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RESEARCH ARTICLE

CAN DOPPLER ULTRASOUND BE USED TO PREDICT THE DEGREE OF ANEMIA IN PATIENTS WITH HOMOZYGOUS SICKLE CELL GENOTYPE?

***Chukwuma Ezeike**

Department of Radiology, Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA), Ebonyi State, Nigeria

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ABSTRACT

Introduction: Sickle cell anemia is characterized by chronic red cell hemolysis which leads to chronic anemia. Patients with sickle cell may be asymptomatic (steady-state) even with anemia. One easy and minimally invasive method of assessing anemia is by using the packed cell volume. **Objective**: The study aimed at determining the steady-state packed cell volume among sickle cell patients and to ascertain if Doppler ultrasound can be used to predict the degree of anemia in sickle cell disease. **Methods:** Fifty males and 50 females aged 0-30years with homozygous sickle cell genotype were recruited in this cross-sectional study. Their packed cell volume and Doppler ultrasound parameters (peak systolic velocity, end-diastolic velocity, pulsatility index, resistivity index and systolic-diastolic ratio) were measured and correlated using SPSS version 22. **Results:** The steady-state mean packed cell volume were 23.54% and 24.92% for the females and males respectively. There was no correlation between packed cell volume and Doppler parameters. **Conclusion:** The steady-state packed cell volume was lower in patients with sickle cell anemia than the expected values for the general population. Doppler ultrasound could not be used to predict the degree of anemia in sickle cell patients.

INTRODUCTION

Homozygous Hemoglobin SS genotype (sickle cell anemia) occurs when there is a sickle cell mutation at both β-globin genes [i] Hemoglobin S (Hb S) is the result of a single base pair change, glutamine (positively charged) is substituted for valine (neutral charge), at the sixth codon of the β-globin gene. This actual amino acid substitution was discovered by Vernon Ingram in 1956 [ii]. The hereditary nature of the sickle cell disease was suspected for many generations but was only demonstrated by Dr. James V. Neel in 1949 [iii]. This follows the simple Mendelian principles. If both parents have homozygous SS, all their offspring will be SS. If one parent has a homogenous SS and the other parent is normal, all the offspring have an equal chance of having the sickle cell trait. If both parents have the heterozygous AS trait, the offspring have a 1:2 chance of having the sickle cell trait, 1:4 chance of having a normal AA genotype and a 1:4 chance of having the homozygous SS genotype. This 1:4 chance is true for every pregnancy irrespective of the result of the previous pregnancies [iv]. One of the earliest presentations of sickle cell anemia is hand-foot syndrome (dactylitis) which typically occurs within the first two years of life, in up to 40% of children. This is followed by bone pain crises in one-fourth of patients. One-fifth of the patients present with splenic sequestration which is associated with reduced pack cell volume [v].

***Corresponding author:* Chukwuma Ezeike,**
Department of Radiology, Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA), Ebonyi State, Nigeria.

Decreased solubility of the blood is the key pathophysiological effect of sickle cell disease. This is worse in a low oxygen medium and it leads to increased viscosity of the blood. The role of deoxygenation in sickling was discovered in the 1920s by Hahn and Gillespie [vi]. In severe deoxygenation, a solid gel or a pseudocrystalline structure known as "tactoids" can be formed following polymerization of the Hb S molecule [vii]. The greater the concentration of the Hb S, the more easily tactoids are formed.**Error! Bookmark not defined.** This leads to an alteration of the shape of the red blood cells, increased rigidity of the red blood cell membrane and hence reduction of its lifespan. Factors that promote sickling include low oxygen tension, decreased pH, increase in temperature, increase in age and increased intracellular Hb S concentration. In vivo observations have shown that red blood cells in sickle cell disease are capable of repeated sickling and unsickling. This depends on the degree of deoxygenation and reoxygenation [viii]. However, after several cycles, some red blood cells lose their ability to return to normal shape even after exposure to oxygen. These red cells are called irreversibly sickled cells (ISCs), which are seen in peripheral blood smears. Some relationship has been demonstrated between the ISC counts and some of the features and complications of the disease [ix]. Like anemia. The ISCs easily undergo hemolysis leading to chronic anemia of sickle cell which may necessitate a repeated blood transfusion. The packed cell volume and

hemoglobin level are indirectly related to the severity of hemolysis [x]. Most of the patients with sickle cell anemia are stable and symptom-free much of the time. This is referred to be a steady-state and this steady-state can be interrupted periodically by a hemolytic crisis. The splenic artery is a very important vessel in sickle cell anemia owing to the spectrum of splenic changes that can occur in sickle cell disease. That is why it is the vessel of choice for this study. The study aimed at prospectively evaluating the Doppler parameters of the splenic artery in patients with sickle cell anemia and correlate it with their packed cell volume (PCV) to determine the possibility of using Doppler ultrasound to predict the packed cell volume. Doppler ultrasound is non-invasive when compared to the traditional methods of testing the packed cell volume which involves needle pricking of the patients.

METHODOLOGY

This was a cross-sectional study was carried out amongst patients with sickle cell anemia who attended the Sickle Cell clinic of Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria from march to august 2019. The patients were scanned in the Radiology Department of the same institution. Ethical approval was obtained from the Hospitals Ethics and Research Committee. Study subjects were selected by simple random sampling from the sickle cell clinic. Informed consent was obtained from older patients while assent was obtained from parents and caregivers of the children who were below 18years. demographic information was collected using structured questionnaires. The inclusion criteria were patients aged 0 to 30years with laboratory-confirmed hemoglobin ss genotype (hemoglobin electrophoresis) who were crises free with no blood transfusion in the past two months. With the aid of a laboratory scientist, specimens for PCV were collected. The PCV was estimated using an automatic hematocrit analyzer.

The Doppler ultrasound scans of the splenic artery were done by the researcher alone to eliminate variability that could arise between different operators. A curvilinear transducer of 3.5MHz of a Mindray diagnostic ultrasound system DC- N6 (Shenzen Mindray Bio-Medical Electronics Co. Ltd, October 2009). The study subjects were positioned in the right lateral decubitus position or the supine position on the examination couch after exposing the abdomen from the xiphisternum and to the pubic symphysis (superior to inferior). The left arm was positioned away from the abdomen and Coupling gel applied. The splenic artery Doppler peak systolic velocity (PSV), end-diastolic velocity (EDV), pulsatility index (PI), resistivity index (RI) and systolic/diastolic ratio (SD) ratio were measured at one centimeter from the hilum of the spleen. Breath-holding methods were employed in some cases. A less than 60 degrees insonation angle was used with a sample volume of two millimeters and a pulse repetition frequency (PRF) of 4kHz. The measurements obtained were documented in the datasheet. The names of the subjects and places of residence of the participants were excluded from the datasheet. Participation was voluntary and counseling was done before the procedure. Information obtained during this study was used for research purposes only. Statistical Package for Social Sciences (SPSS) for Windows, Version 22.0 was used for analysis.

RESULTS

Table 1a. Demographic data

Sex	AGE (years)	
Females	Mean	17.24
	Median	18.00
	Mode	18
	Std. Deviation	7.201
	Range	25
	Minimum	3
	Maximum	28
Males	Mean	16.98
	Median	18.00
	Mode	22
	Std. Deviation	7.787
	Range	26
	Minimum	3
	Maximum	29

P value =0.863

Table 1b. Demographic data

	Females	Males	
Age(years)	N (%)	N(%)	Total N
0-4	3(6)	4(8)	7
5-9	7(14)	7(14)	14
10-14	6(12)	8(16)	14
15-19	13(26)	9(18)	22
20-24	12(24)	12(24)	24
25-29	9(18)	10(20)	19
Total	50	50	100

Table 2a. Summary statistics showing sex and PCV

SEX		PCV (%)
Females	Minimum	14.95
	Maximum	34.01
	Median	24.95
	Range	19.06
	Mean	23.54
	Std. Deviation	4.78
Males	Minimum	15.55
	Maximum	38.34
	Median	25.87
	Range	22.79
	Mean	24.92
	Std. Deviation	5.35
Both sexes	Minimum	14.95
	Maximum	38.34
	Median	25.03
	Range	23.39
	Mean	24.23
	Std. Deviation	5.097

P-value is 0.01

DISCUSSION

Generally, the PCV and the other red cell indices can be related by the following: **Error! Bookmark not defined.** MCHC (g/dL) = (HGB ÷ PCV or HCT) x 100, MCV = (HCT/RBC) x 10, MCH = (HGB/RBC) x 10, PCV = HGB x 3. The mean PCVs from this study were 23.54% and 24.92% for the females and males respectively. The combined mean PCV was 24.23% for both sexes. This is lower than the expected average PCV among healthy males and females in Nigeria [xi]. This is not surprising because of the high rate of hemolysis in sickle cell anemia and the blunted erythropoietin production that occurs in sickle cell [xii]. This is similar to the findings of Akinbami et al where about 60% of their patients had PCV between 20-30% and hemoglobin concentration of 7-10 g/dl [xiii].

Table 3. Summary statistics showing age group and PCV

	Female		Male	
Age (years)	PCV(%)	Frequency (%)	PCV (%)	Frequency(%)
0-4	32.67	6.00	36.65	8.00
5-9	19.13	14.00	18.40	14.00
10-14	18.32	12.00	18.94	16.00
15-19	21.66	26.00	25.30	18.00
20-24	27.27	24.00	26.99	24.00
25-29	25.13	18.00	26.72	20.00
Total		100.00		100.00

Table 4. Showing PCV and the Doppler indices

PCV(%)	N	PSV(cm/s) mean ±SD	EDV(cm/s) mean ±SD	RI mean ±SD	PI mean ±SD	S/D RATIO mean ±SD
15-20	28	73.26±3.51	24.62±2.53	0.66±0.03	1.08±0.12	3.00±0.25
20-25	18	74.66±2.63	25.99±3.77	0.65±0.05	1.13±0.10	2.93±0.45
25-30	44	73.27±2.47	25.78±2.93	0.65±0.03	1.11±0.11	2.87±0.29
30-35	4	71.86±4.67	24.86±6.40	0.66±0.07	1.01±0.04	2.97±0.58
35-40	6	70.97±1.97	23.59±3.63	0.67±0.04	1.08±0.16	3.05±0.38

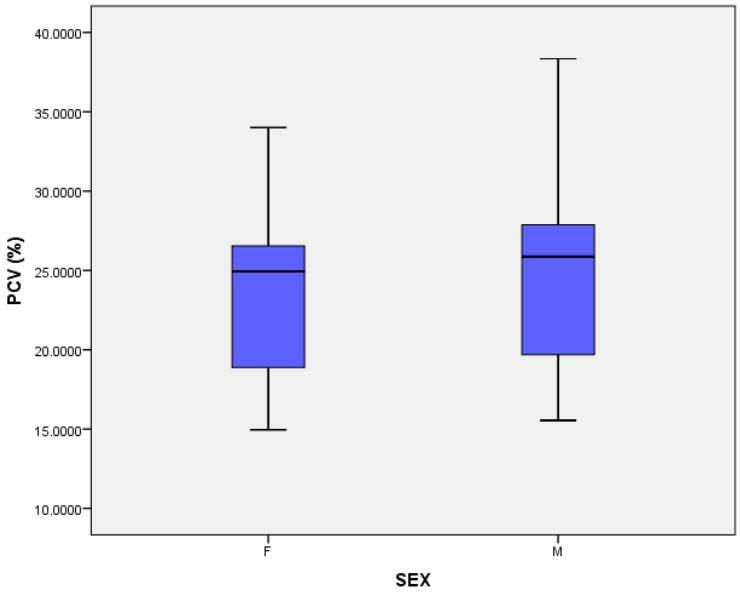

Fig 1. A box of PCV and sex

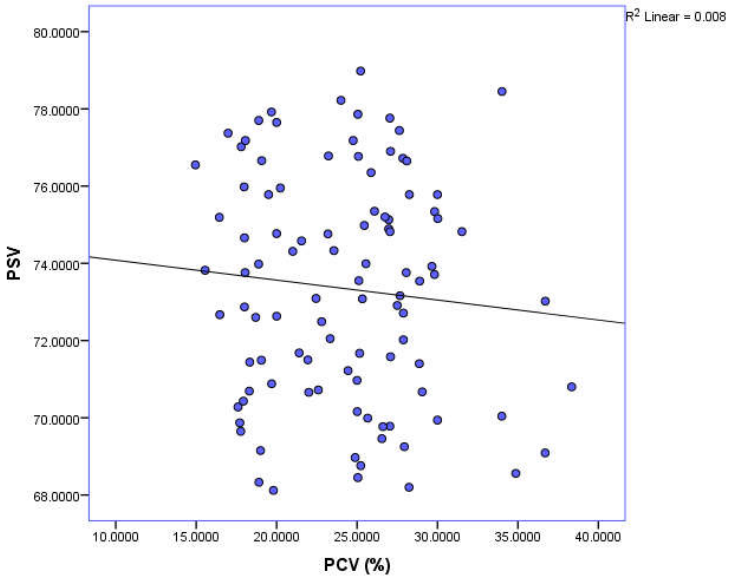

Fig. 2. A scatter plot showing the relationship between PSV and PCV

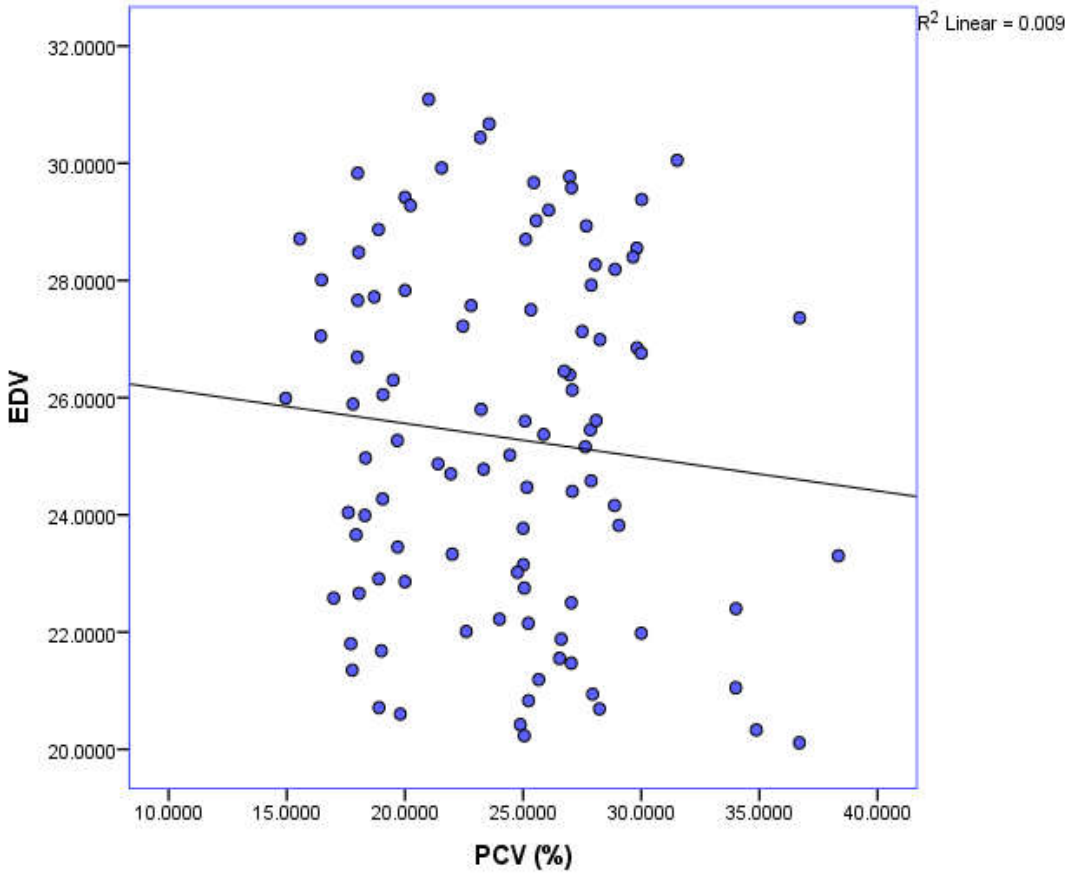

Fig 3. A scatter plot showing the relationship between EDV and PCV

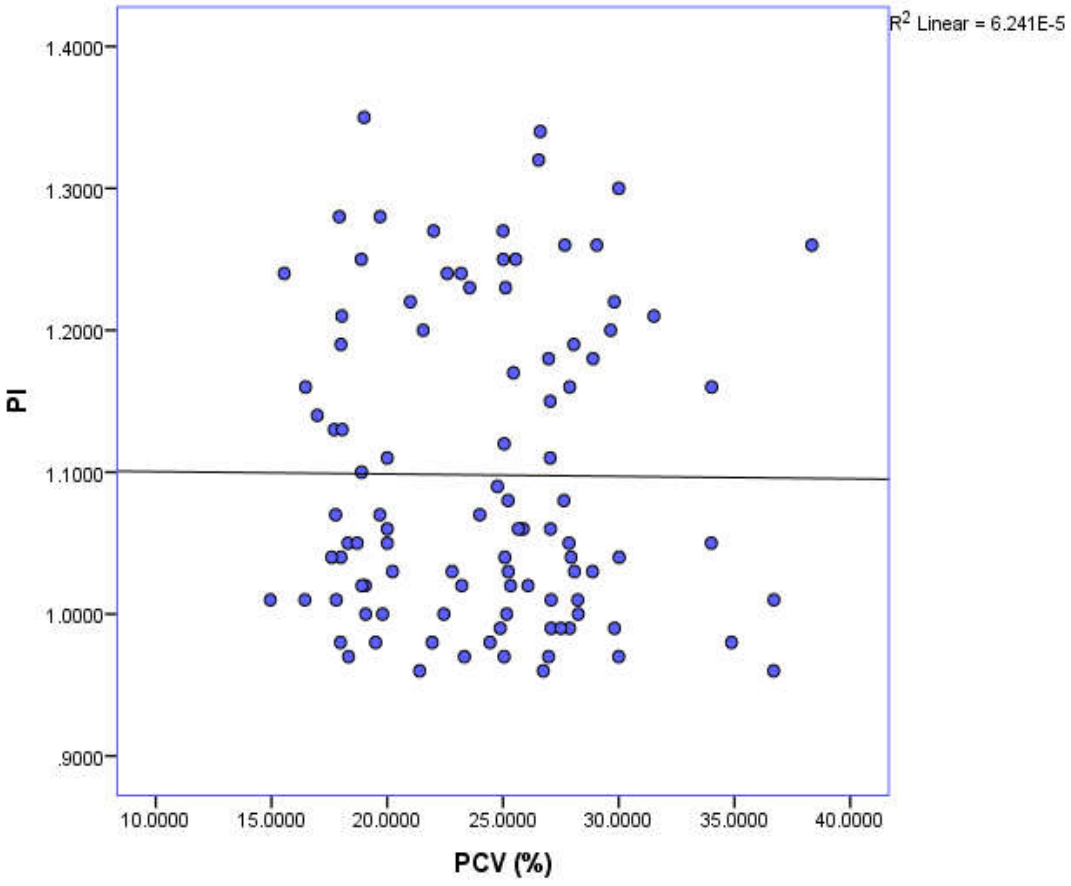

Fig. 4. A scatter plot showing the relationship between PI and PCV

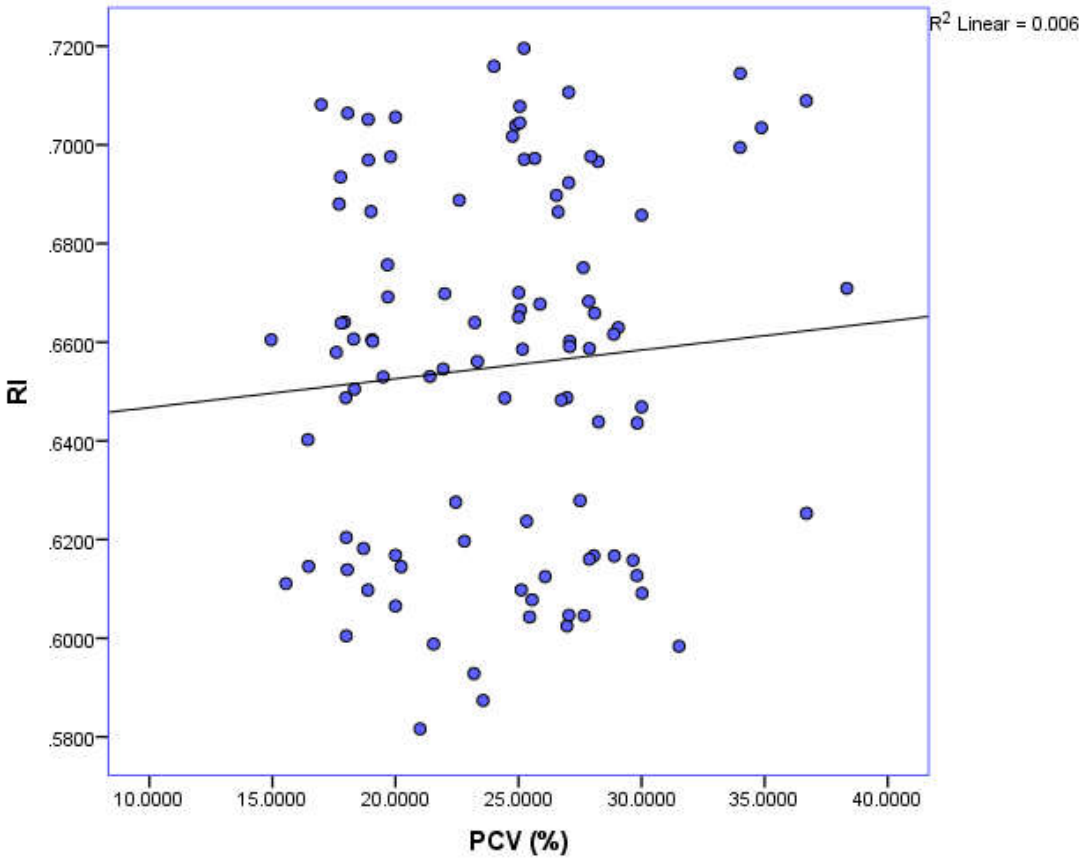

Fig 5. A scatter plot showing the relationship between RI and PCV

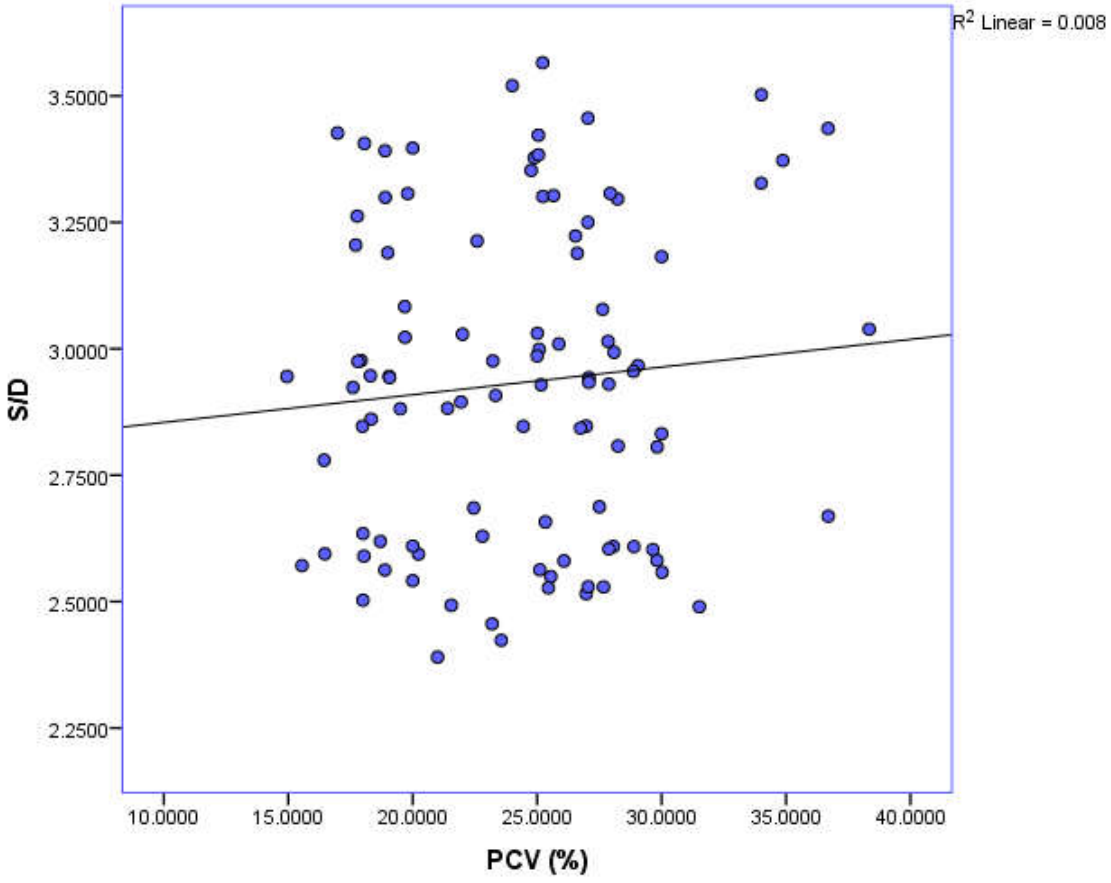

Fig. 6. A scatter plot showing the relationship between SD and PCV

Other previous studies done in Nigeria amongst subjects with sickle cell anemia showed similar findings [xiv,xv,xvi,xvi]. Similar values were obtained from studies outside Nigeria by Antwi-Boasiako et al and Mombo et al [xix]. The highest PCV from this study was among the 0-4year age group with a mean PCV of 32.67% and 36.65% for the females and males respectively. The lowest PCV was 18.32% for the females of 10-14years age group and 18.40% for males of 5-9years age group. The reasons for variations in the severity of sickle cell anemia among different individuals is still debatable up till now. It is generally believed that persistence of the fetal hemoglobin (Hb F) favors higher PCV while some sickle cell haplotypes tend to present with more aggressive symptoms [xx, xxi, xxii].

In this study, the mean PCV in females (23.54%) was lower than the mean PCV in males (24.92%). This is not unexpected. In adult males, androgens cause an increase in erythropoiesis while monthly menstruation and low iron load in adult females are believed to contribute to lower their PCV [xxiii]. In this study, the differences in PCV between the males and females are statistically significant with a p-value of 0.01. From this study, changes in PCV was not associated with statistically significant changes in the values of the Doppler indices in patients with Hb SS. The correlation coefficients are -0.090, -0.097, -0.008, 0.083 and 0.089 for the PSV, EDV, PI, RI and S/D ratio respectively with the P values are 0.373, 0.337, 0.938, 0.412 and 0.378 for the PSV, EDV, PI, RI and S/D ratio respectively. Other studies show a correlation between PCV and Doppler parameters in contradistinction with the findings of this study. Mari et al demonstrated a decrease in the pulsatility index of the renal artery in nine anemic fetuses (less than 30 weeks, menstrual age) soon after intravascular transfusion. Mari et al [xxv] in a pre and post intrauterine transfusion study also found that the fetal middle cerebral artery velocity and pulsatility index are indicators of fetal anemia. In 68 red blood cell iso-immunized pregnancies with the mean velocity of blood in the fetal aorta measured by pulsed Doppler ultrasonography, and the fetal hemoglobin concentration determined in blood samples obtained by cordocentesis, Nicolaides et al [xxvi] found that there was a significant positive correlation between the aortic mean velocity and the hemoglobin deficit. Rightmire DA et al [xxvii] also demonstrated that Doppler blood cell velocities measured in the aortas, inferior vena cavas, and umbilical veins of fetuses are related to the hematocrit levels of the fetal blood determined at fetoscopy. The difference between these studies and the current study could be because they used fetal in-utero subjects with none confirmed as sickle cell anemia, while the current study was in living subjects between 3-30 years who are confirmed sickle cell anemia patients.

Conclusion

The steady-state PCV in patients with homozygous sickle cell genotype in southeast Nigeria is lower than the expected average PCV among healthy Nigerians. Doppler ultrasound could not be used to predict the degree of anemia in patients with sickle cell anemia.

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Conflict of Interest statement: None

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