*ORIGINAL ARTICLE*

# Coinheritance of B-Thalassemia and Sickle Cell Anaemia in Southwestern Nigeria

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***ABSTRACT***

***BACKGROUND: Genes for haemoglobin S are found in high frequencies in Nigeria. However, there is little information on beta thalassemia in sickle cell anaemia in this population. The clinical presentation of HbS- β thalassemia is enormously variable, ranging from an asymptomatic state to a severe disorder similar to homozygous sickle cell disease.***

***MATERIALS AND METHODS: Haemoglobin $A_2$ and HbF were determined in sickle cell anaemia patients attending LAUTECH Teaching Hospital, Osogbo, by elution after electrophoresis and alkaline denaturation methods respectively. Haematological parameters were estimated using Sysmex KX-21N and percentage target cells using Leishman's staining technique.***

***RESULTS: Exactly 6% f the SCA patients were found to have elevated $HbA_2$ (>3.3%) and HbF (>1.3%). These patients also had normal erythrocyte indices, increased platelet count, a significantly higher HCT and an increased % target cell.***

***CONCLUSION: These findings confirm that the frequency of beta thalassaemia in sickle cell patients in Nigeria is higher than previously thought. It is therefore important to consider the possibility of this variant in patients with sickle cell anaemia since their course may differ from that of patients with homozygous sickle cell anaemia.***

***KEYWORDS: β-Thalassemia,Sickle cell anaemia, Haemoglobin $A_2$ , Haemoglobin F***

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## INTRODUCTION

Beta-thalassemia (ß- thalassemia) is one of the most common genetic diseases which involve a diverse group of disorders of hemoglobin synthesis(1).This disorder occurs as a result of reduced output of the ß-chains of adult hemoglobin and this results in reduced Hb in red blood cells (RBC), decreased RBC production and anemia(1,2).

Beta -thalassemia which could be classified as beta thalassemia major ($ß^o$), beta thalassemia minor ($ß^+$) and beta thalassemia intermedia (2) is mostly caused by point mutations or small deletions within the ß-globin gene or its immediate flanking sequences (1). However, in each affected ethnic group, a few common mutations together with a variable number of rare mutations account for most of the cases (3).

Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%) and Sardinia (10.3%) (4). However, its frequency in Africa is variable (5,6). Little is known about the occurrence and manifestations of thalassaemia trait in Nigeria because it is a rare condition, and its diagnosis is considerably more difficult especially in heterozygote subjects. It has also been shown from previous studies that the incidence of beta thalassaemia gene in Africa

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has been variable (5,6). Sickle cell anemia (SCA) is a monogenic disease that characterizes the homozygous state of hemoglobin S (Hb S) (7). The complex pathophysiology includes anaemia, vaso occlusive crises, aplastic crises, sequestration crises and hemolytic crises all of which could be of acute or chronic course and may eventually lead to death (8).

SCA is common in persons of African, Mediterranean, Middle Eastern and Indian ancestry, and in persons from the Caribbean and parts of Central and South America. Hpwever, they can be found in individuals of any ethnic background. In many regions of Africa, the prevalence of the Hb S gene mutation (Glu6Val) (i.e. HbS trait) is as high as 25% - 35%, with an estimated 15 million Africans affected by sickle cell disease (5,9).

Sickle beta-thalassaemia (S/beta-thalassaemia) is a condition, which results from coinheritance of a sickle cell gene and a beta-thalassaemia gene. The clinical phenotype depends on the type of beta-thalassaemia gene (beta (+) or beta (o)) inherited. A definitive diagnosis is required in order to initiate early supportive treatment in patients with homozygous sickle cell disease (SS disease) and to define the later clinical course (10).

The S/beta-thalassaemia can be diagnosed by family history, complete blood count and measurement of Hb A2 and Hb F levels (11). The clinical features of HbS- β thalassemia are tremendously variable, ranging from a completely asymptomatic state to a severe disorder similar to homozygous sickle cell disease depending on the inheritance of each gene (12). This heterogeneity is likely to be due to the presence of different β-thalassemia alleles or interaction with modulating genetic factors like associated α thalassemia and/or a gene for raised HbF production. Haemoglobin beta chain sequence analysis may be used to detect mutations associated with β-thalassemias/hemoglobin variants. Gel electrophoresis or HPLC can differentiate these disorders from heterozygous carriers of the Hb S mutation (Hb AS, Hb SC and others) (13).

There is little information on the coinheritance of beta-thalassemia in sickle cell anaemia patients in Nigeria. This study will therefore provide more information on the existence of coinheritance of beta-thalassemia in sickle cell anaemia in Southwest Nigeria by estimating the haemoglobin variants concentration and red cell indices which are diagnostic criteria for determining the presence of β-thalassemia genes using available methods.

## MATERIALS AND METHODS

This is a cross sectional study in which a total of 100 sickle cell patients were recruited for the study.These patients comprised of 46 males and 54 females attending haematology clinic of the Ladoke Akintola University Teaching Hospital (LTH), Osogbo, Osun State were recruited for period of six (6) months. Subjects in crises, pregnant women and those on the use of herbal medications were excluded from the study in order to prevent variations in HbF or $HbA_2$ concentration which may be induced by physiological stress of pregnancy (14) or by the emerging unevaluated herbal constituent intake in this region (15). The sample size of 100 subjects recruited in this study was obtained using Thumb's rule statistical formula (18).

About 2.5ml of venous blood was collected in an ethylenediamine tetra acetic acid (EDTA) anticoagulated bottle (Becton Dickinson). Complete Blood Count which included the haematocrit (HCT), the red cell distribution width (RDW), the red cell indices (Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC)) and the absolute platelet count was determined using Sysmex KX-2 IN Autoanalyser (16).

Haemoglobin F level was determined by Alkaline Denaturation Method.The method involve preparation of haemoglobincyanide solution which was treated with NaOH and saturated ammonium sulphate solution.The solution was then filtered and measured spectrophotometrically to determine the alkali resistant haemoglobin (Hb F) (17).In addition, the Haemoglobin $A_2$ level was estimated using Elution from Cellulose acetate Method.This is a process in which $HbA_2$ was separated from HbA on cellulose acetate electrophoresis at pH8.9, eluted into buffer and the percentage calculated by

measuring the absorbance of the $HbA_2$ eluate (6,18 ).

The coexistence of elevated $HbA_2$ (>3.5%) and HbF (>1%) was considered indicative of β-thalassemia trait (19).The Blood Film Appearance by Leishman's Stain was used to determine the percentage target cells.

All analysis were performed with commercially available software (EPI Info version 3.5.3.). Significance of differences and comparison of means between groups was determined using the independent t-test while Chi square was used to compare proportions. Probability values of ($P< 0.05$) were interpreted as significant.

## RESULTS

The 100 subjects comprised of 54 men and 46 women. Their haematocrit ranged from 13.0 – 36.9% with a mean haematocrit of 0.25 ± 0.009L/L. The mean $HbA_2$ among the subjects was 3.0 ± 0.8% with a range of 2.0–4.1%. Thirteen (13%) of the subjects had $HbA_2$ values higher than 3.3% while 2 (23%) had $HbA_2$ values greater than 3%.

The mean HbF was 1.0 ± 0.6% with a range 0.9–2.8%.Ten (10%) and 19% of the patients had HbF values greater than 1.3% and 1% respectively. The mean platelet count and percentage target cells were 329.19 ± 9.37 $x10^6$/L and 14.31± 2.08% respectively.

Six percent (6/100) of the subjects had both elevated $HbA_2$ (>3.3) and HbF (>1.3) displaying a statistical significance at $p<0.005$ as represented in Table 1. These six subjects also had an increased mean % Target cells (27 vs.13.4 %, $p=2.05x10^{-5}$), Platelet count (468.1 vs. 322.1 $x10^6$/L, p=0.04), HCT (28.8 vs. 25.1%, p=0.05) and a weak band of HbA on electrophoresis compared with other SCA subjects. This is indicated in Table 2.

Table 1: Cross tabulation of HbF versus $HbA_2$

| | | HbF (%) | | Total | P-value |
|---|---|---|---|---|---|
| | | **<1.3** | **>1.3** | | |
| | **<3.3** | 83(92.2) | 4(40) | 87 | |
| **$HbA_2$ (%)** | | | | | 0.0002 |
| | **>3.3** | 7(7.8) | 6(60) | 13 | |
| **Total** | | 90 | 10 | 100 | |

Table 2: Differences between subjects with Sickle-Beta Thalassemia and Homozygous Sickle cell anaemia

| | HCT(L/L) | MCV(fl) | MCH(pg) | MCHC(g/dl) | PLT($10^6$/L) | %TC* | RDW |
|---|---|---|---|---|---|---|---|
| **HbF<1.3; $HbA_2$<3.3(%) (n=94)** | 0.25 | 77.21 | 24.20 | 31.24 | 322.10 | 13.40 | 54.10 |
| **HbF>1.3; $HbA_2$ >3.3(%) (n=6)** | 0.28 | 79.75 | 25.43 | 31.90 | 468.10 | 27.00 | 54.70 |

***%TC- Percentage Target Cells**

## DISCUSSION

The clinical and hematologic features in HbS/β thalassemia inheritance are quite variable: some are severe while some are less significant. The clinical severity largely depends upon the nature of the β thalassemia mutations inherited. HbS/β thalassemias are classified as HbS/$\beta^0$ thalassemia, having absence of HbA with a severe clinical course similar to homozygous Hb S disease and HbS/$\beta^+$ thalassemia usually associated with 20-30% of HbA with a milder clinical course (12).

However, studies showed that HbS-$\beta^+$ thalassemia cases with the IVS 1-5 (G→C) or IVS II-745 mutation had low HbA levels (3–5%) which orchestrated the conclusion that this level of

HbA does not influence the clinical expression of the disease compared to sickle cell disease in the community (20). This can be observed in this present study with the display of a weak band of HbA on electrophoresis.

In addition, a study by Murkerjee *et al* indicated that the HbS/$\beta^+$ thalassemia patients had quite variable clinical presentation.This could be partially explained by the association of α thalassemia (9/11) and XmnI polymorphism in HbF production, either in homozygous or heterozygous state(21). This findings was corroborated by an earlier study which reported that sickle homozygous individuals from Maharashtra and Gujarat regions in Western India have shown that α thalassemia is the major modulator of the severity of the disease.This was because it was observed to be more prevalent among tribals with a milder disease than among nontribal with more severe manifestations (22).

This study which showed that beta-thalassaemia trait in Nigeria is higher than previous thoughts was based on elevated $HbA_2$ and HbF levels as well as increased % target cells. Out of the 100 subjects in this study, six participants (comprising of four males and two females) currently managed as sickle cell anaemia were discovered to have increased HbF (>1.32%) and $HbA_2$ (>3.32%) and the presence of a less significant concentration of HbA electrophoretic band.

This was much higher than figures reported in a previous study (1.2%) which investigated the presence of β-thalassemia in sickle cell anaemia with increased level of HbF (>1.5%) and $HbA_2$ (>3.5%) in Benin, Nigeria (6). A similar study by Kotila et al in Ibadan, Nigeria, showed much higher figures of the population (25%) with increased HbF and $HbA_2$ levels in healthy individuals (23).

It was observed in this study that about a third of subjects with increased HbF (30%) were associated with increase in % target cell (>24%).This corresponds with reports of several studies that target cells along with other poikilocytes are present in sickle cell anaemia and thalassemia although the precise percentage was not stated (24). Also, Omoti C.E in a similar study reported that peripheral blood film revealed the presence of target cells and occasional microcytes apart from the sickled cells (6).

Elevated HbF compared with the red cell indices in this study indicated that the HbF does not have a significant effect on the red cell indices ($P<0.05$). This was observed by Beutler in 1990 that individual values for MCV, MCH and MCHC occasionally overlapped with those in the normal population thus casting doubt on the adequacy of these criteria alone in identifying all cases of the heterozygous β-thalassaemia in SCA patients (25).

Also, red cell indices in iron deficiency and alpha thalassaemia trait can give a similar picture as found in beta thalassaemia trait. It may therefore not be as informative in an environment in which the three pathologies exist concurrently (25).

The mean difference in HbF and $HbA_2$ in this study shows that populations with higher HbF had a higher mean $HbA_2$ ($p=4.64\ X10^{-6}$).This is concurrent with the conclusion of Omoti C.E. that the possibility of coinheritance of the S/beta thalassaemia gene occurs in about 1.2% of Nigerians with SCA (6).

Populations with diagnostic features of S/β-thalassemia where compared with those subjects with sickle cell anaemia (Table 2), and statistically significant differences were observed in the mean % Target cells , Platelet count and HCT with the mean value in S/β- thalassemia subjects higher than the homozygous sickle cell subjects. In contrast, a related study by Bashir *et al,* in North Jordan observed that total haemoglobin value was not significantly different (P greater than 0.05) in the two types of S/β-thalassemia (S/β° and S/$\beta^+$) (26). However, platelet count was discovered to be usually normal in β-thalassemia, except in cases of splenomegaly (27). In addition, this significant increase in platelet count and HCT may be due to the interaction of the sickle cell gene with mild forms of ß-thalassaemia (S/ ß+thal) which may be quite innocuous compared with patients with haemoglobin S.

Expectedly, the % target cells in S/β-thalassemia doubled those for SCA in this study. This is justified by the outcome of a study which showd features of anisocytosis, microcytosis, reticulocytosis and high target cells in SCA subjects with $\beta^+$-thalassemia gene (28).

It should be noted that several methods are available for the quantification of $HbA_2$, but electrophoresis is still the conservative method of choice ,nevertheless, it is slow and labour

intensive. HPLC which is a better and superior technology is very expensive in this locality.

In conclusion, this study demonstrated that the coinheritance of beta-thalassaemia trait in sickle cell anaemia in Nigeria is about 6%. Therefore, it is important to consider beta-thalassaemia trait as a differential diagnosis in sickle cell anaemia patients who present with reduced or continuous haemolytic crises in this environment.

Future studies using molecular methods on those classified as S/β-thalassemia patients is highly recommended.This will enable the determination of the exact type of mutation and classification of the common mutations in this population.

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