ORIGINAL ARTICLE

When The Inheritance Of Two Heterozygote States Become A Diagnostic Problem: Misdiagnosis Of The Sickle Cell Trait.

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Abstract

BACKGROUND: *The sickle cell trait is a benign asymptomatic condition that should not ordinarily be associated with clinical manifestations of a haemoglobinopathy.*

METHOD: *This is a case control study of sickle cell trait patients who presented with symptomatology of a haemoglobinopathy. HbA_2, HbF and HbS levels as well as the haematocrit and the peripheral film pictures of 10 symptomatic individuals (patients) with the sickle cell trait were compared with those of 20 asymptomatic individuals (controls) with the sickle cell trait.*

RESULTS: *The mean HbA_2 of the cases was 4.9% compared to the mean of 2.2% for the controls ($p<0.0001$). Nine of the patients and none of the controls had a raised HbA_2 (> 3.5%). The mean HbF of the patients was 5.6% with a range of 1.2-14.0% while the mean of the control was 2.0% and a range of 0.7-8.4% ($p=0.006$). Six (30%) of the controls had normal HbF level (<1%) while none of the patients had a normal HbF level. The mean haematocrit of the patients and controls were 0.33 and 0.37 respectively ($p=0.009$). HbS level was below 40% in both groups. Pregnancy did not significantly affect the mean HbF, the mean HbF for pregnant and non-pregnant cases were 2.1% and 3.8% respectively.*

CONCLUSION: *These findings suggest that the prevalence of the thalassaemia trait may be higher among Nigerians than previously thought with the clinical severity masked by the co-inheritance of other genes like thalassaemia that occur frequently in the same population.*

KEY WORDS: *haemoglobins, thalassaemia trait, Africa, double heterozygote*

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Introduction

There have been anecdotal reports in the past suggesting that the sickle cell trait is associated with increased morbidity and mortality but these findings have not been substantiated[1,2.] There are, however, certain accepted pathologic conditions which are known to be associated with the sickle cell trait, these include renal papillary necrosis with gross haematuria, bone pains and splenic infarction when traveling in unpressurized aircraft[3,4]. When an individual with the sickle cell trait shows symptoms suggestive of sickle cell disease, it is necessary to exclude other sickle cell abnormalities by quantifying HbA_2, HbF and HbS after other possible causes of the said symptoms have been excluded before attributing such symptoms to the sickle cell trait, which ordinarily is a benign asymptomatic condition. Failure to do this may create an erroneous belief that the sickle cell trait is a disease state. This is particularly important in a country as Nigeria where the sickle cell carrier (HbAS) rate is 25% of the population and 2-3% of the population have sickle cell anaemia (HbSS)[5].

In this regard it was observed that some patients with the sickle cell trait presented at the Haematology Clinic of the University College Hospital, Ibadan, Nigeria, with symptoms suggestive of a haemoglobinopathy. Symptoms observed included history of dactylitis, bone pains, recurrent jaundice with anaemia and avascular necrosis of the femoral head. The majority of the patients with recurrent jaundice and anaemia presented during pregnancy and two of them had an associated history of haemoglobinuria. It was therefore deemed necessary to ascertain if these symptoms were due purely to the sickle cell trait or due to co-inheritance of other heterozygote state especially the beta thalassaemia trait.

The study was therefore designed to investigate for laboratory features of ß thalassaemia trait among this group of symptomatic sickle cell trait individuals as well as among a group of asymptomatic sickle cell trait individuals. HbA_2 and HbF were used to diagnose ß thalassaemia trait in these patients because a raised level of HbA_2 is the hallmark for the diagnosis of the ß thalassaemia trait especially in the presence of an elevated level of HbF[6]. Red cell indices are useful in the diagnosis of the thalassaemias but the pitfall is that they are not applicable in pregnant women, children and in iron deficiency[7] . In addition, they may not be useful in a population with an equally high level of alpha thalassaemia as seen in the Nigeria.

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Subjects and Methods

Patients: Ten symptomatic (patients) and twenty asymptomatic (controls) sickle cell trait individuals were studied. The patient and control groups were matched for age and sex. Four of the patients were pregnant at the time of the study and their controls were also matched by parity and gestational age. All individuals studied were Nigerians.

Eligibility: All HbAS patients who were referred to the department during a one year period with symptoms like recurrent history of bone pains, previous history of dactylitis or avascular necrosis of the femoral head, recurrent history of jaundice and/or haemoglobinuria or other symptoms which ordinarily would have been attributed to sickle cell disease. Patient who had been transfused in the last three months before presentation were either excluded or given a later appointment. Ethical approval was obtained from the institutional review board while each participant gave an informed consent.

Methods: The packed cell volume was determined using the microcentrifugation method. A peripheral blood film stained with Leishman was examined in all the subjects. Haemoglobin A_2 level was determined by elution after electrophoresis on cellulose acetate strips (6) and HbF was determined by the alkali denaturation method of Betke (8). Haemoglobin S level was quantified by elution after electrophoresis on cellulose acetate strips (9).

Statistical methods: Because of the small number of study patients and controls, non-parametric statistics were used to analyze the data. The Mann-Whitney U test was used to test for differences between cases and controls in HbA_2, HbF, HbS and haematocrit levels. A p value of <0.05 was considered significant.

Results

The age range of the symptomatic individuals (patients) was between 14 and 35 years, and they comprised nine females and one male. Five of the patients had mild jaundice; two had moderate (5cm, 8cm) hepatomegaly while one had mild (2cm) splenomegaly (Table I). A total of seven spontaneous abortions were noted among the nine female patients while only two was observed among the 18 female controls. The peripheral blood film showed the presence of hypochromia, microcytosis and anisopoikilocytosis to the same degree in both the patients and the controls. Target cells and hypersegmented neutrophils were noted more in the control group than the patient group.

ABLE I: CLINICAL FEATURES OBSERVED IN SYMPTOMATIC SICKLE CELL TRAIT PATIENTS.

Clinical features		No (%)
History	Bone pains	7 (70)
	Blood transfusion	4 (40)
	Haemoglobinuria	2 (20)
Physical Examination		
	Jaundice	5 (50)
	Splenomegaly	1 (10)
	Hepatomegaly	2 (20)

The mean HbA_2 of the patients was 4.9% compared to the mean of 2.2% for the controls ($p<0.0001$). Nine of the patients and none of the controls had a raised HbA_2 (3.5%). The mean HbF of the patients was 5.6% with a range of 1.2-14.0% while the mean of the control was 2.0% and a range of 0.7-8.4%. The difference between the mean HbF of both groups was statistically significant ($p=0.006$). Six (30%) of the controls had normal HbF level (<1%) while none of the patients had a normal HbF level. The mean haematocrit of the patients and controls were 0.33 and 0.37 respectively ($p=0.009$). Table II shows the distribution of the haematocrit of both patients and controls. Both patients and controls had HbS levels below 40%.

Discussion

The elevated levels of HbA_2 and HbF observed in the patients suggest the presence of the beta thalassaemia trait since a raised HbA_2 is the hallmark of the classic ß thalassaemia trait[6]. The raised level of HbF in the controls is more likely to be genetic rather than acquired since acquired causes of a raised HbF like myeloproliferative disorders is unlikely because the patients are healthy. The presence of the thalassaemia trait in some of the controls will not be unusual since this is a disorder that could be clinically silent[10] with 30-50% of individuals with this trait having HbF levels of 1-3%. High persistence of fetal haemoglobin (HPFH) will give an HbF level of 20% or more and the red cells will show a pancellular distribution of HbF.

Adult male or female carriers of either or thalassaemia trait are not usually severely anaemic[10, 11]. All but one of the symptomatic individuals in this study had haematocrit of $\geq$ 0.30, the mean haematocrit among patients and controls were, however, significantly different ($p=0.009$) with the control subjects having higher haematocrit (Table II).

TABLE II: HbA_2, HbF AND HbS VALUES AMONG PATIENTS AND CONTROLS.

	Patients n=10 Median (IQ range)	Controls n=20 Median (IQ range)	Mann-Whitney U	P value
HbA_2 (%)	4.9 (4.0-6.2)	2.2 (1.8-2.5)	10.5	<0.0001*
HbF (%)	5.1 (1.9-13.3)	1.4 (0.9-2.5)	41.0	0.009*
HbS (%)	31.0 (25.8-33.0)	33.0 (29.0-36.0)	61.0	0.085
Haematocrit (/L)	0.35 (0.32-0.36)	0.38 (0.35-0.39)	17.5	0.009*
*p< 0.05				

TABLE III: HAEMATOCRIT OF SYMPTOMATIC AND ASYMPTOMATIC SICKLE CELL TRAIT INDIVIDUALS.

Haematocrit (/L)	Patients	Controls
0.25-0.29	1	0
0.30-0.34	3	1
0.35-0.39	5	12
≥0.40	0	3

Four of the nine female patients were pregnant when they started experiencing symptoms, which supports the fact that stressful periods like pregnancy may worsen the clinical states of these patients[10], carriers for thalassaemia are usually symptom free but may have mild degree of anaemia (9-11g/dl) with red cells which are hypochromic and microcytic[11]. The high frequency of spontaneous abortions among the symptomatic individuals in this study suggests that there may be a higher tendency to fetal wastage in maternal thalassaemia that may require close monitoring in pregnancy[12].

Recent publications have alluded to the fact that sickle cell trait is a cause of sudden unexpected death[13,14]. In one, of the publications[13], undiagnosed sickle cell-beta thalassaemia syndrome associated with splenic sequestration was found to be responsible for the unexpected death. This underscores the need to rule out a thalassaemic syndrome before attributing unexpected death to sickle cell trait, which expectedly is a benign condition.

The combination of sickle cell trait and thalassaemia trait results in an HbS concentration of more than 60%[14]. This was not observed in this study where both patients and controls had HbS concentration that was less than 40%. There are two plausible explanations for this. Firstly, megaloblastic haemopoesis evidenced by the presence of hypersegmented neutrophils in the peripheral blood film is a possible reason since it has been observed that megaloblatic haemopoesis suppresses the production of HbS more than that of HbA[15,16]. Secondly, studies have also shown that coincidental inheritance of and thalassaemia genes may be responsible for the different levels of HbS found in sickle cell trait individuals[17,18]. Even though the thalassaemia status of the patients in this study was not determined, the frequency of the thalassaemia gene in West African is 30-40%[19]. It is therefore possible that the coinheritance of thalassaemia with the thalassaemia trait might be partly responsible for the depressed synthesis of HbS in these patients. Alpha thalassaemia is also a reason for not using red cell indices such as MCV/ MCH to screen for the thalassaemia trait in populations where thalassaemia is prevalent, since significant proportion of carriers may be missed.

If a person has both sickle cell trait and the á thalassaemia trait the HbA_2 may be slightly raised but such individuals are not usually symptomatic and HbF level will be in the normal range[15]. The alpha thlassaemia status also correlates with the amount of HbS in sickle cell trait individuals, individuals who do not have thalassaemia have HbS value of 35-40% while those with 2 thal status will have values as low as20-28%[16].

Another known ameliorating factor is the coinheritance of the glucose-6-phosphate dehydrogenase (G6PD) defect, which has also been known to produce a haematologic picture of larger and more completely haemoglobinzed red blood cells than seen in majority of thalassaemia heterozygotes[16]. G6PD occurs in 25% of male subjects[21] in this population, its effect on the severity thalassaemia therefore calls for further studies.

In summary, this study suggest that the prevalence of the thalassaemia trait may be higher than 1% among Nigerians as formerly reported[22] with the clinical severity masked by the co-inheritance of genes for thalassaemia and G6PD, which are equally high in the same population. It is also important to always quantify the HbA_2 and HbF levels in symptomatic sickle cell trait patient before attributing these symptoms to the benign heterozygous state.

CONCLUSION

A population-based study to quantify HbS, F and A_2 will also be of utmost importance in confirming the findings of this study.

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