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Original Article

A Retrospective Analysis of the Significance of Haemoglobin SS and SC in Disease Outcome in Patients With Sickle Cell Disease and Dengue Fever☆,☆☆

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ABSTRACT

Background: Little is known about the significance of haemoglobin genotype in dengue fever severity. This study was undertaken to determine the case fatality ratio and the impact of genotype in patients with sickle cell disease and confirmed dengue fever.
Methods: This retrospective analysis included 40 patients with confirmed dengue and sickle cell disease, during the study period (2010–2012).
Findings: There was a significantly higher case fatality ratio, 12.5% among patients with either haemoglobin SC disease or homozygous SS disease when compared to that of the general population 0.41% ($p < 0.0001$). The unadjusted odds of dying among those with haemoglobin SC disease compared with the group with homozygous SS disease was OR = 4.4 (95% CI 0.6 to 31.7). The predictors of mortality independent of sickle cell disease genotype were haemoglobin concentration at presentation OR = 0.57 (95% CI, 0.35 to 0.94) and the change in haemoglobin concentration from steady state OR = 0.59 (95% CI, 0.37 to 0.94). Adjusting for haemoglobin concentration at presentation increased the risk of death for the SC genotype relative to SS genotype OR = 13.4 (95% CI 1.1 to 160.3).
Interpretation: The risk of fatal dengue may be higher among patients with a relatively mild genotype (haemoglobin SC).

1. Introduction

Sickle-cell disease (SCD) is considered a major global public health problem (Serjeant, 2014). There are approximately 250,000 births annually (Hannemann et al., 2011). Africa, bears the highest burden of the disease (Makani et al., 2013). However many cases occur in the United States of America, the Caribbean, and Northern Europe (Hannemann et al., 2011). In Jamaica the β-globin chain abnormalities β^S and β^C occur with gene frequencies of 0.055 and 0.019 respectively (Serjeant, 1981). Differences in the characteristics of haemoglobin C and S have been reported, including distinctly more noticeable K^+ loss and dehydration in haemoglobin SC red cells and this contributes to differences in the phenotypic manifestations between haemoglobin SC disease (HbSC) disease and homozygous haemoglobin SS (HbSS) disease (Hannemann et al., 2011). However whether there are phenotypic differences in response to dengue fever between the genotypes is unclear. In Jamaica, the Sickle Cell Unit (SCU) is the only comprehensive care facility that provides care for SCD with more than 5000 individuals registered at the facility.

Many countries with a high SCD burden are also known to have outbreaks of dengue fever (DF) which is a mosquito borne, viral illness (Brown et al., 2009). In fact, like SCD, dengue fever is also a major public health concern (Limonta et al., 2009) and is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. It is said that more than one-third of the world's population live in these high risk areas. In the tropics and subtropics, DF is a leading

☆ Research in context: Jamaica, like many other countries with a high burden of Sickle Cell Disease (SCD) also suffers from epidemics of dengue fever. The two most common types of SCD are homozygous haemoglobin SS (HbSS) and haemoglobin C (HbSC) disease. SCD has been observed to be a risk factor for fatal dengue. This study adds to literature as we observed that the less severe disease, HbSC, may be associated with a higher risk of death from dengue fever. There are currently no vaccines for dengue fever. Hence determining risk factors will inform public health campaigns aimed at risk reduction.

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cause of death (Centers for Disease Control and Prevention, 2013). The World Health Organization (WHO) estimates that 50 to 100 million dengue infections occur yearly, including 500,000 cases of dengue hemorrhagic fever (DHF) and 22,000 deaths annually, mostly among children and during the last 50 years, the incidence has increased 30-fold (World Health Organization, 2014).

Indeed, there have been widespread epidemics of DF in Caribbean countries including Cuba and Jamaica. The two most recent outbreaks in Jamaica were in 2010 and 2012, where 3202 and 5903 suspected cases were reported, respectively (National Surveillance Unit, Ministry of Health, Jamaica). In Jamaica, DF is a class 1 reportable disease (Ministry of Health Kingston Jamaica, 2012).

According to Sickle Cell Unit clinic guidelines, DF is suspected in persons with SCD if the patient presents with a fever and two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia or rash (Asnani et al., 2008). Patients were either treated as outpatients or admitted to hospital depending on their clinical condition. During epidemics, individual patients suspected of having DF are not always specifically tested but in keeping with Ministry of Health reporting guidelines, are designated as likely cases based on clinical criteria.

This study was undertaken to determine the case fatality ratio and the significance of genotype (HbSC and Hb SS) in patients with sickle cell disease and dengue fever.

2. Materials and Methods

2.1. Study Design

After approval by the University of the West Indies Ethics Committee, a retrospective review of charts of consecutive patients suspected to have had DF during the period January 1, 2010 to December 31, 2012 was carried out. The above dates included 2 dengue fever epidemics that occurred in 2010 and 2012. The privacy rights of patients included in this study were strictly observed.

Persons were eligible to be included in the study if they were registered patients at the SCU with a diagnosis of HbSS or HbSC and had clinical features suggestive of DF (Asnani et al., 2008).

2.2. Diagnosis of DF

Patients with HbSS disease, or HbSC seen at the SCU who had clinical findings consistent with DF and dengue serology positive for dengue viral antigen (NS1Ag) and or dengue-specific immunoglobulin M (IgM) were classified as confirmed DF while, those with both Ag and dengue specific IgM negative were classified as unlikely DF. Suspected cases were classified as possible DF if there were no available results or if the results available were equivocal.

The IgM Elisa kit used was the dengue fever virus Capture DxSelect (Focus Diagnostics, Cypress, PA, USA) (World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR), 2009). This IgM assay detects dengue-specific immunoglobulin M as early as day 3 to day 5 after the onset of an illness (World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR), 2009; Peeling et al., 2010). The Dengue NS1 antigen (SDBIOLINE Dengue DUO NS Ag, Standard Diagnostics) is detectable up to nine days (Wang and Sekaran, 2010) after the onset of illness. This is a lateral flow (immunochromatographic) assay. All serological investigations for DF, haemoglobin and other laboratory assays were performed on the day of presentation which ranged from 1 to 10 days (median 5 days) after the onset of illness.

Data from the Ministry of Health, Jamaica documented that the epidemic which occurred in 2010 was attributable to serotype 2 and that in 2012 was attributable to serotype 1 (National Surveillance Unit, Ministry of Health, Jamaica).

2.3. Definition of Steady State Blood Count

At the SCU this is defined as the average of all complete blood counts (CBCs) done after the age of four years at "well" or "routine visits" (that is no ill health at or within 2 weeks of the visit). If patients are less than four years, the last well CBC is used for comparison (Asnani et al., 2008).

2.4. Death Ascertainment

Patients registered at the SCU who died were identified through an existing surveillance system instituted by the Ministry of Health, Jamaica and the SCU. Additional clinical information was obtained from hospital records. The number of deaths among confirmed cases of DF in the general population was obtained from the National Surveillance Unit, Ministry of Health, Jamaica.

2.5. Statistical Analysis

The sample for analysis consisted of persons with HbSS or HbSC with laboratory confirmed DF. Summary measures were expressed as means with standard deviation or geometric means with 95% confidence intervals, as appropriate. Differences in mean values between groups were tested using independent t-tests or Wilcoxon rank-sum test as appropriate. Highly skewed continuous variables were Napier transformed prior to inferential testing by t-test. Associations between categorical variables were tested using the chi-square statistic.

To assess the risk factors for death in the study sample, logistic regressions were performed with the outcome being dead or alive and the candidate predictors being genotype, haematological variables and age. To reduce bias due low frequency of events and small sample size a penalized likelihood estimation of the logistic regression was used (Kosmidis and Firth, 2009). The Stata statistical software version 12 for Windows™ (College Station, TX 77845) was used for analysis. Significance was taken as p value of <0.05.

3. Results

During the study period, January 1, 2010 to December 31, 2012, 111 patients with either HbSC or HbSS disease registered at the SCU were suspected of having DF. Of these 40 subjects (36.0%) had definitive serological evidence of current dengue fever (Confirmed DF) and comprised the analytical sample. Twenty-seven subjects (24.3%) had serology that was not in keeping with dengue fever (Unlikely DF), while 44 subjects (39.6%) had serological data that was either equivocal or unavailable (Possible DF) (Fig. 1).

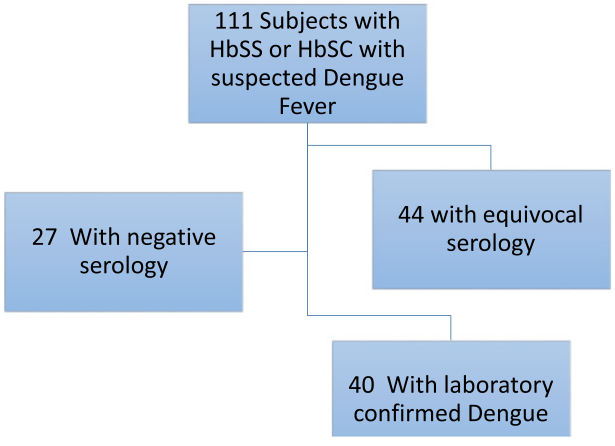

Fig. 1. Flow chart of study participants.

3.1. Clinical Characteristic

Of the 40 persons with laboratory confirmed DF 18 had HbSC and 22 had HbSS. There was no significant difference in the gender distribution between the groups or in mean age (HbSC 13.9 + 11.8 years vs HbSS 15.3 + 14.8 years). Similarly, there was no difference in the proportion of patients hospitalized or the proportion receiving intensive unit care, hydroxyurea and clinical symptoms between HbSC and HbSS groups. However, in subjects with SC disease 63% (10 of 16 subjects) had a palpable spleen on admission $p < 0.01$ and this was significantly greater than in proportion in HbSS 18% (3 of 17) (Table 1).

3.2. Mortality

There were 7 confirmed non sickle cell dengue related deaths in the general population among 1716 laboratory confirmed cases yielding a case–fatality ratio of 0.41% or 4.1 per 1000. On the other hand there were 5 deaths among 40 laboratory confirmed cases with HbSS or HbSC yielding a case–fatality ratio of 12.5% (125 per 1000). The case fatality ratio was significantly greater than in the general population ($p < 0.0001$) Additionally, there were more deaths in SC group than the SS genotype group resulting in higher case fatality ratio 222 per 1000 vs 45 per 1000 but the difference in proportion was not statistically significant ($p = 0.09$).

Within the HbSC group there were certain patterns observed. The persons who died were generally older with lower haemoglobin on admission and with a greater fall in Hb from their documented steady state. However, higher white cell counts were observed in this group and as expected a greater proportion of them had intensive care admission. However these differences were not statistically significant. There was only one death in the HSS group who was a child (Table 1).

Logistic regressions were used to determine the major predictors of mortality. The unadjusted odds of dying in SC compared with SS was OR = 4.4 (95% CI 0.6 to 31.7). Adjusting for haemoglobin concentration increased the risk of death for the SC genotype relative to SS genotype OR = 13.4 (95% CI 1.1 to 160.3) (Table 2). In contrast adjusting for change in haemoglobin concentration from steady state, did not significantly affect the odds of death in HbSC relative to HbSS. Similarly adjusting for acute white cell or platelet counts or change in white cell or platelet counts from steady state did not significantly affect the odds of death for HbSC (data not shown).

The OR for haemoglobin concentration on presentation was 0.57 (95% CI, 0.35 to 0.94) and the OR for change in the haemoglobin concentrations from steady state was 0.59 (95% CI, 0.37 to 0.94). These were predictors of mortality independent of SCD genotype even after adjusting age (Table 2). Thus, among individuals with confirmed dengue a higher haemoglobin concentration in the acute phase decreased the risk of dying. That is, a higher haemoglobin concentration of 1 g/dl in the acute phase decreased the probability of dying by 36%, adjusting for genotype and 38% adjusting for genotype and age. While, when the acute haemoglobin was compared to the steady state, it was found that a decrease of 1 g/dl from the steady state increased the probability of dying by 63% adjusting for genotype and 62% adjusting for genotype and age.

4. Discussion

In this study we sought to determine the mortality experience of persons with HbSS and HbSC with laboratory confirmed dengue, as well as to ascertain whether there were differences in morbidity. Our findings of a thirty fold increased mortality among persons with SCD, is supported by previous reports mainly from Cuba, which have suggested that SCD is a risk factor for severe DF. Bravo et al. (1987), reporting on the 1981 Cuban epidemic, stated that chronic diseases such as bronchial asthma, diabetes mellitus and sickle cell anaemia (Hb SS disease) were risk factors for severe disease. Limonta et al. (2009) reported two persons who died in the 2001–2002 Havana dengue epidemics, both with SCD. The genotypes of the affected persons were however not stated. Moesker et al. (2013) reported two cases from Curacao of fatal DF in persons with SCD (one case had Hb SC disease, the other Hb SS disease).

The higher mortality in sickle cell disease compared to the general population may be due to heighten inflammatory response in sickle cell disease relative to subjects without sickle cell disease. For example recent advances have shown that specific antibodies and T cells directly influence a cytokine imbalance in dengue that eventually leads to

Table 1
Clinical characteristics of sample.

Variables	HbSC		HbSS	
	Survived (N = 14)	Died (N = 4)	Survived (N = 21)	Died (N = 1)
Age, years (mean, SD)	11.0 (7.1)	23.7 (20.2)	15.8 (14.9)	4.3
Acute haemoglobin conc., g/dl	9.5 (1.3)	6.2 (4.1)	6.6 (1.6)	3.7
Steady state haemoglobin conc., g/dl	10.0 (1.0)	10.3 (0.4)	7.9 (1.2)	7.9
Change in haemoglobin conc.[a], g/dl	−0.5 (1.5)	−4.1 (4.4)	−1.1 (1.3)	−4.2
Red cell count, $\times 10^{12}$	3.9 (0.4)	1.6 (0.8)	2.6 (1.0)	2.8
Acute platelet count, $\times 10^{12}$/l	170.1 (85.6)	217.2 (149.0)	273.2 (154.6)	304.0
Steady state platelets, $\times 10^{12}$/l (mean, SD)	273.1 (140.7)	288.9 (73.3)	409.7 (131.7)	304
Change in platelet counts[a], $\times 10^{12}$/l (mean, SD)	−96.1 (161.2)	−55.6 (113.4)	−127.1 (153)	0
Acute white cell count[b], $\times 10^{9}$/l (mean, SD)	6.2 (4.1 to 9.5)	15.6 (4. To 61.7)	14 (10.3 to 19.1)	11.6
Steady state white cell count, $\times 10^{9}$/l (mean, SD)	8.8 (3.2)	10.5 (2.6)	11.3 (3.4)	12.0
Change in white cell count[a], $\times 10^{9}$/l (mean, SD)	−0.4 (7.2)	6.4 (10.3)	7.6 (14.1)	−0.4
Gender (M:F)	7:7	3:1	9:12	0:1
Hospital admission (Y:N)	11:3	4:0	16:5	1:0
Intensive care (Y:N)	1:13	3:1	1:20	1:0
Hydroxyurea use (Y:N)	0:0	0:0	2:19	0:1
Gastrointestinal symptoms (Y:N)	12:2	3:1	14:7	0:1
Presence of spleen, N = 33 (Y:N)	7:6	3:0	3:14	
Rash (Y:N)	2:8	1:3	1:11	0:1

Abbreviations: conc = concentrations; values are counts or means with SD. HbSC — sickle cell haemoglobin C disease; HBSS — homozygous S sickle cell disease. Steady state — defined as the average of all complete blood counts (CBCs) done after the age of four years at "well" or "routine visits" (that is no ill health at or within 2 weeks of the visit). If patients are less than four years, the last well CBC is used for comparison.

[a] Change is difference between values measured during DF and steady state.

[b] Values are geometric means with 95% confidence interval.

Table 2
Predictors of death in sample.

	Model	HbSC	Acute haemoglobin concentration g/dl	Change in haemoglobin concentrations g/dl	Age years
Unadjusted model	1	4.4, 0.6 to 31.7	–	–	–
Haematological adjusted models	**2**	13.4, 1.1 to 160.3	0.57, 0.35 to 0.94	–	–
	3	3.4, 0.4 to 28.9		0.59, 0.37 to 0.94	–
Age & haematological adjusted models	4	11.2, 0.94 to 133.4	0.60, 0.36 to 0.99	–	1.02, 0.95 to 1.1
	5	2.9, 0.37 to 22.9		0.61, 0.38 to 0.97	1.01, 0.94 to 1.1

Values are odds ratio with 95% confidence intervals. Model 2 bold significance is the estimate of the effect (odds ratio), for the variable HbSC is significant ($p < 0.05$).

severe disease and vascular damage (Remy, 2014). Additionally, several studies in patients with SCD have shown excessive inflammatory cytokines in both steady-state and during a vaso-occlusive crisis and therefore the existence of ongoing inflammation (Musa et al., 2010). Taken together these observations may indicate an amplified inflammatory response in SCD during a dengue infection resulting in increased morbidity and mortality.

Also, in this study, subjects within each genotype with lower haemoglobin or greater fall in haemoglobin from steady state had higher case fatality. This within group difference is probably reflective of the extent of haemorrhage at presentation. Surprisingly, when genotypes are compared, the HbSC genotype, which as a group, characteristically have higher haemoglobin counts than HbSS, had a higher case fatality. There are several possible pathophysiological mechanisms that could be responsible for this observation.

Firstly, it may be that subjects with HbSC are more susceptible to fatal dengue due to the increased susceptibility of the red blood cell in HbSC to dehydration based on K^+ loss (Hannemann et al., 2011). This leads to a greater propensity for dense cell formation when compared to HSS. These dense cells have an increased intracellular haemoglobin concentration hence higher mean cell haemoglobin concentration (MCHC). The higher MCHC causes both an increase in HB S polymerization and also decreases the time to sickling (Nagel et al., 2003). Perhaps the dengue virus either alters the HbSC RBC rheology by triggering RBC dehydration or affects the endothelial adhesivity resulting in a massive vascular leak syndrome and intravascular dehydration. This in turn would result both in intravascular dehydration and massive sickling.

Another possible mechanism which could explain a higher morbidity in HbSC disease is neo-angiogenesis. It has been reported that neo-angiogenesis and retinopathy are more frequent in HbSC patients (Banu et al., 2013). It is possible that blood vessels produced by neo-angiogenesis are more permeable in response to inflammatory cytokines such as High mobility group box 1 (HMGB1) protein which increases vascular permeability of endothelial cells in a dose dependent manner in in-vitro experiments (Ong et al., 2012). The HMGB protein is increased in dengue fever infection and sickle cell vaso-occlusive crises (Xu et al., 2014) and is thought to play an important role in dengue shock syndrome (Ong et al., 2012). This could contribute to a massive vascular leak syndrome, intravascular dehydration and shock.

The study was limited by its retrospective design: inconsistencies in the reporting methods of various laboratories led to some cases being classified as possible DF rather than definitively confirmed DF. Despite this, four of five deaths with confirmed DF occurred in SCD patients with HbSC genotype. The authors were not able to determine primary from secondary infections as methods such as IgG/IgM antibody ratios were not available. However, based on past medical history recorded in the patient notes, only 3 patients were documented as having DF previously. They did not contribute to the deaths.

5. Conclusion

In conclusion, DF was associated with a much higher morbidity in patients with SCD. We therefore recommend that persons with SCD, in particular Hb SC disease, who present with symptoms compatible with DF during an epidemic, should be treated as high risk, and carefully managed in an inpatient setting.

Author Contributions

Angela E Rankine — MB; BS, MRCPCH, Research Fellow Sickle Cell Unit, Tropical Medicine Research Institute, University of the West Indies, Mona Kingston 7 Jamaica — Study design, submission to ethics, data collection, literature search, data analysis, data interpretation, writing, preparation of drafts, manuscript revisions, approval of final draft and agreement to be accountable for all aspects of work. No conflicts of interest.

Marvin E Reid — MB; BS, PhD, Professor/Director Tropical Metabolism Unit, Tropical Medicine Institute University of the West Indies, Mona Kingston 7 Jamaica — Conceptualization, study design, literature search, data analysis, data interpretation, figures, critical review of manuscript revisions, approval of final draft and agreement to be accountable for all aspects of work. No conflicts of interest.

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