

ASSESSMENT OF THE HAEMATOLOGICAL INDICES OF HbAS AND STEADY STATE HbSS SUBJECTS RESIDENT IN ABUJA, NIGERIA.

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ABSTRACT

Sickle cell trait (HbAS) has been regarded as a benign state, whereas there have been reported cases of morbidity associated with HbAS individuals inform of complications and crises. There is an apparent lack of awareness of the likely morbidity of these individuals. This study was conducted to compare the similarities in the hematological Indices of subjects with hemoglobin genotypes AS and Steady State SS. An outpatient population-based study was carried out in Maitama District Hospital, Maitama, Abuja and Kubwa General Hospital, Kubwa, Abuja. The HaemoglobinSS (HbSS), Red Blood Cell count (RBC), Pack Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular HaemoglobinSS (MCH) and Mean Corpuscular HaemoglobinSS SS Concentration (MCHC) and platelet (PLT) were measured. 168 subjects in the age group 10-40 participated in the study, which included 56 HbAS, 46 steady state HbSS and 66 HbAA control. There was no significant differences between the hematological indices of subjects with hemoglobin genotypes AS and Steady State SS in this study. The result of this study will channel the focus of clinicians and researchers to

the likelihood of HbAS being a diseased state and further pay more attention to the health status of HbAS patients.

Key words: HbAS, steady state HbSS, Abuja, hematological indices, assessment.

INTRODUCTION

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates. The steady state HbSS refers to a condition where the sickle cell patient at that point in time and within a period of four weeks is not experiencing an acute painful crisis or any changes due to therapy. Amongst the haemoglobin phenotype that has the makeup haemoglobin S, (HbS), the most common are Haemoglobin AS,(HbAS) and haemoglobin SS (HbSS) (Rees et al., 2010).

Sickle cell trait, HbAS also known as the heterozygous form of HbS, is a haemoglobin morphology where one normal pair, HbA and one abnormal pair of the haemoglobin beta gene, S is inherited. HbSS is a homozygous form of HbS where both beta genes encode haemoglobin S which makes it abnormal. The shape of sickle cells that are meant to be flexible are almost rigid and the red blood cells that are influenced become inflexible, leading to microcirculation block (De Montalembert, 2008). This non-deformability and other adverse effects characterized with sickle cells rarely affect the heterozygous individuals, HbAS, except in rare and extreme conditions such as severe hypoxia. Steady state sickle cell anemia, SCA patients, are known to be free of sickle cell crises or clinical complications. The absence of infection, acute clinical symptoms or acute complicating

factors or crises in at least three month does not occur or present itself in steady state SCA patients (Bookchin and Lew, 1996). A sickle cell anaemia(SCA) patient is said to be in steady state when there is absence of acute complicating factors or acute clinical symptoms, infection and crisis for at least three months. Early recognition and subsequent clinical and haematological assessment of the disease are important and are greatly facilitated by awareness of the patient's steady state. (Bookchin and Law, 1996). Three quarters of sickle-cell cases occur in Africa. An estimation of about 150,000(2%) of newborns are affected by sickle cell anemia. The carrier frequency ranges between 10% and 40% across equatorial Africa, decreasing to 12% on the North African coast and <1% in South Africa. In Nigeria, carrier prevalence is about 20 to 30% (Uzoegwu and Onwurah, 2003), while sickle cell disease which tends to be the major concern affects 2 to 3% of the Nigerian population of more than 160 million (Fleming *et al.*, 1979). A recent large retrospective study in Benin City, South- South Nigeria revealed an SCD prevalence of 2.39% and a carrier rate of about 23% (Nwogoh *et al.*, 2012).

Sickle cell disease is an unhealthy occurrence in individuals with abnormal homozygous haemoglobin variant, HbSS. It can present as Sickle cell crises, which include: Vaso-occlusive crisis, anemic crisis, hemolytic crisis. It has been observed that some individuals with the heterozygous hemoglobin phenotype, HbAS, tend to present with some complications associated with individuals with abnormal homozygous hemoglobin variant, HbSS. This is with regards to the high rate of some conditions found to be associated with HbAS. These include sudden deaths, discovered in athletes

(Quattrone, 2015;Kark, 1987).Other complications have also been found to be associated with HbAS, without affecting the life span of the individual (Shaw and Sharpe, 2010). They include; Diabetes mellitus (Bredrzycki et al., 2006), Renal disorders (Davis Jr, et al., 1995), Vaso-occlusive crises (Saxena et al., 2015).

Contrary to the belief that 'sickle cell trait is generally regarded as a 'benign condition' studies have been able to establish the fact that sickle cell traits may have rare complications(Roach, 2005). These complications are not known to occur in normal state of HbAS patient except in pathological conditions that cause hypoxia, acidosis, dehydration, hyperosmolarity, hypothermia or elevated erythrocyte 2,3-DPG.These imbalances, when exhibited in a HbAS patient can transform silent sickle cell trait into a syndrome resembling sickle cell disease with vaso-occlusion due to rigid erythrocytes. This study, therefore, was carried out to assess the similarities in hematological indices of HbAS and steady state HbSS subjects that leads to both conditions behaving alike in unusual conditions.

Specific Objectives:

- ❖ To confirm the presence of the various hemoglobin phenotypes. This will be by performing hemoglobin electrophoresis for sample participants and checking for the hemoglobin phenotypes of other participants in their hematology profile medical records.
- ❖ To assess the demographic (age and gender) characteristics of subjects.
- ❖ To evaluate all the hematological parameters of hemoglobin AS and steady state hemoglobin SS which

include hemoglobin (HB), red blood cell (RBC), packed cell volume (PCV), mean corpuscle volume(MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC), white blood cell (WBC), neutrophils, eosinophils, basophils, monocytes and platelets using an automated hematological analyzer.

- ❖ To compare the reference values of hemoglobin AS and steady state hemoglobin SS based on their gender, to check for significant similarities.

LIMITATIONS

Some subjects were reluctant to participate in the study due to the involvement of blood sample requirement. In addition, HbSS subjects were reluctant to participate due to stigma attached to the condition, especially subjects that were minor, their parents/guardians were not interested in giving approval for their lads to participate in the research. Also, some medical files that were used did not contain hematology profile results that includes the whole parameters to be used for this study.

In addition, it was difficult to get steady state HbSS patients in the hospital setting since they are usually disease free during that period.

MATERIALS AND METHODS

The study was carried out in Maitama District Hospital, Maitama Abuja and Kubwa General Hospital, Kubwa, Abuja. The patients in this study were from residents of Abuja, Nigeria. The study was approved by Health and Human Services Secretariat, Area 11, Garki, Abuja. Written informed consents were obtained from subjects prior to the study. Consent was also sought from the parents/guardians of

the children recruited into the study by signing a written informed consent agreement. A total of 168 subjects in the age group 10-40 participated in the study, which included 56 HbAS, 46 steady state HbSS and 66 HbAA control.

SAMPLE SIZE

Sample size of 168 subjects were selected using Fisherman's sampling method. Also, considering the limited timeline of the research project practical and low turnout of steady state HbSS in hospitals except they have sickle cell clinic, 46 HbSS were selected for the research and 56 of HbAS were used to compare, while 66 HbAA were used as controls. Patients with pathophysiological states and hematological disorders that alter hematological parameters were excluded from the research.

Hemoglobin phenotypes aside HbAS, and steady state HbSS were also excluded.

INSTRUMENT OF DATA COLLECTION

Tourniquet, syringes, 3parts Helena Titan III hemoglobin electrophoresis machine,
Abacus 380 haemoglobin analyzer, EDTA bottles, gloves, haematology profile medical results/records

METHOD OF DATA COLLECTION

The respondents were classified according to gender and hemoglobin AS, steady state SS and hemoglobin AA individuals. Under all aseptic precautions, 2-3 ml of blood samples were collected by clean venepuncture via the antecubital vein using a plastic syringe with minimum stasis, into commercially prepared concentrations of sequestrene Ethylene Di-amine Tetraacetic Acid (EDTA) bottles. Helena

Titan III Hemoglobin electrophoresis machine was used to determine complete blood counts (CBC) within 2 hours of collection. This is a three-part auto analyzer which analyzed the following parameters Haemoglobin (Hb), Red Blood Cell count (RBC), Pack Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) and platelet (PLT) parameters.

The rest of the data were collected from respondents 'haematology profile medical results/records

STATISTICAL ANALYSIS

Data collected were analyzed using SPSS version 20. Data analysis included descriptive statistics, appropriate test for association, Cross tabulation was done and chi square test was used for association between variables at 5% level of significance. P-value less than 0.05 was considered to be significant.

RESULTS

The demographic parameters of the male and female participants are shown in table-1. Hb concentrations and PCV were lower in steady state HbSS patients in both sexes. The MCV was comparatively high in steady state HbSS patients as compared to HbAS and HbAA participants ($P < 0.05$). MCHC was found to be low in steady state HbSS patients as compared to HbAS and HbAA. However, the difference was not statistically significant. The WBC of steady state HbSS was comparatively higher compared to HbAS and HbAA. The MCH of HbAA, HbAS and steady state HbSS participants fell within a normal range. In HbAS and HbAA subjects, PCV is slightly lower in both sexes. Hb counts of HbAS and HbAA

patients were normal though that of HbAS individuals were slightly lower than HbAA. However, the difference was not statistically significant. The platelet count of HbAS, most HbAA and some steady state HbSS fell within the normal range while few HbAA and some HbSS have an abnormal platelet count. We found low Hb concentration and PCV in steady state HbSS participants as well as HbAS participants as compared to HbAA participants of both sexes. The haematological parameters of male and female participants with HbAA were not statistically significant. The haematological parameters between male and female participants with HbSS were not statistically significant.

Table 1. Demographic Parameters

Variables	Frequency (n= 168)	Percentage (%)	
Gender	Male	60	35.7
	Female	108	64.3
Age (years)	10-15	71	42.3
	16-20	21	12.5
	21-25	27	16.6
	26-30	30	16.7
	31-35	12	7.1
	36-40	8	4.8
Haemoglobin (Hb)	AA	66	39.3
	AS	56	33.3
	SS	46	27.4

Table 1 shows that 60 (35.7%) of the study population were male, while 108 (64.3%) were female. Most of the study population fall within 10-15(42.3%) years age bracket the least was 36-40 (4.8%). Sixty six (39.3%) of the participant were HbAA, 56 (33.3%) HbAS and HbSS was 46 (27.4%)

Assessment of the Haematological Indices of HBAS and Steady State HbSS Subjects Resident in Abuja, Nigeria.

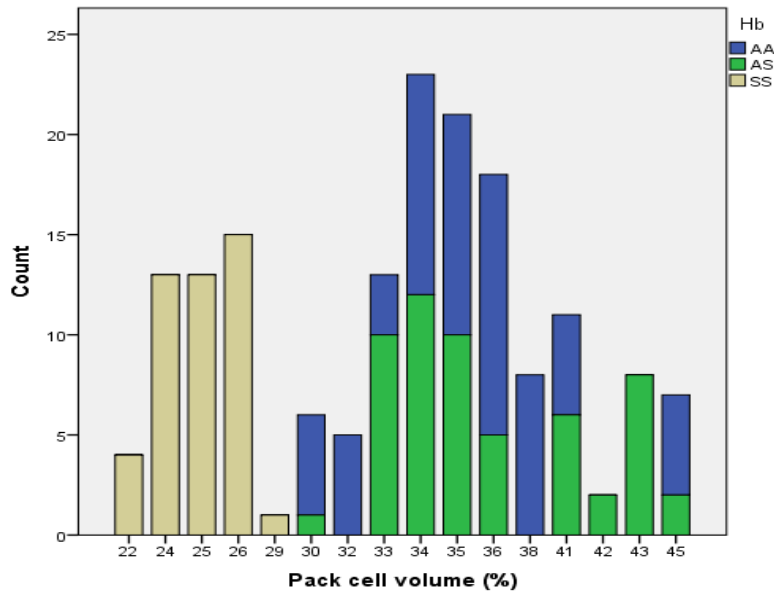


Figure 1 shows the Pack cell volume (PCV) of Haemoglobin (Hb) genotype AA, AS and SS.

The PCV of HbSS participants were 22-29% while HbAA and HbAS participants were from 30-35%.

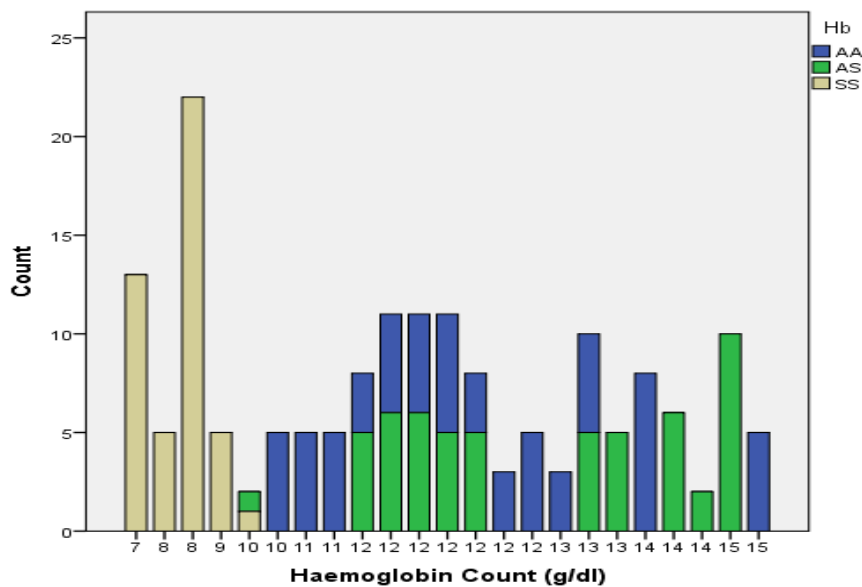


Figure 2: shows the haemoglobin counts of haemoglobin genotype AA, AS and SS.

Most of the participant with HbSS have haemoglobin count of 7-9g/dl while HbAA and HbAS participants have haemoglobin count of 10-15g/dl

Table 2: Platelet Counts in steady state HbSS, HbAS and the control subjects (HbAA).

Variable	Haemoglobin genotype AA	Haemoglobin genotype AS	Haemoglobin genotype SS
Platelet count (x10 ⁹ /L)	Frequency	Frequency	Frequency
<150	8 (4.8%)	0 (0%)	0 (0%)
150-450	58 (95.2%)	56 (100%)	22 (47.8%)
>450	0(0%)	0 (0%)	24 (52.2%)

Eight (4.8%) of the HbAA participants have platelet count of <105 x10⁹/l, 58 (95.2%) have platelet count of 150-450 x10⁹/l and 56(100%) of HbAS participants have the same, 22 (47.8%) of HbSS participants have platelet count of 150-450 x10⁹/l the least 24 (52.2%) platelet count were >450 x10⁹/l.

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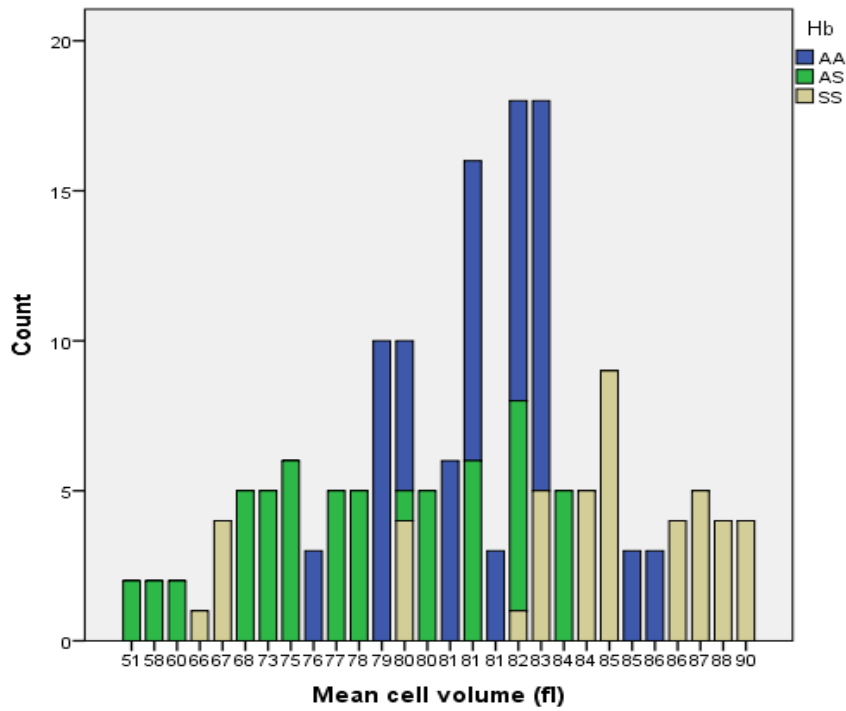


Figure 3 shows the MCV of haemoglobin genotype AA, AS and SS.
Most of the HbSS participants have high MCV.

Assessment of the Haematological Indices of HBAS and Steady State HbSS Subjects Resident in Abuja, Nigeria.

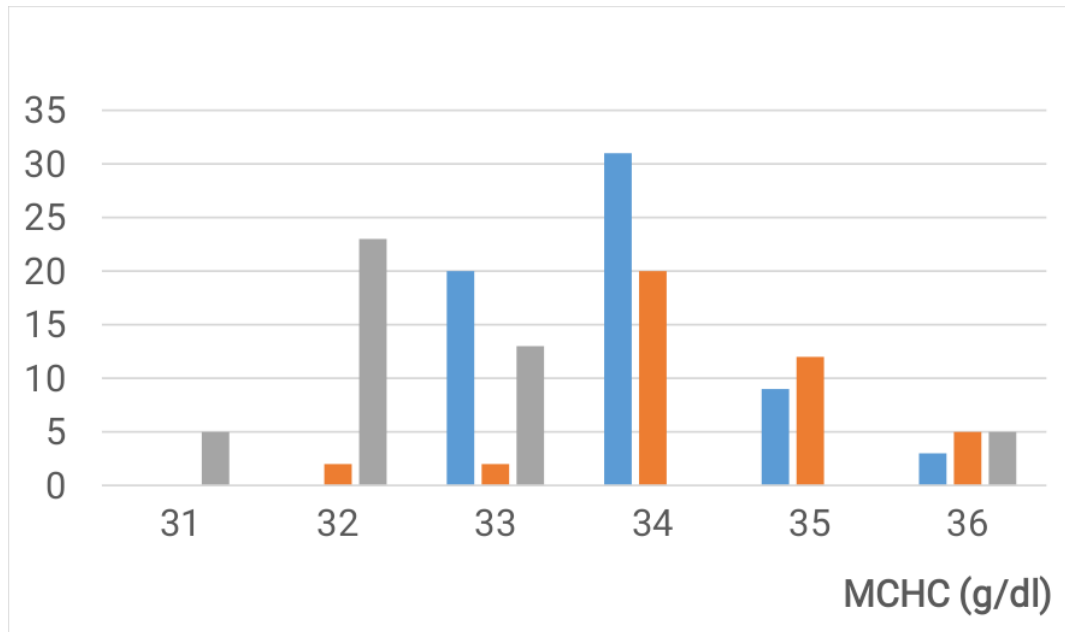


Figure 4 shows the mean cell haemoglobin concentration of haemoglobin genotype AA, AS and SS. Most of HbSS participants have MCHC of 32g/dl while most HbAA and HbAS participants have MCHC of 34g/dl

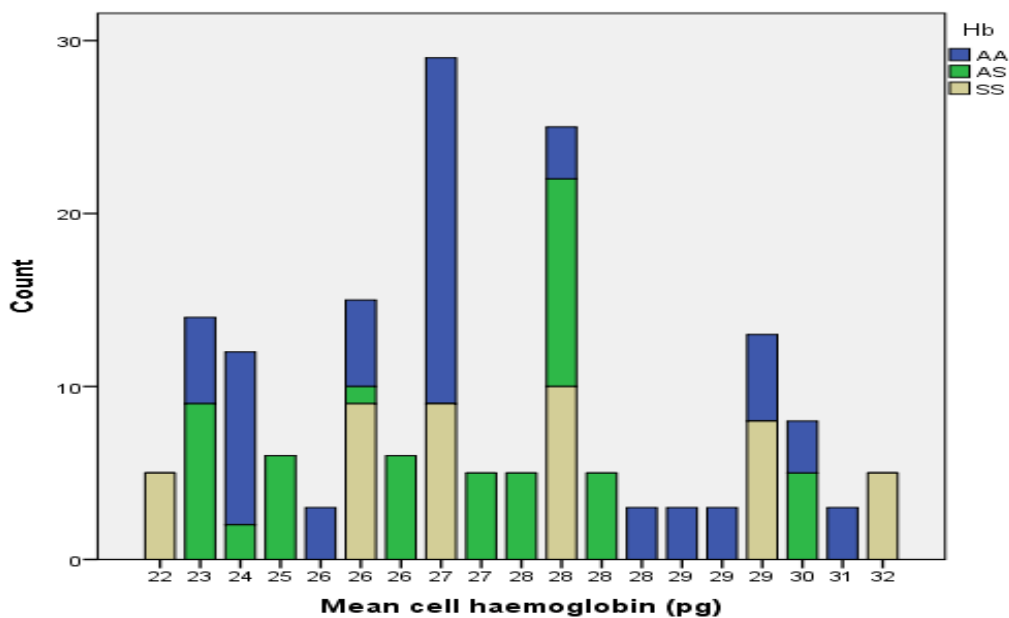


Figure 5 shows the MCH of the participants, HbAA, HbAS and HbSS participants MCH falls within 22-32pg.

DISCUSSION

Sickle cell anemia(SCA) patients are known to experience serious complications and morbidity than sickle cell carriers, also known as HbAS, who are generally believed to live a normal life. Though, several studies have established the classification of HbAS as a diseased state.

The present study is a systematic and honest attempt to analyze the similarities in the hematological indices of HbAS and steady state HbSS, using HbAA as control from residents of Abuja, Nigeria. The results showed variations among the different Hb genotypes according to the various parameters analyzed.

The both sexes of steady state HbSS patients had lower Hb concentrations and PCV, though the Hb concentration and PCV were low in female steady state HbSS patients as compared to male. The sex variation was not statistically significant ($P > 0.05$). In HbAS and HbAA subjects, PCV is slightly lower in both males and females. Hb counts of HbAS and HbAA patients were normal though that of HbAS individuals were slightly lower than HbAA. However, the difference was not statistically significant ($P > 0.05$). Androgens in males may have a relative effect in increased erythropoiesis, which is responsible for higher levels of hemoglobin and erythrocyte count in males, while blood loss in females during menstruation causes decrease in erythropoiesis. These results are in agreement with those of Khan, et al.,(2010) . The hemoglobin concentration, packed cell volume and the relative proportion of haemoglobin fractions in sickle cell anemia patient are dependent on the degree and severity of haemolysis during oxygenation and deoxygenation process associated with

recurrent infections in sickle cell anemic patients (Brittenham et al., 1985). These cells are misshaped, therefore the red blood cells are destroyed causing anemia. This is known as hemolysis. Despite the replenishment of the destroyed red blood cells by the bone marrow, the new created red blood cells do not match the rate of destruction (Kumar et al., 2009). A blunted response to erythropoietin secretion in sickle cell anemia could also be the cause. The extent of its elevated secretion may not be proportional to the degree of anemia. The multisystemic nature of the HbSS patients' bone marrow and kidney can have an adverse effect leading to significant decrease in PCV.

The mean MCV was comparatively high in steady state HbSS patients as compared to HbAS and HBAA participants. However, the MCV was significantly high ($P < 0.05$). MCHC was found to be low in steady state HbSS patients as compared to HbAS and HbAA. However, the difference was not statistically significant. These results are in agreement with those of Shukla and Solanki, (1985), Serjeant and Serjeant, (2001), and Mohanty et al., (2008). The MCH of HbAA, HbAS and steady state HbSS participants fell within a normal range. No gender related difference was seen in MCV, MCH and MCHC values. There is critical balance in maintaining vitamin B12 and folic acid of HbSS patients. Chronic hemolysis or pregnancy in females leads to increase demand in erythropoiesis and causes deficiency which results in macrocytosis (Hayes et al., 1985).

The WBC and differential counts of steady state HbSS were comparatively higher compared to HbAS and HBAA. This result is related to the reports from Ahmed et al., (2006),

Akinbami et al.,(2012). Oxidative stress could be the cause of the increase in WBC..The platelet count of HbAS, most HbAA and some steady state HbSS fell within the normal range while few HbAA and some HbSS have an abnormal platelet count. Underlying chronic inflammation, autosplenectomy and hyposplenism in HbSS patients can be responsible for possible splenic sequestration, absence or reduction of spleen which are the indications of high platelet counts (de Franceschi et al.,2011). This result is in line with the work of Freedman and Karpatkin,(1975) and Omoti, (2005) who recorded high platelet counts HbSS patients both in steady and unsteady state.

Comparing steady state HbSS and HbAS we found low Hb concentration and PCV in steady state HbSS participants as well as HbAS participants as compared to HbAA participants of both the sexes. The hematological parameters of HbAS and steady state HbSS patients were not statistically significant. The hematological parameters of male and female participants with HbAA were not statistically significant. The hematological parameters of male and female participants with HbSS, were not statistically significant.

CONCLUSION

The results of this study shows non-statistical significant difference in the hematological indices of steady state HbSS and HbAS.Sickle cell carriers, HbAS and the steady state HbSS subjects showed mild to moderate anemia. Regular health check-ups and monitoring of hematological profile of HbAS and steady state HbSS may help to guide the clinician to prevent, manage and control morbidity, complications, crises and mortality. The result of this study will channel the

focus of clinicians and researchers to the likelihood of HbAS being a diseased state and further pay more attention to the health status of HbAS patients. It will also prompt researchers to further study on other factors that cause the morbidity in HbAS by using other parameters and procedures. Just as there are steady state and non-steady state HbSS, there is a likelihood of having steady state HbAS and non-steady state HbAS. Therefore, further large cohort studies are needed to determine; the differences between these two states, the clinical complications of the unsteady state and the changes in the hematological parameters along with the factors involved.

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