**THE HAEMATOLOGY OF JAMAICANS:**

**RED CELL INDICES IN HbAA, HbAS, HbAC, and HbA-HPFH GENOTYPES**

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**Abstract**

Based in the parish of Manchester in central Jamaica, the Manchester Project offered free detection of haemoglobin genotype to senior classes in 14 secondary schools between 2008-2013. Restricting the database to 15,103 students aged 15.0-19.9 years provided an opportunity to examine the red cell characteristics of the different haemoglobin genotypes, including normal (HbAA) in 85.0%, the sickle cell trait (HbAS) in 9.7%, HbC trait (HbAC) in 3.5% and hereditary persistence of fetal haemoglobin (HbA-HPFH) in 0.4%. Compared to the normal HbAA phenotype, HbAS had significantly increased mean cell haemoglobin concentration (MCHC), red cell count (RBC), and red cell distribution width (RDW) and decreased mean cell volume (MCV) and mean cell haemoglobin (MCH), these differences being even more marked in HbAC. Compared to HbAA, the HbA-HPFH had significantly increased RDW but there were no consistent differences in other red cell indices, and there were no significant differences in haematological indices between the two common deletion HPFH variants, HPFH-1, and HPFH-2. Although these changes are unlikely to be clinically significant, they contribute to an understanding of the haematological spectrum of the common haemoglobin genotypes in peoples of African origin. (187 words)

**Introduction**

The Manchester Project in central Jamaica, was designed to determine whether knowledge of haemoglobin genotype influenced choice of partner and reproductive outcome (Serjeant et al. 2017). Over a 6-year period (academic years 2007-2013), haemoglobin genotypes were determined in 16,612 secondary school students mostly aged 15-19 years (Mason et al. 2016). Since the studied population had no known ascertainment biases, this database also afforded an opportunity to describe the haematological characteristics of normal (HbAA) and the common abnormal haemoglobin genotypes in Jamaica. The sickle cell trait (HbAS) occurred in 9.7% of Jamaicans, the HbC trait (HbAC) in 3.5%, and since the trait for hereditary persistence of fetal haemoglobin (HbA/HPFH) was unexpectedly common at 0.4%, the red cell characteristics of this condition were also examined.

Published data have reported the HbAS genotype to be microcytic in non-anaemic adult African-American males attending the Veterans Administration for abnormal haemoglobin states (Sheehan & Frenkel 1983), in non-anaemic Sicilian males (Maggio et al. 1984), and in a screening programme including both genders and a mean age of 19.5 years in Washington DC (Castro & Scott 1985). Haematocrits were lower and mean cell haemoglobin concentration (MCHC) higher among African-American HbAS children compared to HbAA children (Rana et al. 1993). In a study of haemoglobin C disorders, Smith and Krevans (1959) noted that HbAC had increased target cells and decreased osmotic fragility. A study of 15 subjects with HbAC found mild anaemia, a reduced red cell mass and evidence of shortened red cell survival (Pringle and McCurdy 1970). Other studies have shown that HbAC has a higher MCHC (Ballas et al. 1987; Hinchliffe et al. 1996), and tends to be more microcytic than normal (Sheehan & Frenkel 1983; Maggio et al. 1984). Rana et al. (1993) found lower haematocrit and higher MCHC in HbAC, compared to HbAS.

These reports on the red cell characteristics of both HbAS and HbAC are generally based on small numbers, undefined biases in subject ascertainment, and have not been adequately controlled for age or gender. The current database has allowed a more extensive analysis of red cell indices in three common haemoglobin genotypes, HbAA, HbAS and HbAC and has been extended to the red cell characteristics of carriers for hereditary persistence of fetal haemoglobin (HbA-HPFH) which appears relatively common in this Jamaican population.

**Material & Methods**

*Subject Ascertainment* Manchester is a parish in central Jamaica where, in collaboration with the local Ministry of Health and Ministry of Education, screening was offered to the senior students of 15 secondary schools distributed throughout the parish with two in the south (Winston Jones, Cross Keys), two immediately south of the regional capital, Mandeville (May Day, Victor Dixon), four in Mandeville (Manchester, DeCarteret College, Bishop Gibson, Belair), two in the east (Bellefield, Porus) one in the centre (Mile Gully), and four in the north (Christiana, Spalding, Holmwood, Knox)(Figure). The entire dataset included 16,612 but after excluding two schools with sometimes atypical admission criteria (Belair and Victor Dixon High Schools) and restricting to subjects aged 15.0-19.9 years, there was a total of 15,103 students or 91% of those screened.

*Logistical Considerations* After approval by the school Principal, discussions were held with the school staff and Parent-Teachers Associations, and illustrated lectures on sickle cell disease and its genetics were given to the students. All students were given letters for their parents outlining the objectives and the option of declining for their child to be tested. Several weeks after these lectures, the guidance counsellors and school nurses were contacted and arrangements made to offer screening at sites within the schools, which were usually the sick bay, wellness centres, vacant classrooms or auditoria. Teachers were informed of the programme and asked to invite their classes to attend at a time convenient to their schedule. The screening was voluntary and free and conducted at times requested by the schools but usually between 9-11am. At pre-arranged times, a physician, clerical assistants and 3–4 phlebotomists visited the school, the students completed a form providing personal details and one 5 ml EDTA sample was taken by venepuncture. The turn-round time for individuals was about 20 minutes and 150–250 students could be screened within a 2–2½ hour period. Each school was visited 2-5 times in each of the 6 academic years (2007-2013) of the programme.

*Laboratory Methods*. Following sample collection at schools, the team returned to the laboratory, sorted samples alphabetically and assigned serial unique ID #’s. Samples were then analysed by alkali haemoglobin electrophoresis on cellulose acetate and bands in the position of HbS were confirmed by the slide sickle tests and all electrophoretic variants were assessed by acid agar gel electrophoresis. Haematological indices were measured in electronic analysers (Cell-Dyn 1700, Sysmex XS1000i). Subjects with an AA phenotype and MCH ≤ 26 pg/dL and a red blood cell distribution width (coefficient of variation) [RDW(CV)] < 18.0 were considered candidates for the beta thalassaemia trait and HbA2 levels were measured by high performance liquid chromatography (HPLC) and DNA sequencing performed in those with values ≥ 3.5%. When confirmed, carriers for the beta thalassaemia trait were excluded from the group considered to have the AA phenotype. When a clearly visible band of HbF occurred in subjects with an apparent HbAA phenotype, HbF and HbA2 were estimated by HPLC and if HbF exceeded 8%, samples were analysed for Mendelian forms of HPFH using DNA diagnostics, for deletional and non-deletional forms of HPFH. For technical details see supplemental file and Craig et al 1994. and Clark & Thein 2004.

*Distribution of results and counselling* All tested students received laminated cards bearing personal details (name, school, date of birth), laboratory ID#, and the haemoglobin genotype with interpretation. Normal subjects (AA phenotype) received a card stating that they were not at risk of a baby with sickle cell disease. Carriers of abnormal genes received a card stating the genotype, for example AS (sickle cell trait) and the notation that ‘This will not affect your health but you could pass it on to your children. If your partner is normal, you cannot have a child with sickle cell disease’. The reverse side of the card for those with abnormal genes had a simple pedigree as an example and contact information for the screening laboratory. Students with abnormal genotypes were contacted by the screening team, given the cards and offered genetic counselling which could be taken either individually or in small groups as preferred. The normal genotype cards were distributed by the teacher or guidance counsellor.

*Statistical Analysis* Preliminary analysis indicated that all indices were approximately normally distributed except for RDW values, and HbF values in the HPFH trait so these were log transformed for analysis to make them more symmetrically distributed.  A mixed model was used to allow for the hierarchical school-based structure of the data, though for no outcome did the intra-class correlation exceed 0.01, so the values are not reported.  Each outcome was adjusted for sex and for age, separately for each sex.  Binary terms for HbAS, HbAC and HbA-HPFH were used to estimate and test the difference of each outcome in these groups from the normal group.

**Results**

Compliance with screening over the 6 years, increased from 56% to 92%, and females accounted for 57%. The distribution of genotypes/phenotypes is summarised in Table 1. Haematological indices and the significance of any deviation from the HbAA phenotype in selected indices is shown in Table 2. Haemoglobin levels did not differ in the HbAS subjects but were marginally higher in HbAC due to a stronger effect in males. Both HbAS and HbAC showed significant increases in MCHC, red blood cell count (RBC) and red cell distribution width (RDW) and decreases in mean cell volume (MCV) and mean cell haemoglobin (MCH), the differences being more marked in HbAC.

Of the 53 subjects with HPFH, there were three with point mutations in the promotor region (two G -202 C>G; one A -198 T>C), 48 with deletional HPFH (HPFH-1 in 14; HPFH-2 in 34), and two were unidentified for technical reasons. Of the latter, one had a HbF level of 30% but there was a technical failure in sequencing and the other had a HbF band of similar strength but the sample was lost; neither could be contacted for repeat sampling. The haematological features of those with deletional HPFH (Table 3) showed the expected gender differences but red cell indices did not deviate consistently and significantly between the common deletional mutations or from the AA phenotype.

**Discussion**

The primary objective of the Manchester Project was to identify carriers of abnormal haemoglobin genotypes (Serjeant et al. 2017), but the resulting database has provided a large body of information on red cell characteristics of the more common genotypes/phenotypes among healthy Jamaican students. The current study confirms that HbAS tends to have higher MCHC and RDW and lower MCV and MCH compared to age-sex matched controls with a normal HbAA phenotype and that these differences are even more marked in HbAC. These findings are consistent with previous observations which were usually based on undefined selection criteria. It is unlikely that these differences are clinically important and limited data showed normal red cell survival in the sickle cell trait (Barbedo & McCurdy 1974). However, all 6 patients with HbAC in one study had a reduced red cell mass and shortened red cell survival (Pringle & McCurdy 1970) raising the possibility of increased haemolysis and an increased risk of cholelithiasis.

Alpha thalassaemia status was not available in the present study but in the subjects with HbAA in the Jamaican Cohort, heterozygous alpha thalassaemia () occurred in 30% and homozygous alpha thalassaemia () in 2%. Although a potential cause of lowered MCV, the prevalence of alpha thalassaemia in HbAA, HbAS and HbAC in the present study is unlikely to deviate from this and confound the conclusions. Furthermore, an element of microcytosis may have been attributable to iron deficiency which was common among the females in the Manchester Project as judged by the response to oral iron supplementation (Mason et al. 2014). These are potential weaknesses of the current data but the strengths are the large numbers, limitation to a narrow age range (15.0-19.9 years), correction for gender, and without the potential biases inherent in the previously published data.

The variant for HPFH is believed to be more common among people of African origin with estimates of approximately 1 in a 1000, but the prevalence in central Jamaica was 3-4 per 1,000. The diagnosis was based on the persistence of high levels of HbF into adult life, usually higher than 15% and confirmed by molecular studies. The two deletions are of 85kb and 84kb respectively deleting the delta and beta chains and associated DNA; the HPFH-2 deletion commencing approximately 5kb on the 3’ side of that for HPFH-1. The HPFH-2 gene accounted for 71% of the identified 48 subjects with deletional HPFH’s and a similar dominance of HPFH-2 was noted in a report of 30 cases with HbS-HPFH (Ngo et al. 2012). As might have been predicted from the similarity of deletion length and content, there were no significant and robust differences between the red cell indices of HPFH-1 and HPFH-2. Compared with HbAA there was some evidence of significantly higher haematocrit and RDW and lower MCV. The clinical relevance of the HPFH variant is that when it is co-inherited with the sickle cell gene, it produces HbS-HPFH with high levels of HbF distributed evenly throughout the red cells population. This renders the red cells more resistant to sickling, and their red cell indices are usually normal or mildly microcytic and they do not have the symptoms of SS disease. In particular, there is no evidence of early loss of splenic function and pneumococcal prophylaxis may not be necessary.

It is unlikely that the observations related to red cell indices in HbAA, HbAS, and HbAC have clinical significance but awareness of these characteristics may prevent unnecessary investigation and contribute to a greater understanding of molecular mechanisms in abnormal haemoglobins. (1,991 words)

#### **Figure 1 Legend**

Map showing position of Manchester parish and distribution of the school groups.

#### **Declarations**

***Funding*** School screening was supported by the National Health Fund of Jamaica and Jamalco.

***Conflicts of interest/Competing interests***: Serjeant GR, Serjeant BE, Mason KP, Gibson F, Osmond C, Thein SL, Happich M, Kulozik A declarethat they have no conflict of interest.

***Availability of data and material*** Data are available

***Code availability*** (software application or custom code)

***Authors' contributions*** GRS, BES, KM conceived the study, GRS, BES, KM, FG analysed haematology and electrophoresis, CO performed statistical analysis, SL analysed the HPFH variants. All approved the submitted version.

***Ethics approval*** From the Ministry of Health and the Ministry of Education, Jamaica

***Consent to participate*** All students were given letters for the parents allowing exclusion from blood tests. All participation of the students was voluntary.

***Consent for publication*** Approved by the Southern Region Health Authority of the Ministry of Health, Jamaica.

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**Supplemental File**

DNA was extracted from EDTA blood samples using the QIAsymphony®, automated DNA extraction instrument, (Qiagen Ltd, UK) using the DNA Midi Kit according to the manufacturer’s recommendations. Samples were eluted into 200 µl Qiagen buffer. Detection of the HPFH deletionsutilized Gap PCR in which two primers were used to amplify across the breakpoints For HPFH2 - HPFH2-F: 5’-GCCACTTACCATTTGATAGCTCTG-3’ and HPFH2-R: 5’-TGGGTTAGATTGACTCCTGGGTA-3’ and a control primer to amplify the normal allele Control-R:5’- CTTGCTATGCCAACTCACTACCC-3’. For HPFH-1, the primers were: HPFH1-F: 5’-AGAATGTCACACTTAGAATCTG-3’ and HPFH1-R: 5’- CACTTTAATTCTGGTCTACCTGAA-3’, and control: 5’-ACTGTGATGTTGGAAATGGAC-3’. Qiagen Multiplex reaction buffer was used, cycling 95°C for 30 sec, 58°C for 1 min and 72°C for 30 sec. Products were visualised on an ethidium bromide stained gel, a 292 bp HPFH-2 product and a 462 bp product representing the normal allele, for HPFH-2; and 1193 bp HPFH-1 product and a 1616 band for the normal allele.

Non-deletional HPFH involves specific amplification of each gamma globin promoter region (A or G) in a single amplicon covering the entire region. A 3’ primer (HBG-R) is used in combination with the A specific 5’ (HBG1-F) forward primer to amplify the A Gamma promoter region. For the G Gamma promoter region, the 5’ forward primer (HBG2-F) and the same 3’ reverse primer used for A Gamma (HBG-R) are used. Qiagen Multiplex reaction buffer was used, cycling 95°C for 10 mins x1, 95C for 40 sec, 55°C for 40 sec and 72°C for 40 sec for 35 cycles, and final 72C for 5 mins. These γ A and G gene specific PCR products are then used as a template for a Big Dye Terminator (Applied Biosystems, Ltd) cycle sequencing reactions using the same primers. The cycle sequencing products are then analysed on the ABI Genetic analyser 3130xl.

**Table 1**

**Principal Genotypes/Phenotypes in 15,103 subjects aged 15.0-19.9 years**

Genotype/ Males Females Total

phenotype n n n (%)

HbAA 5,580 (5,579) 7,251 (7,219) 12,831 (84.96)

HbAS 632 830 (827) 1,462 (9.68)

HbAC 226 301 527 (3.49)

HbA-HPFH 24 30 54 (0.36)

Beta thalassaemia trait 66 71 137 (0.91)

HbSS 7 15 22 (0.15)

HbSbeta+ thalassaemia 2 7 9 (0.06)

HbSbetao thalassaemia 0 1 1 (0.01)

HbSC 13 19 32 (0.21)

HbS-HPFH 2 1 3 (0.02)

HbSVariant (Hb Korle Bu) 0 1 1 (0.01)

HbCC 3 4 7 (0.05)

HbCbetao thalassaemia 1 0 1 (0.01)

Variant traits – alpha chain 2 4 6 (0.04)

- beta chain 5 5 10 (0.07)

6,563 (43.5%) 8,540 (56.5%) 15,103 (100.00)

HPFH – hereditary persistence of fetal haemoglobin.

CC disease should be considered as a phenotype since these were not further investigated

Bracketed figures in male/female column refers to those with haematological indices, the difference representing finger-prick samples.

**Table 2**

**Selected red cell indices (mean, SD) in HbAS and HbAC with significance of difference from a normal AA phenotype**

Phenotype (n) Hb (g/dL) Hct (L/L) MCHC (g/dL) RBC (x1012/L) MCV (fL) MCH (pg) lnRDW

Males

HbAA (5579) 14.47, 1.16 42.96, 3.47 33.72, 1.42 5.13, 0.44 83.96, 5.72 28.31, 2.32 13.68, 1.25

HbAS ( 632) 14.50, 1.09 42.79, 3.19 33.92, 1.49 5.26, 0.47 81.64, 5.67 27.70, 2.29 14.09, 1.32

HbAC ( 226) 14.75, 1.14 42.96, 3.21 34.40, 1.78 5.46, 0.47 79.00, 6.26 27.09, 2.31 15.11, 1.48

Females

HbAA (7219) 12.45, 1.24 37.34. 3.50 33.36, 1.56 4.51, 0.39 82.96, 7.04 27.70, 2.82 14.06, 1.98

HbAS ( 827) 12.51, 1.20 37.24, 3.40 33.61, 1.47 4.65, 0.40 80.39, 7.13 27.05, 2.80 14.48, 2.19

HbAC ( 301) 12.58, 1.27 36.91, 3.66 34.08, 1.80 4.80, 0.39 77.02, 6.93 26.27, 2.67 15.51, 2.07

**Significance of phenotype differences – difference / (95%CI) / p value**

Males 0.04 -0.12 0.20 0.13 -2.30 -0.60 0.03

HbAS:HbAA (-0.05, 0.14) (-0.40, 0.16) (0.08, 0.32) (0.10, 0.17) (-2.77, -1.83) (-0.79, -0.41) (0.02, 0.04)

0.35 0.41 <0.01 <0.01 <0.01 <0.01 <0.01

HbAC:HbAA 0.28 0.03 0.67 0.33 -4.95 -1.22 0.10

(0.13, 0.43) (-0.42, 0.48) (0.48, 0.86) (0.28, 0.39) (-5.72, -4.19) (-1.53, -0.91) (0.09, 0.11)

<0.01 0.90 <0.01 <0.01 <0.01 <0.01 <0.01

Females 0.06 -0.11 0.26 0.13 -2.58 -0.64 0.03

HbAS:HbAA (-0.03, 0.14) (-0.36, 0.14) (0.15, 0.37) (0.11, 0.16) (-3.09, -2.08) (-0.84, -0.44) (0.02, 0.04)

0.22 0.38 <0.01 <0.01 <0.01 <0.01 <0.01

0.13 -0.41 0.73 0.29 -5.94 -1.42 0.10

HbAC:HbAA (-0.01, 0.28) (-0.81, -0.01) (0.56, 0.91) (0.25, 0.34) (-6.75, -5.13) (-1.75, -1.10) (0.08, 0.11)

0.06 0.04 <0.01 <0.01 <0.01 <0.01 <0.01

RDW is skewed in distribution and log transformed for the tests for difference. RDW-CV expressed in arbitrary units.

SD = standard deviation; CI = confidence interval; diff = difference derived from the multilevel model adjusting for school effects.

**Table 3**

**Red cell indices (mean, SD, range) in HbA-HPFH**

**according to deletion variant HPFH-1 and HPFH-2**

**Indices by Gender within Variants**

HPFH-1 n Hb MCHC RBC MCV MCH RDW HbF (%) HbA2 (%)

Males 6 13.73, 1.12 33.6, 1.8 5.11, 0.58 79.9, 7.0 27.3, 3.8 16.1, 2.7 26.4, 4.6 2.1, 0.3

range (n) 12.1-14.8 30-35 4.42-5.91 70-92 21-33 13.0-20.6 22.5-33.5 (5) 1.8-2.3 (3)

Females 8 12.88, 1.39 33.6, 0.9 4.67, 0.47 81.8, 3.7 27.7, 2.0 14.4, 1.5 27.1, 5.3 2.2, 0.2

range (n) 10.3-14.8 32-35 3.78-5.13 75-86 24-30 11.2-15.8 20.8-34.7 (7) 2.0-2.4 (5)

Both sexes 14 13.24, 1.31 33.6, 1.3 4.86, 0.55 81.0, 5.2 27.5, 2.8 15.2, 2.2 26.8, 4.8 2.3, 0.2

HPFH-2

Males 16 14.71, 1.12 33.3, 1.2 5.53, 0.49 80.0, 3.9 26.6, 1.6 16.1, 1.5 28.9, 4.9 2.2, 0.2

range (n) 12.8-16.5 31-35 4.33-6.24 74-88 24-30 12.8-18.3 22.1-39.4 (13) 2.0-2.5 (10)

Females 18 12.94, 1.36 33.2, 1.2 4.90, 0.37 79.7, 7.3 26.4, 2.4 16.0, 1.7 26.3, 5.9 2.2, 0.2

range (n) 10.0-15.0 31-35 4.06-5.61 60-92 19-30 13.6-19.7 14.7-38.3 (18) 2.0-2.6 (15)

Both sexes 34 13.77, 1.52 33.2, 1.2 5.20, 0.53 79.9, 5.9 26.5, 2.0 16.1, 1.6 27.4, 5.54 2.2, 0.2

Discrepancies between these numbers and those in Table 1 are due to other HPFH variants.

RDW represents RDW-CV expressed in arbitrary units. Other units defined as in Table 2

There were no statistically significant differences between indices in HPFH-1 and HPFH-2 but compared with the AA phenotype, Hb and Hct levels in HPFH were lower in females, and MCV was lower in males.

**A picture containing diagram

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**Haematological Indices**

Figure Legend

Outline of Jamaica showing position of Manchester parish and the distribution of schools