Volume regulatory hormones and plasma volume in pregnant women with sickle cell disorder

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Abstract

Background: Sickle cell disease (haemoglobin SS (HbSS)) mainly affects those of West African origin and is associated with hypervolaemia. Plasma volume rises by up to 50% in normal pregnancy but was previously found to be paradoxically contracted in late sickle cell pregnancy. The renin–angiotensin–aldosterone system is activated very early in human pregnancy to support the plasma volume expansion. We hypothesised that activation of the renin–angiotensin–aldosterone system would be blunted in pregnant women with sickle cell disease.

Materials and methods: We measured plasma volume and concentrations of plasma renin, angiotensinogen, aldosterone and other volume-related hormones in a cross-sectional study of pregnant and non-pregnant Nigerian women with HbSS or HbAA.

Results: Plasma volume was higher in non-pregnant HbSS than HbAA women, but had not risen by 16 weeks, unlike plasma volume in HbAA women. The concentration of plasma renin also rose significantly less by 16 weeks in HbSS; angiotensinogen and aldosterone concentrations increased.

Conclusions: The lower plasma renin concentration at 16 weeks with HbSS could be either primary or secondary to vasoconstriction related to inadequate vasodilator activity. The contracted plasma volume might then stimulate aldosterone synthesis by non-angiotensin II dependent stimulation. Studies of vasodilators such as nitric oxide, vasodilator eicosanoids or the PlGF/VEGF/sFlT-1 axis in pregnant HbSS and HbAA women will test this hypothesis.

Keywords
Sickle cell disease, sickle cell anaemia, plasma volume, volume regulatory hormones, renin–angiotensin–aldosterone system (RAAS), pregnancy

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Introduction

Sickle cell disease (SCD) is a disorder of haemoglobin and those afflicted, who are mainly of West African origin, suffer from chronic anaemia and ‘crises’ as a result of recurrent haemolysis and the resultant small vessel occlusion and end organ ischaemic necrosis.1,2 When affected women become pregnant, there is an increased incidence of vaso-occlusive crises, infections, and maternal and perinatal morbidity and mortality.3 The increase in perinatal mortality is associated with low birthweight and intrauterine growth restriction.4

Plasma volume rises by up to 50% in normal pregnancy5,6 and this rise has been shown to begin as early as

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six weeks after the last menstrual period. The initiating factor for plasma volume expansion in pregnancy is now thought to be the early peripheral arterial vasodilation. Arguments for this include the fact that systolic and diastolic blood pressures fall during the first trimester of pregnancy, despite an increase in blood volume. Activity of the renin–angiotensin–aldosterone system (RAAS) also increases in pregnancy and this is noticed from as early as the sixth week of gestation. This supports arterial vasodilation as the primary stimulus as opposed to volume expansion per se as the RAAS is normally stimulated when there is a reduction in peripheral vascular resistance.

This increase in plasma volume in pregnancy is a positive phenomenon and has been found to be one of the biggest physiological determinants of birthweight. A study conducted about 30 years ago found that pregnant haemoglobin SS (HbSS) women had a reduced plasma volume by late pregnancy, compared with their HbAA counterparts and compared with early pregnancy. The reason for this was not known but it was postulated that examining the RAAS in these women might be useful.

Women with pre-eclampsia also have a reduced plasma volume at the time of diagnosis and associated intrauterine growth restriction; under-activity of the RAAS has been found in such women as well. Pregnant HbSS women have been reported to have a higher incidence of pre-eclampsia although not all studies have found this association.

Other hormones may play a role in volume control in pregnancy. Progesterone (PROG), prolactin (PRL) and arginine-vasopressin (AVP) have all been reported to be of importance in various studies. High PROG was found as early as 18–21 weeks in women who later developed pre-eclampsia, although not all studies have found this association.

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Apart from the sodium control of extracellular volume, there is also volume control of water excretion. A rise in extracellular fluid (ECF) volume inhibits AVP secretion while a decline in ECF increases AVP secretion. AVP is also increased by an increase in osmotic pressure but volume stimuli override the osmotic stimulation of AVP secretion. Plasma osmolality falls by ~10 mOsm/kg in early pregnancy, in the face of maintained AVP secretion, but the hypothalamic ‘osmostat’ is then reset, to respond normally to volume depletion or expansion. Relaxin is another hormone that might be responsible for resetting the osmostat in pregnancy and is also thought to play a role in the early vasodilation of normal pregnancy via endothelin and nitric oxide mediation.

We hypothesised that activation of the RAAS would be blunted in pregnant women with SCD. We therefore decided to undertake plasma volume measurements in pregnant and non-pregnant HbSS women and HbAA controls, and to examine some of their volume regulatory hormones so as to detect any abnormalities, thus adding to the sparse literature on the physiology of volume control in HbSS pregnancy. We also hoped to identify any parallels with pre-eclampsia.

**Methods**

The study was approved by the Research and Ethics Committee of the Lagos University Teaching Hospital, Lagos, Nigeria (reference number ADM/DCST/221/Vol. 9). Written, informed consent to participation was given by all women. A prospective, cross-sectional study was performed in women attending the antenatal and sickle cell clinics of the Lagos University Teaching Hospital. The groups studied were as follows:

1. Pregnant women with HbSS at 16 weeks’ gestation.
2. Pregnant women with HbSS at 36 weeks’ gestation.
3. Pregnant women with HbAA at 16 weeks’ gestation.
4. Pregnant women with HbAA at 36 weeks’ gestation.
5. Non-pregnant HbSS controls.
6. Non-pregnant HbAA controls.

Inclusion criteria: Women aged between 18 and 44 years with regular and known menstrual periods who were non-smokers and without known renal, metabolic or cardiac disease.

Exclusion criteria: Women who had received or donated blood or with a history of sickle cell crises in the three months prior to the study were excluded. Those with known hypertension and those on prostaglandin-based medication were also excluded.

It was calculated that 15 pregnant SCD women and 15 age and parity matched pregnant HbAA controls would give an 80% power of reproducing the difference in plasma volume between both groups of about 1 litre, seen at 36 weeks’ gestation in a previous study at the 5% significance level. We had originally planned to study the same women at 16 and 36 weeks’ gestation, which would have been a considerably stronger design. However, recruitment proved difficult, as many women approached did not wish to undergo plasma volume measurements at all, and most
women who agreed to participate were reluctant to commit to two measurements of plasma volume, so the study was re-designed as a cross-sectional one.

An intravenous cannula was inserted into a large forearm vein and a three way cannula was attached. Venous blood was taken into three plain serum separator gel tubes, two ethylenediamine tetra-acetic acid tubes and a lithium heparin tube for the various substances that were assayed. The tubes were then centrifuged at 4°C and the plasma was separated and stored at −20°C until assay or shipment to the UK.

**Measurements**

Plasma volume was measured using the Evans blue dye dilution method using an ultraviolet spectrophotometer with a visible range (Agilent 8453) to measure the absorbances at a wavelength of 610 nm. Known concentrations of Evans blue were prepared using a 1 mg/ml stock solution. Fifteen millilitres of Evans blue dye was injected into the cannula within 1 min and the cannula was flushed thereafter. Five-millilitre samples were then withdrawn at 10, 20 and 30 min after injection, using the same venous line technique previously validated by el-Sayed et al.

Concentrations of plasma renin (expressed as plasma renin concentration (PRC)), plasma angiotensinogen (Aogen), aldosterone (ALD), PROG, PRL and AVP were also measured. All measurements in non-pregnant women were made in the follicular phase of the menstrual cycle.

**Assays**

**Radioimmunoassay.** Plasma samples were air freighted on dry ice to Nottingham, UK for assay of PRC and Aogen concentrations using established radioimmunoassays. Samples were assayed in duplicate, and all samples from individual patients were run in the same assay. The inter- and within-assay coefficients of variation (CoVs) were, respectively, 14.8% and 5.6% for PRC and 13% and 5.1% for Aogen.

**Enzyme immunoassay (EIA).** Serum ALD was assayed with an ALD ELISA kit (catalogue number 1875, Alpha Diagnostic International, Texas, USA). The inter-assay CoV was 6.2% (n=5) and the intra-assay CoV was 3.4% (n=20). The minimal detectable concentration was 15 pg/ml (n=10). AVP was assayed with an EIA kit (catalogue number 900-017, Assay Designs, Michigan, USA). The inter-assay and intra-assay CoVs were 7.8% (n=5) and 6.6% (n=10) respectively. PROG was assayed with an EIA kit (Catalogue number Prol-96, Teco Diagnostics, California, USA). The intra- and inter-assay CoVs were 3.8% (n=10) and 4.7% (n=6) respectively. PRL was assayed with an EIA kit (Catalogue number PROL-96, Teco Diagnostics). The intra- and inter-assay CoVs (n = 10, 10) were 4.5% and 8.7%. Sodium and potassium were analysed by flame photometry using the Jenway Flame Photometer (Jenway, Essex, UK). The intra- and inter-assay CoVs (n = 10, 10) for sodium and potassium were 2.3% and 4.2% and 3.9% and 4.8% respectively.

The power supply in Lagos is unfortunately not reliable even to hospitals, and several power cuts occurred during the course of this study, resulting in the thawing of some stored samples. We analysed samples only where we were sure that they had not been at risk of thawing/refreezing.

**Data handling**

Data were tested for normality of distribution using the frequency distribution analysis. If they were not normally distributed, they were normalised by logarithmic transformation or non-parametric tests were used as necessary. Normally distributed data are summarised and presented as means ± standard deviation, with subsequent analysis by Student’s t-test or analysis of variance (ANOVA) as appropriate. Pearson’s correlation coefficient was used for measurement of association between variables or Kendall’s tau (τ) for trend across groups. The Mann–Whitney U test and Kruskal–Wallis ANOVA were used for non-parametric data, which are presented as median and interquartile ranges.

**Results**

Basal demographic data for HbAA and HbSS women are shown in Table 1. The observed differences in the baseline characteristics of the women were as expected with respect to their haemoglobin phenotypes and pregnancy status.

**Plasma volume**

**Non-pregnant women.** There is discussion as to the most appropriate way of expressing plasma volume in subjects of different build. Non-pregnant women of genotype HbSS had significantly higher plasma volumes than women of HbAA regardless of the correction factor used, that is, per kg (body weight), per unit body mass index (BMI) or per unit body surface area (calculated as described by Mosteller) (Table 2).

**Pregnant women.** As expected, plasma volume rose significantly from the non-pregnant state during pregnancy in HbAA women (Table 2; p=0.003 Kendall’s τ). However, in HbSS women, there was no significant change in plasma volume in pregnancy compared with their non-pregnant counterparts (p=0.4). Since plasma volume rose in HbAA
women, the difference in plasma volume between HbAA and HbSS women was lost during pregnancy from 16 weeks’ gestation.

**Hormone and electrolyte measurements**

The concentrations of measured circulating hormones and plasma sodium and potassium concentrations are shown in Tables 3 (HbAA) and 4 (HbSS). PRL concentrations were significantly lower ($p<0.05$) in non-pregnant HbSS women than in HbAA; none of the other observed small differences between the non-pregnant groups were statistically significant.

PRC, Aogen, PROG and PRL concentrations had all risen significantly by 16 weeks’ gestation in HbAA women, and continued to rise to term (Table 3), when PRC was four-fold higher than in non-pregnant women (Figure 1(a)). Plasma ALD rose significantly by mid-pregnancy in HbSS women, but showed no further rise, unlike in HbAA (Figure 1(a)). The other hormones measured in HbSS women showed a similar pattern of pregnancy change to those seen in HbAA women (Table 4); again, AVP concentrations did not alter, but for logistic reasons (see Methods), numbers measured were small. Plasma sodium concentration fell markedly by the third trimester in HbSS women ($p<0.001$; Figure 1(b)), but not in HbAA women. Plasma potassium concentrations were higher at all times in HbSS women (Tables 3 and 4; $p<0.006$).

### Table 1. Baseline characteristics of the non-pregnant and pregnant study women.

| HbAA (N=39) | Non-pregnant (N=19) | 16 weeks pregnant (N=10) | 36 weeks pregnant (N=10) |
|-------------|---------------------|--------------------------|--------------------------|
| Age, years  | 25.1 ± 4.3          | 28.8 ± 4.7$^a$           | 30.4 ± 4.9$^a$           |
| Parity      | 0 (0, 0)            | 1 (0, 2)$^a$             | 0 (0, 1)$^a$             |
| Weight, kg  | 60.5 ± 9.0          | 74.0 ± 10.3$^a$          | 78.6 ± 10.6$^a$          |
| Height, m   | 1.63 ± 0.06         | 1.66 ± 0.05              | 1.64 ± 0.04              |
| BMI, kg/m$^2$| 22.7 ± 3.3          | 27.0 ± 3.3$^a$           | 29.3 ± 3.4$^a$           |

| Age in years, body weight, height and BMI are reported as mean ± standard deviation. Parity is reported as median and interquartile range in parentheses. |
|-----------------------------------------------------------------------------------------------------------------------------------|
| $^a$Comparison of pregnant groups with non-pregnant, $p<0.05$.                                                                 |
| $^b$Comparison of haemoglobin SS women with haemoglobin AA, $p<0.05$.                                                            |
| N: number studied in each group; BMI: body mass index.                                                                              |

### Table 2. Comparison of plasma volume in pregnant and non-pregnant women.

| HbAA | Non-pregnant (N=19) | 16 weeks pregnant (N=10) | 36 weeks pregnant (N=10) |
|------|---------------------|--------------------------|--------------------------|
| PV, ml | 2165 ± 497         | 2911 ± 1020$^a$         | 3089 ± 1035$^a$         |
| PV/body weight, ml/kg | 36.1 ± 8.3         | 40.1 ± 14.4             | 40.6 ± 16.1             |
| PV/BMI, ml per kg/m$^2$ | 97.1 ± 24.4        | 108.9 ± 37.8            | 107.8 ± 40.0            |
| PV/BSA, ml/m$^2$ | 1308 ± 281         | 1593 ± 564              | 1659 ± 603              |
| HbSS | Non-pregnant (N=25) | 16 weeks pregnant (N=11) | 36 weeks pregnant (N=11) |
| PV, ml | 2714 ± 499$^b$     | 2758 ± 913              | 3003 ± 1382             |
| PV/body weight, ml/kg | 51.1 ± 16.8$^b$    | 50.2 ± 19.7             | 48.9 ± 25.3             |
| PV/BMI, ml per kg/m$^2$ | 131.4 ± 42.8$^b$  | 126.5 ± 51.9            | 129.5 ± 66.0            |
| PV/BSA, ml/m$^2$ | 1762 ± 593$^b$     | 1768 ± 623              | 1801 ± 879              |

Data are reported as mean ± SD.

$^a$Comparison with non-pregnant, $p<0.05$.

$^b$Comparison between HbAA and HbSS, $p<0.005$.

N: number of women studied; Hb: haemoglobin; PV: plasma volume; BMI: body mass index; BSA: body surface area.
Plasma volume in relation to hormone concentrations

Non-pregnant. Plasma volume was significantly correlated with log10 PRC in HbAA women (Figure 2; \( r = 0.583; p = 0.018 \)), but not in HbSS women (\( p > 0.8 \)). Non-pregnant plasma volume was not significantly correlated with any of the other hormones measured.

Pregnant. In HbAA women, plasma volume was only significantly correlated with log10 Aogen (Figure 3; \( r = 0.608; p = 0.010 \)), whereas in HbSS women, plasma volume was significantly correlated only with log10 ALD (\( r = 0.579; p = 0.019 \)).

Pregnancy outcome

HbAA women delivered later than HbSS women (38.6±0.3 compared with 37.3±0.4 weeks \( p = 0.01 \)). The birthweight of babies born to HbAA women was, as expected, higher than that of HbSS women (3.3±3.0 compared with 2.7±0.10 kg; \( p < 0.001 \)). Since both gestation age at delivery and maternal BMI were significantly lower in HbSS women, corrected birthweight centiles were calculated using the GROW Bulk Centile Calculator (v. 6.7.7.1) software with the West African coefficient (https://www.gestation.net/cc/about.htm). The mean birthweight centile was lower, but not significantly so, in babies of HbSS mothers (45±6 compared with 59±7; \( p >0.1 \)). Birthweight was inversely correlated overall with plasma volume in babies of HbAA and HbSS women (\( r = -0.504; p = 0.017 \)); the association was strengthened if plasma volume/kg was used (Figure 4; \( r = -0.570; p = 0.007 \)). Birthweight was also positively correlated with PRC (\( r = 0.570; p = 0.013 \)). Univariate ANOVA confirmed the impact of plasma volume/kg and PRC on birthweight (overall \( p = 0.009 \)); genotype did not independently influence birthweight (\( p >0.2 \)).

Discussion

We found that there was increased plasma volume in non-pregnant women with SCD, which did not rise during pregnancy. The hypothesis underlying our work, that this could be related to an abnormality in the RAAS or its response to pregnancy, has been supported by our data. The early rise in PRC was blunted by comparison with HbAA women, and no further increase occurred. The smaller rise in PRC at 16 weeks’ gestation in the HbSS women could be the result of a primary under-activity of renin synthesis or release, so that PRC never rose in response to the challenge of pregnancy, or of an earlier rise, subsequently suppressed by another factor. It is also possible that the already-expanded plasma volume of HbSS women counter-balanced the early stimulus of pregnancy. However, as PRC was raised at 16 weeks, albeit to a lower extent than in AA pregnancy, it is likely that there is an initial sensed arterial under-filling of pregnancy in HbSS women in the early stages, as in HbAA women.17 This reduction in peripheral resistance may have activated the RAAS initially leading to a rise in PRC. As pregnancy progressed, the PRC then failed to rise further. This may be
similar to that which occurs in pre-eclampsia where a deficiency of prostacyclin and/or nitric oxide and/or an imbalance in the PIGF/VEGF/sFlt-1 axis has been identified, contributing to vasoconstriction and the reduction in renin concentration.18

PROG rises markedly from the beginning of pregnancy. Its structural similarity to ALD results in competition for binding sites in the renal distal tubule, and the increased concentrations would cause sodium loss were ALD concentrations not to rise. This is usually secondary to increased renin release in response to sodium load at the macula densa. ALD concentrations were very similar in HbAA and HbSS women throughout, but HbSS women had significantly lower plasma sodium concentrations in the third trimester (Tables 3 and 4), implying an inadequate RAAS response at this time. There may be non-angiotensin II (AngII) dependent stimulation of ALD synthesis and/or release in pregnant HbSS women. This has also been suggested in established pre-eclampsia where although both ALD and renin are relatively low,40 some authors have found the ALD reduction to be proportionally less than that of renin. However, Aogen concentrations were very similar at all stages in HbAA and HbSS women. Aogen can become rate-limiting in human pregnancy,41 but under conditions of hypoxia, such as occur in pre-eclampsia, the proportion of oxidised:reduced Aogen is increased, allowing more effective cleavage by cell-bound renin.42 Hypoxia is a common feature of SCD. A recent systematic review and meta-analysis of more than 26,000 women with SCD and an equivalent number of controls showed that women with SCD had a significantly greater risk of developing pre-eclampsia (relative risk 2.43; 95% confidence interval 1.75, 3.39).22 None of our study subjects developed pre-eclampsia.

Small size at birth is associated with problems both in infancy and in adult life – the ‘Developmental origins of adult disease’ concept.45 Babies can be absolutely small (clinically, usually regarded as being below 2.5kg) or small relative to such factors as the mother’s parity, ethnicity and build and gestation age at delivery. As is usually reported, the babies of the HbSS mothers were smaller, in absolute terms, than those of HbAA mothers. However, when the birthweights were corrected to centiles for the West African population, using well-established software that takes into account the mother’s build and parity as well as the usual obstetric and neonatal factors, the HbSS babies were not significantly smaller. This is not to overlook the impact of small absolute size at birth on neonatal outcome, but does suggest that the foetus of the HbSS mother is relatively protected while in utero. The significant inverse correlation between plasma volume and birthweight (Figure 4) was unexpected, since an expanded plasma volume is regarded as necessary for adequate foetal growth. However, the positive association between PRC and birthweight and PRC and plasma volume (Figure 2) may indicate a more complex interaction that cannot be unravelled without a considerably bigger sample size.

**Figure 1.** (a) Plasma renin concentration rose throughout gestation in haemoglobin AA (HbAA) women ($p = 0.0001$; Kendall’s τ); it had risen by 16 weeks’ gestation in HbSS women, but rose no more. (b) Plasma sodium was not changed in pregnancy in HbAA women ($p >0.6$), but fell significantly in HbSS women ($p = 0.018$ Kendall’s τ).
In conclusion, we have shown that, in HbSS women, plasma volume is expanded before pregnancy, and fails to expand further during pregnancy. This is associated with a blunted renin release, but maintained Aogen concentrations, which might be sufficient, under the oxidative stress of SCD, to maintain circulating AngII concentrations, and hence account for the maintained ALD concentrations. It is also possible that pregnant HbSS women have a generalised increase in peripheral resistance in late pregnancy leading to a reduction in renin synthesis or secretion, but do not reduce their plasma volumes from their pre-pregnant supranormal

Table 4. Hormone concentrations in haemoglobin SS women.

|                      | Non-pregnant             | 16 weeks pregnant       | 36 weeks pregnant       |
|----------------------|--------------------------|-------------------------|-------------------------|
| PRC, ng/ml per h     | 11.81 (7.18, 18.20)      | 21.63 (12.39, 35.17)    | 21.97 (9.27, 34.97)     |
|                       | N=23                     | N=10                    | N=9                     |
| Aogen, μg/ml          | 0.98 (0.46, 1.48)        | 2.26 (1.18, 3.87)       | 3.17 (1.37, 4.46)       |
|                       | N=24                     | N=10                    | N=8                     |
| Aldosterone, ng/ml    | 62.7 (42.6, 104.0)       | 114.6 (71.6, 141.1)     | 186.3 (126.4, 253.4)    |
|                       | N=24                     | N=11                    | N=5                     |
| Progesterone, ng/ml   | 0.6 (0.3, 1.0)           | 22.9 (19.0, 33.8)       | 76.9 (61.9, 83.3)       |
|                       | N=23                     | N=11                    | N=5                     |
| Prolactin, ng/ml      | 35.0 (16.3, 104.3)       | 126.6 (74.6, 168.2)     | 305.2 (227.0, 434.9)    |
|                       | N=25                     | N=11                    | N=5                     |
| AVP, pg/ml            | 3.80 (3.40, 4.73)        | 3.60 (3.40, 4.68)       | 3.17 (1.37, 4.46)       |
|                       | N=22                     | N=6                     | N=4                     |
| Plasma sodium, mmol/l | 142.2±6.2                | 142.1±6.8               | 134.9±5.1               |
|                       | N=22                     | N=9                     | N=10                    |
| Plasma potassium, mmol/l | 4.0±0.5                 | 4.0±0.8                 | 4.1±0.5                 |
|                       | N=22                     | N=9                     | N=10                    |

Data are reported as median (interquartile range).

*p<0.05.

Refers to comparison with non-pregnant, p<0.05.

Refers to comparison with non-pregnant, p<0.01.

Refers to comparison with non-pregnant, p<0.005.

Refers to comparison with non-pregnant, p<0.001.

N: number of women studied; PRC: plasma renin concentration; Aogen: angiotensinogen; AVP: arginine vasopressin

Figure 2. The plasma volume was positively correlated with log_{10} plasma renin concentration (PRC) in non-pregnant haemoglobin AA (HbAA) women (r = 0.583; p = 0.018) but not in HbSS women (p>0.8).

Figure 3. Overall in pregnancy, plasma volume/kg was positively correlated with log_{10} angiotensinogen (logAogen) in haemoglobin AA (HbAA) women (r = 0.608; p=0.01) but not in HbSS women (p>0.9).
levels. There may be an abnormality at either the hypothalamo–pituitary level or in the renal sodium sensing system. To test this latter hypothesis we would need to measure vasodilatory substances such as prostacyclin and nitric oxide metabolites, and also explore the renal function of these women in late pregnancy.

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