually positive when pre-marital genotype is positive. E.g. Premarital genotype found 2 positives, and the 2 were actually positives (by HPLC). That is, the pre-marital genotype is positive and it was truly positive

PPV: 100%.

Negative predictive value is the probability that it is actually negative when pre-marital genotype is negative. E.g. Pre-marital genotype found 11 negatives, and only 1 was actually negative (by HPLC). That is, the premarital genotype is negative and it was truly negative.

NPV: 9.1%;(95% confidence interval, C.I: 7.20% to 11.41%)

Accuracy is the overall probability that a genotype is correctly classified. E.g. Out of the 13 genotype results, 3 were correctly classified (2 for positive and 1 for negative). That is, probability that both pre-marital genotype and HPLC has the same result.

Accuracy: 23.1%;(95% confidence interval, C.I: 5.04% to 53.81%).

Table 5: The 18 positive post-marital genotype reports were also positives (by HPLC). Probability of post-marital genotype correctly detecting positive (AS/AS)

Sensitivity: 100%; 95% C.I: 81.5% to 100%

Specificity: can't compute because there was no negative

PPV: 100%

NPV: can't compute because there was no negative

Accuracy: 100.0%.

From Table 6: Among the 19 parent pairs who had HPLC analysis, the prevalence of HbF in females was 26.3%; HbC in females, 0%. The prevalence in males was 15.8% for HbF and 5.3% for HbC.

From Table 7: majority of the parents were aware of the mode of inheritance; females (70.0%) and males (67.5%).

DISCUSSIONS

The present study having compared self-reported and HPLC determined parental genotype status, observed 76.9% discordance and 23.1% concordance for premarital 'being AA/AS or AA/AA' and a 0% discordance and 100 % concordance for post-marital 'being AS/AS'. In contrast Bean and colleagues[1] on comparing self-reported and genetically determined SCD reported 94.1% discordance for self-reported 'SCD' and a 100% concordance for self-reported 'no SCD'. These investigations imply that self reported SCD status are rather unreliable. The genetic evaluation, a definitive diagnosis of haemoglobinopathies is not readily available in our setting thus our use of HPLC. In an earlier study, Eneh C.I.[4] documented high discordance () between self-reported parental premarital and post marital status. This seems to lend credence to the greater unreliability of earlier (premarital) self reports than latter (postmarital) reports. A fact re-enforced by the observed higher accuracy of post marital reports than premarital reports in this study. One wonders if the high concordance between post marital AS/AS HPLC and self reports is a pointer to better recollection of the more recent post marital reports or a more concerted and knowledge driven attempt to test at more reliable sources such as tertiary health centers. The above investigations[1,4] like the present study show that efforts have been made towards validating 'self-reported' SCD status.

The present study has shown that premarital reports are more specific for 'not being AS/AS' but predict 'being AS/AS' more than 'not being AS/AS' than . Conversely the post marital self-report are more sensitive and while predicting the truly positive ('being AS/AS') are more accurate than the premarital reports. Thus in ambigous cases and in the absence of HPLC, one may rely on a post-marital self report for diagnosis. In a previous study to determine the accuracy of expectant parents' self-reported sickle cell status and its reliability for identifying high-risk newborns for targeted screening, Burnham-Marusich and colleagues[5] observed that self-report accuracy obtained by comparison with the more presumptive cellulose acetate paper results was significantly higher for those who reported not having SCT or SCD (86%) than individuals who reported having SCT or SCD (61%), with an overall self-report accuracy of 50%. The higher (100%) accuracy of our post marital self reports may be attributed to a much smaller sample size. The much lower accuracy of the premarital reports may likely be to poor recollection of remote events by these parents. The 7.5% frequency of premarital HbAS/AS (figures 1-3), although higher than (2.8%) found in Nnaji's study and 2.3% found in Turkey by Altay et al.,[23] is low compared to 52.5% (AA/AS, AA/AA) found in the present study. These findings coupled with the high percentage discordance (76.9%) between premarital self reported (AA/AS and AA/AA) and HPLC determined (AS/AS) genotype are indications that poor diagnostic techniques mar personal knowledge of sickle cell status. These low frequencies of premarital HbAS/AS[3,4,23] coupled with the finding of a high rate of respondents who would call-off the marriage if both couples were carriers of SCD (HbAS) has been cited as an indication that awareness of the transmission of SCD among premarital couples was high.[3,4,23] The above observations imply that awareness of transmission mode seems confounded by poor knowledge of one's personal status.

This study found only Hb C and HbF as other variants of haemoglobinopathies. This finding agrees with views expressed in many literature that other variants of haemoglobinopathies were very rare or absent.[3] This is in contrast to studies from the Mediterranean region.[24,25] Nigeria as a resource limited country uses mainly sickling/solubility test and alkaline electrophoresis with their high degree of inaccuracy[26] and inability to separate co-migratory variant such

as Hb S, D, G and Hb A, C, E and O-Arab.[26] This drastically limits our knowledge of the Hb variants present in our population. Recent studies however have shown HPLC as the method of choice for quantifying haemoglobin variants and because of its reliability and reproducibility of results has replaced alkaline electrophoresis as primary screening forhaemoglobinopathies[8,26,27,28] in some parts of the country. The result of this study lend evidence in support of the use of HPLC as a screening tool for haemoglobinopathies at least at point of care. However, the proprotion of parents who declined based on inability to pay for the analysis is clinically significant as it implies that most of the self reports were not of HPLC determined genotype.

CONCLUSIONS

(1) Self-report accuracy obtained by comparison with HPLC results is significantly higher for post marital (AS/AS i.e SCT) reports than premarital (AA/AS or AA/AA i.e not SCT) among parents of children with SCA.
(2) Knowledge of personal sickle cell status seems marred by poor access to proper diagnostic tools.
(3) HPLC where available should be used for screening or at least be used for confirmation of premarital ambiguous cases

Recommendations: Additional studies are needed to establish the efficacy of a reliable, low-cost, rapid and simple 'point of care' screening assay[29,30] practical in resource-limited clinical settings as a mandatory premarital screening tool since the use of HPLC in population-wide screening in resource limited countries may be impractical.

Authors' contribution: C.I E conceived and designed the study, wrote the clinical protocols and the Manuscript, obtained approvals and supervised sample collection. C.N. and C.A enrolled patients and collected samples, All 3 authors worked together to document the data for analysis, as well as proof-read and approve the final version of the manuscript for publication.

Funding: This work was partly supported by the authors and Synlab laboratories who gave a discounted price for the HPLC analysis.

Competing interest: The authors declare no competing interests.

Availability of data: Not applicable

Acknowledgements: We acknowledge E.B of Synlab laboratory who performed the experiment and N.U who analyzed the data. Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

1. Bean C.J, Hooper W.C, Ellingsen D, DeBaun M.R, Sonderman J, Blot W.J. et al. Discordance between self-report and genetic confirmation of sickle cell disease status in African-American adults. Public Health Genomics. 2014;17:169-172.
2. Guideline- The laboratory diagnosis of haemoglobinopathies. Br J Haematol.1998;101(4):783-92. doi: 10.1046/j.1365-2141.1998.00809.x. PMID: 9674756.
3. Nnaji G.A, Ezeagwuna D.A, Nnaji I, Osakwe J.O, Nwigwe A.C, Onwurah O.W et al. Prevalence and pattern of sickle cell disease in premarital couples in Southeastern Nigeria. Niger J Clin Pract. 2013;16:309-14.
4. Eneh Chizoma I. Discordance between self-reported premarital and post-marital parental sickle cell status. Indian J Child Health. 2020; 7(8):324-327
5. Burnham-Marusich A.R, Ezeanolue C.O, Obiefune M.C, Yang W, Osuji A, Ogidi A.G et al. Prevalence of Sickle Cell Trait and Reliability of Self-Reported Status among Expectant Parents in Nigeria: Implications for Targeted Newborn Screening. Public Health Genomics. 2016;19:298-306.
6. Moorchung N, Phillip J, Sarkar R.S, Prasad R, Dutta V. Is high pressure liquid chromatography an effective screening tool for characterization of molecular defects in hemoglobinopathies?. Indian J Pathol Microbiol.2013;56:36-9.
7. Bender M.A, Hobbs W. Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, Sickle Cell Disease. In: editors. Gene reviews. Seattle (WA)1993.
8. Bravo-Urquuiola M, Arends A, Montilla S, Velasquez D, Garcia G, Alvarez M, et al. Advantages in the use of high performance chromatography technique for screening hemoglobinopathies in Venezuela. Invest Clin.2004;45: 309-15.
9. Nankanja R, Kiyaga C, Geisberg M, Serrao E, Balyegyusa S. Implementation of a sickle cell disease screening initiative in Uganda with Hemo Type SC™ [abstract]. Blood. 2018;132(suppl 1). Abstract LBA-3.
10. Nnodu O.E., Sopekan A., Nnebe-Agumadu U, Ohaeri .C., Adeniran A., Shedul G. et al. Implementing newborn screening for sickle cell disease as part of immunisation programmes in Nigeria: a feasibility study. Lancet Haematol. 2020; 7: e534-e540
11. Nigeria Demographic and Health Survey 2018. National Population Commission, Abuja 2019.
12. Makani J, Cox S.E, Soka D, Komba A.N, Oruo J, Mwamtemi H, et al. Mortality in sickle cell anemia in Africa: a prospective cohort study in Tanzania. PloS one. 2011;6(2):e14699. Epub 2011/03/02. pmid:21358818; PubMed Central PMCID: PMC3040170.
13. Head C.E, Conroy M, Jarvis M, Phelan L, Bain B.J. Some observations on the measurement of haemoglobin A2 and S percentages by high performance liquid chromatography in the presence and absence of alpha thalassaemia. J Clin Pathol. 2004;57(3):276–80. Epub 2004/03/03. pmid:14990599; PubMed Central PMCID: PMC1770250.
14. Clarke G.M, Higgins T.N. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000;46(8 Pt 2):1284–90. Epub 2000/08/06. pmid:10926923.
15. Jenkins M.A, Ratnaike S. Capillary isoelectric focusing of haemoglobin variants in the clinical laboratory. Clin Chim Acta. 1999;289(1–2):121–32.

16. Piety N., George A, Serrano S. A Paper-Based Test for Screening Newborns for Sickle Cell Disease. Sci Rep. 7, 45488 (2017). https://doi.org/10.1038/srep4548814.
17. Sachdeva R, Dam A.R, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol. 2010;53:57-62.
18. Fowora M.A. Adherence to Self-Care Management of Sickle Cell Disease Among Caregivers submitted in partial fullfillment of the doctoral dissertation of Walden University 2016.
19. McGann P. T. A prospective newborn screening and treatment program for sickle cell anemia in Luanda, Angola. Am. J. Hematol 88, 984–989, doi: 10.1002/ajh.23578 (2013).
20. Piel F.B, Hay S.I, Gupta S, Weatherall D. J., Williams T. N. Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions. PLoS medicine 10, e1001484, doi: 10.1371/journal.pmed.1001484 (2013).
21. Hayeems R.Z, Bytautas J.P, Miller F.A: A systematic review of the effects of disclosing carrier results generated through newborn screening. J Genet Couns. 2008;17:538-549.
22. Odunvbun M.E, Okolo A.A, Rahimy C.M: Newborn screening for sickle cell disease in a Nigerian hospital. Public Health.2008;122:1111-6.
23. Altay C, Yilgor E, Beksac S, Gurgey A. Premarital screening of haemoglobinopathies: A pilot study in Turkey. Hum Hered. 1996;46:112-4.
24. Ohls R.K, Christensen R.D. Development of Haematopoeitic system. In: Behram RE, Kliegman RM, Jenson HB, editors. Nelson Textbook of Paediatrics. 17th ed. Philadelphia: Saunders; 2004. p. 1601-4.
25. Taiwo I.A, Oloyede O.A, Dosumu A.O. Frequency of sickle cell genotype among the Yorubas in Lagos: Implications for the level of awareness and genetic counseling for sickle cell disease in Nigeria. J Community Genet. 2011;2:13-8
26. Adeyemo T., Ojewunmi O., Oyetunji A. Evaluation of high performance liquid chromatography (HPLC) pattern and prevalence of beta-thalassaemia trait among sickle cell disease patients in Lagos, Nigeria. Pan Afr Med J. 2014; 18: 71.=HPLC LAGOS
27. Joutovsky A, Hadzi-Nesic J, Nardi M;A. Retention time as a diagnostic tool for haemoglobin variants and haemoglobinopathies: A study of 60,000 samples in a clinical diagnostic laboratory. Clinical Chemistry. 2004;50(10):1736–47.
28. Mohammed-Nafi'u R.1, Audu L.I, Ibrahim. M, Wakama T.T, Okon E.J. Pattern of haemoglobin phenotypes in newborn infants at the national hospital abuja using high performance liquid chromatography. Niger Postgrad Med J.2020;27(3):190-195.
29. Kanter J, Telen M.J, Hoppe C, Roberts C.L, Kim J.S, Yang X. Validation of a novel point of care testing device for sickle cell disease. BMC Med. 2015;13: 225.
30. Piety N.Z., Yang X, Kanter J., Vignes S.M., Alex George A., ShevkoplyasS.S et al.(2016)Validation of a Low-Cost Paper-Based Screening Test for Sickle Cell Anemia. PLoS ONE 11(1): e0144901.https://doi.org/10.1371/journal.pone.0144901.6.

Table I: Demographic Characteristics of the Study Participants **n = 40**

	Frequency	Percent	Range	M±SD
Age			1-17	7.70±4.30
- 1- 5	16	40.0		
- 6-10	13	32.5		
- 11 +	11	27.5		
Sex				
- Male	21	52.5		
- Female	19	47.5		
Female (Mothers) age			22-58	35.92±8.01
- ≤ 30	10	25.0		
- 31-40	18	45.0		
- 41 +	11	27.5		
- *Not indicated*	*1*	2.5		
Male (Fathers) age			28-65	43.62±8.67
- ≤ 30	3	7.5		
- 31-40	10	25.0		
- 41 +	24	60.0		
- *Not indicated*	3	7.5		
SEC				
- 2	17	42.5		
- 3	13	32.5		
- 4	8	20.0		
- 5	2	5.0		

Table II: Summary of Genotype results **n = 40**

All	Father & Mother	Father or Mother
Pre-marital genotype	24(60.0)	16(40.0)
Post-marital genotype	31(77.5)	9(22.5)
HPLC genotype	19(47.5)	21(52.5)
HPLC genotype (n = 19)	**Had both**	**Only HPLC**
Pre-marital & HPLC	13(68.4)	6(31.6)
Post-marital & HPLC	18(94.7)	1(5.3)
Female (Mothers) with HPLC (n = 19)		
Pre-marital & HPLC	14(73.7)	5(26.3)
Post-marital & HPLC	18(94.7)	1(5.3)
Males (Fathers) with HPLC (n = 19)		
Pre-marital & HPLC	13(68.4)	6(31.6)
Post-marital & HPLC	18(94.7)	1(5.3)

Table III: Concordance and Discordance between Pre- and Post-Marital Genotype and HPLC Genotype

	Frequency	Percent
Pre-marital & HPLC *(n = 13)*		
Concordance	3	23.1
Discordance	10	76.9
Post-marital & HPLC *(n = 18)*		
Concordance	18	100.0
Discordance	0	0.0

Table IV: Sensitivity and Specificity for Pre-marital self reported parental genotypes

	HPLC Genotype		
Pre-marital genotype	Positive (AS/AS)	Negative (AA/AS or AA/AA)	Total
Positive (AS/AS)	2	0	2
Negative (AA/AS or AA/AA)	10	1	11
Total	12	1	13

Table V: Sensitivity and Specificity for Post-marital and HPLC

	HPLC Genotype		
Post-marital genotype	**Positive (AS/AS)**	**Negative (AA/AS or AA/AA)**	**Total**
Positive (AS/AS)	18	0	18
Negative (AA/AS or AA/AA)	0	0	0
Total	18	0	18

Table VI: Prevalence of HBC and HbF Haemoglobin Variants **n = 19**

	Frequency	Percent
Females		
- HbF	5	26.3
- HbC	0	0.0
Males		
- HbF	3	15.8
- HbC	1	5.3

Table VII: Awareness of mode of inheritance of SCD **n = 40**

	Frequency	Percent
Female (aware of mode of inheritance)		
Yes	28	70.0
No	11	27.5
No indicated	1	2.5
Male (Aware of mode of inheritance)		
Yes	27	67.5
No	11	27.5
No indicated	2	5.0

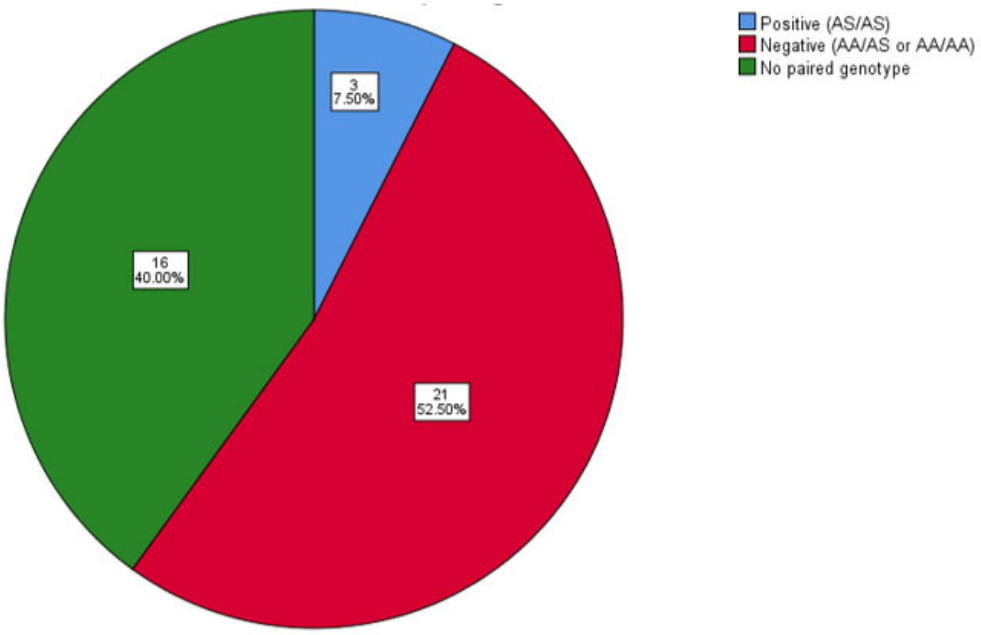

Figure I: Pre-marital paired genotype.

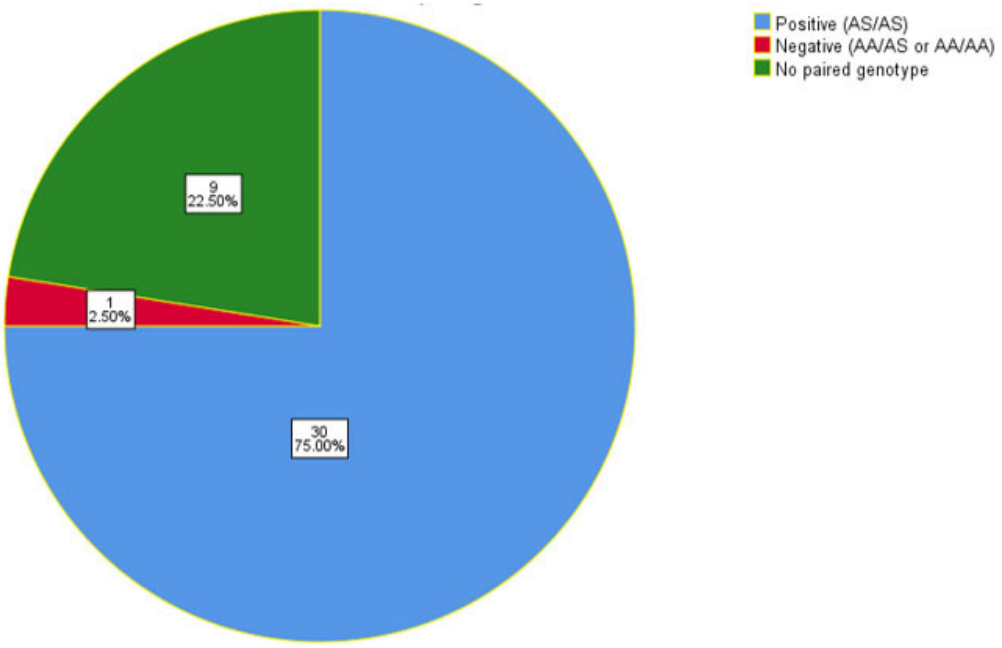

Figure II: Post-marital paired genotype.

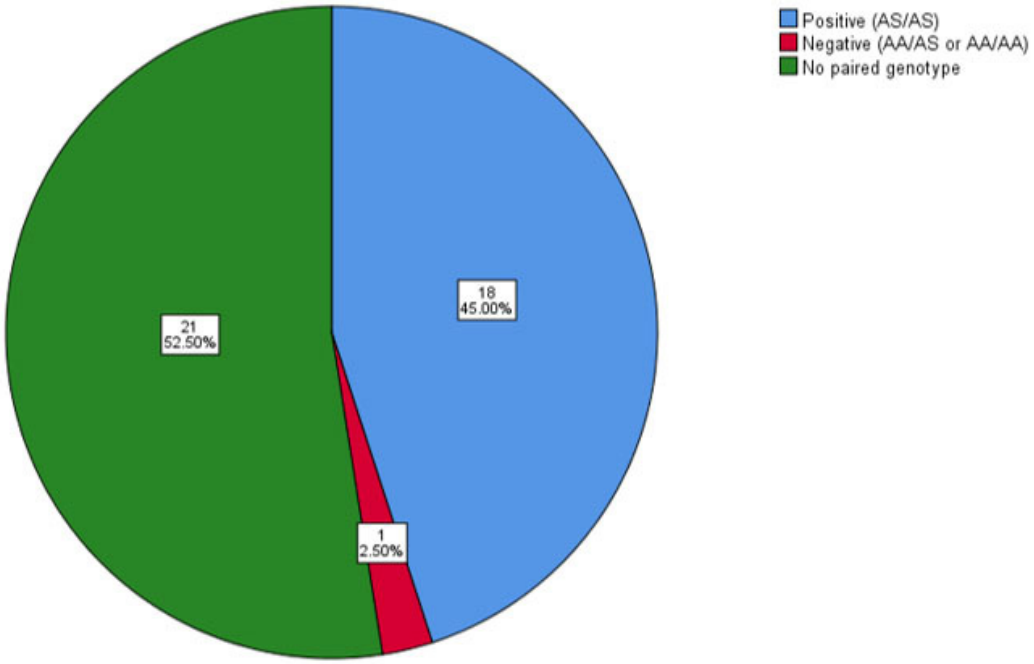

Figure III: HPLC paired genotype.