International Journal of Scientific Research and Management (IJSRM)
||Volume||12||Issue||08||Pages||1119-1129||2024||
Website: www.ijsrm.net ISSN (e): 2321-3418
DOI: 10.18535/ijsrm/v12i08.mp01

Sickle Cell Disease Pain and Zinc, Any Link? A Case-Control Study among Patients With Sickle Cell Disease In Korle-Bu

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Abstract

Background: Over some few decades, markers of pain have been studied, of which some trace elements are of no exception. Yet, there is paucity of data on trace elements in SCD pain. Here, zinc levels and its relation to SCD vaso-occlusive pain was shown.

Aim: To determine [zinc] and its relation to pain in sickle cell disease patients.

Methodology: The case-control study was located at the Ghana Institute of Clinical Genetics (sickle cell clinic). After obtaining ethical clearance from College of Health Sciences (CHS-Et/M.1-P5.12/2023-2024), a validated pain assessment questionnaires were used for data collection. Ten (10) mls of blood was collected, six (6) mL was placed in a serum separating tube (SST) for the determination of zinc and four (4) mL into EDTA tube for full blood count and Hemoglobinopathy on cellulose acetate electrophoresis. The data analysis was done using the Statistical Package for the Social Science (SPSS) version 21 and Microsoft Excel 2016.

Results: Average zinc levels for the entire SCD VOC, was 2.569±1.073(39) and this was relatively low during comparison. [Zn] in SCD subjects who consume alcohol yielded p – value of 0.0001 while in patients experiencing VOC had a p-value = 0.000. The odds ratio for zinc exposure seemed to have protective effect on sickle cell disease either in steady state or vaso-occlusive state. [Zn] stratifications also related statistically to blood pressure readings, SCD pain, hemoglobin, red blood cells, white blood cells, platelets and body mass index.

Conclusion: The average [Zn] in SCD individuals experiencing VOC was relatively low (p-value = 0.000).

Keywords: zinc, trace elements, Sickle cell disease, atomic absorption spectroscopy.

Introduction

Pain is among the common complications of sickle cell disease (SCD) and the major reason SCD patients attend the emergency department or hospital to seek medical care around the world and this is of public health concern. There has been an alarming incidence over several decades till date [1].

Using information from the published SCD worldwide prevalence map by the World Health Organization, about 20-25 million people worldwide have homozygous SCD with approximately 12-15 million in Sub-Saharan Africa [2]. More than 200,000 babies are born in Africa with sickle cell disease being 80% of all the children born in sub-Saharan countries such as Ghana, Nigeria and Gabon [3]. In West Africa, Ghana has the second highest prevalence of SCD. SCD affects about 2% of all Ghanaian newborns annually while the sickle cell trait affects 25-30% of the population [4].

SCD is hereditary and it is recognized by chronic hemolysis with acute or chronic painful vaso-occlusive crises following hypoperfusion and organ failure [5]. These events challenge clinical settings as clinicians try managing total pain in SCD patients. Based on some unmet challenges of bone pain in SCD, it is now evident that zinc levels determination in SCD patients could provide an added clue when explored extensively.

Studies have indicated how zinc supplements [6] decrease the frequency of infections [7, 8] and painful crises in SCD; while other studies have well indicated that SCD patients are likely to be deficient in zinc [5], thus benefitting from the supplement. Zinc deficiency is suggested to be widespread among SCD patients and has been linked to increased demand, chronic hemolysis and use as well as secondary excretion in the urine. Despite suggestions that zinc therapy may lessen the discomfort of painful crisis, zinc deficiency has not been definitively linked to the onset or intensity of Vaso-occlusive crisis [9].

Studies have showed that zinc supplements decrease vaso-occlussive crises in SCD [6]. Routine oral zinc supplementation lowers the prevalence of diarrheal disease and diarrhea-related mortality in individuals at high risk of zinc deficiency [10]. The increased incidence of infections in individuals with SCD with zinc deficiency may be due to the impairment of T-helper cell function which leads to a reduction in IL-2, there may also be an impaired B-cell function [11]. However, a randomized control trial conducted by Namazzi et al., [12] showed that children with sickle cell anemia (SCA) receiving low doses (10mg per day) of zinc or placebo experienced the same rate if there were serious or invasive illnesses such as Sepsis, malaria and tonsillitis.

As it is now, mixed evidences exist on the beneficial role of zinc supplementation in people with sickle cell disease. Evidence from some studies show that provision of zinc supplements for about one year increased the zinc levels however there was no significant difference in hemoglobin levels and body mass index of both the study population and control group [6]. Another study conducted by Ugwu et al., [13] also suggests that there is a positive association between zinc and hemoglobin. In a systematic review by Lui et al., [14] indicated zinc supplement improve growth outcomes such as height, weight and head circumference but not in pregnancy. The presence of these mixed evidences warrants the need for further research to elucidate the direct association between zinc and sickle cell disease pain.

Aim

To determine zinc levels and its relation to acute vaso-occlusive pain crisis in sickle cell disease patients.

Methodology

Study Design

Case-control study; since the focus was on ‘pain’ and ‘no pain’ status.

Study Site

The study was performed at the Ghana Institute of Clinical Genetics (sickle cell clinic), Korle-Bu, Accra.

Study Population

The study involved sickle cell disease patients attending the Ghana Institute of Clinical Genetics (sickle cell clinic) and control subjects at the blood bank, Korle-Bu.

Inclusion Criteria

- Patients with ages 13 years and above were included as well as those in their steady state (based on Ballas [15] classification) and those with acute vaso-occlusive pain crisis.

Exclusion Criteria

The following were excluded from this study:

- Patients who had blood transfusion 3 months ago and or declined to respond to the questionnaire were excluded from the study.

Sample Size Determination And Sampling Strategy

For precision level in outcome of interest, Cochran formula was used; $n = [z^2(p)(1-p)]/e^2$. At 95% confidence interval, z =1.96, desired precision ‘e’ of 0.05, and SCD prevalence ‘p’ being 0.02, the minimum sample size was calculated.

For hypothesis testing among groups, the formula below was used:

$$n = (\frac{r+1}{r})\ \frac{\sigma^2(Z_\beta + Z_{\frac{\alpha}{2}})^2}{(difference)^2}$$

Where:

n= sample size

r+1/r = ratio of the controls to the cases

σ = standard deviation of the outcome variable

Z_β = Desired power (typically 0.84 for 80% power)

$Z_{\alpha/2}$ = level of statistical significance (typically 1.96)

difference = effect size

therefore, if:

r+1/r = 0.37 since the ratio of the controls to the cases was about 1:2.7

Difference in mean=3.2, σ= 12.54, then n= 44.55 samples in each group.

A 10% factor of lost by attrition was included (4.45 samples), making anticipated total sample size of 48.99. However, on the field, seventy-two (72) controls were obtained. Also, all other factors and prior confounding variables were controlled by stratification. Therefore, the case population was supposed to be 132.3, but on the field, 158 samples were obtained, while the control population was 72, who were gender - matched, hence giving a total sample size of 230.

Sampling Strategy
A convenient sampling method was used for data collection.

Data Collection, Tools And Methods
After obtaining ethical clearance from College of Health Sciences (CHS-Et/M.1-P5.12/2023-2024) and patient's consent, visual analogue pain rating (that allowed subjects to rate their pain on a 10-point scale) as well as venous blood samples were taken for analysis of full blood count, hemoglobin electrophoresis and assessment of zinc levels. These are briefly described below:

Blood processing
Four mL of blood for complete blood count was collected into EDTA tube which was used to determine the full blood count of participants while the other 6mL of blood for zinc determination was taken by laboratory assistants at the study sites using a 10ml syringe into serum separating tube (SST). The blood samples were safely transported in an ice chest packed with ice to the laboratory**.** After allowing the SST blood to coagulate at room temperature, it was centrifuged for five minutes at 3000rpm. The serum was kept in a refrigerator at -22℃ while awaiting analysis.

Cellulose acetate electrophoresis
This involved the migration of hemoglobin on cellulose acetate membrane (57mm x 140mm 0.22um) in a buffer in an electric field at an alkaline pH. EDTA whole blood sample was centrifuged; red blood cells obtained and washed 3 times in 0.85% saline and once in distilled water. Cellulose acetate membrane was marked and made wet with the buffer prior to the start of the experiment. Red blood cells were placed at vantage points on the membrane; voltage and time were then set for the electrophoretic run. The migration of hemoglobin was determined by comparison to a reference hemoglobinopathy.

The Atomic Spectrophotometer for assessment of zinc levels

The atomic absorption spectrophotometer was based on the principle that atoms in a sample absorb specific wavelengths of light, allowing for the quantitative analysis of elements by measuring the amount of absorbed radiation.

In carrying out this study, the atomic spectrophotometer at the Ecolab in the University of Ghana, Geography Department was used to determine the concentration of zinc in both patients' samples and control. Ecolab standards of operation protocol was carefully adhered to the latter.

Data Handling, Analysis And Presentation

The data taken was treated as PHI (protected health information) and analyzed using the software Statistical Package for the Social Science (SPSS) version 21 and Microsoft Excel 2016. The mean and standard deviation for numerical data was determined and student T-test and Analysis of Variance (ANOVA) were used for statistical association between means and three or more mean values respectively. The odds ratio was also determined during the analysis of data.

Results

Study Participants

The study investigated 158 sickle cell disease (SCD) patients and 72 control group (Fig. 1, Fig. 2).

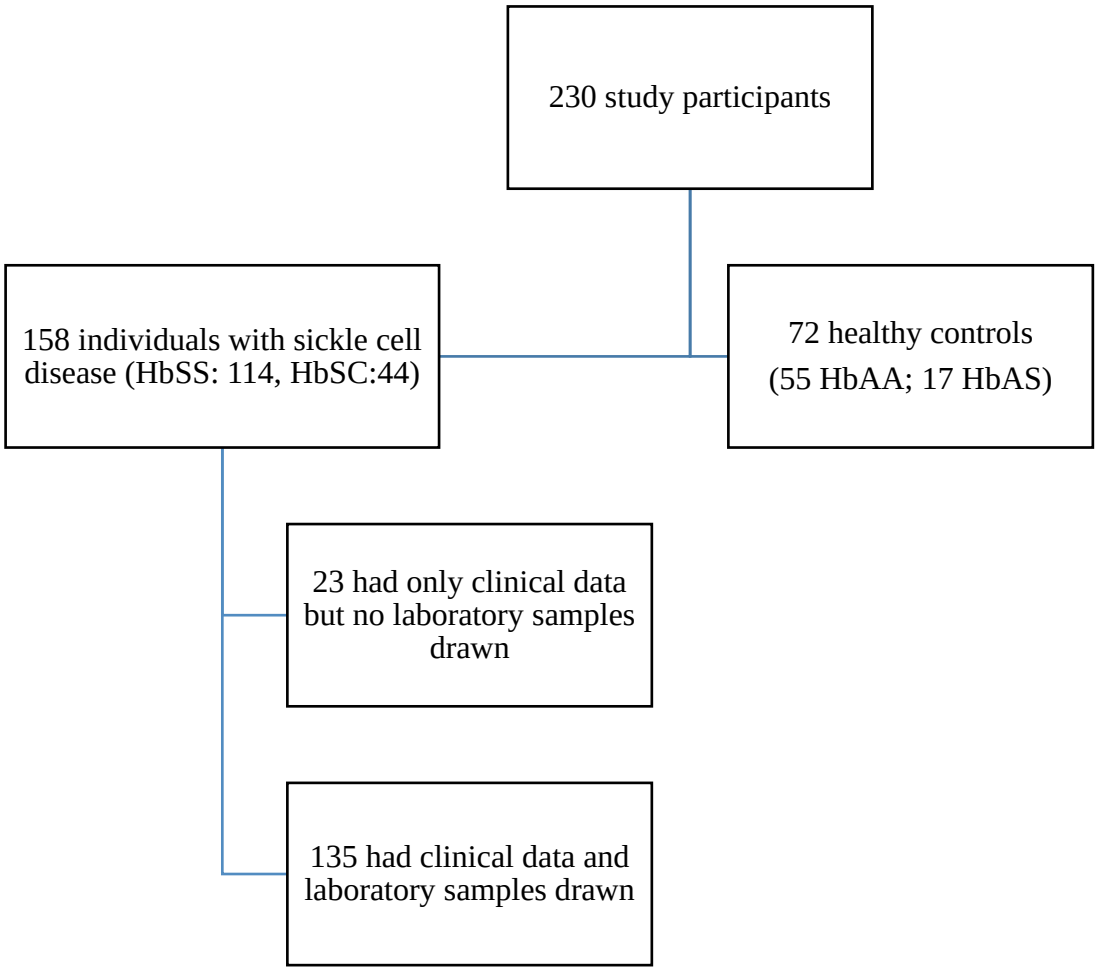

Fig. 1: Flowchart for study participants used in data analysis.

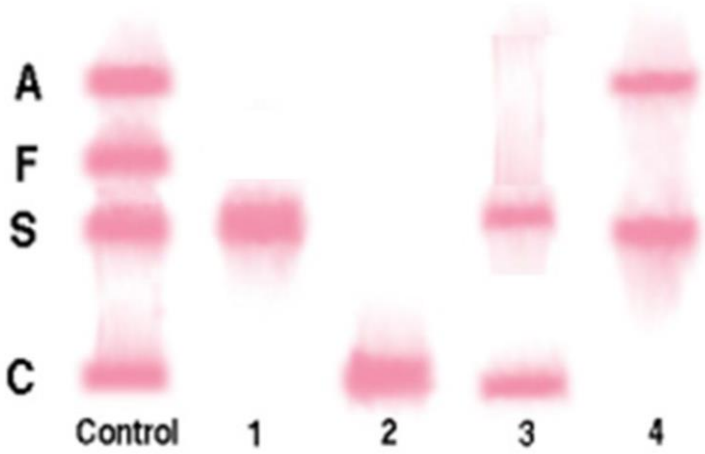

Fig. 2 Cellulose acetate electrophoretogram for the hemoglobinopathy identified in this study

Socio-Demographics

Females (62.7%) and tertiary level of education (49.4%) were prominent findings. The mean age for the SCD group was 29.65±12.20(158) while the mean age for the control group was 26.31±9.04(72). Majority of subjects in both SCD and controls never smoked. However, few SCD subjects (18.7%) have taken alcohol before.

Zinc Levels

Average zinc levels for the entire SCD VOC, SCD steady state and control subjects were 2.569±1.073(39), 2.503±1.072(91) and 1.403±1.390(72) µg/ml respectively while the average [Zn] in SS individuals experiencing VOC was 2.357±2.121(28) µg/ml. Reference range for [Zn] was 0.7 to 1.8 µg/ml (Yokokawa et al., 2020).

General Characteristics

Statistical significance was seen in heart rate, pain rating, blood pressure readings, hemoglobin levels, red blood cells and white blood cells after zinc level stratification (Table 1) and gender matched analysis (*supplementary Tables 1 and 2*). Unique statistical finding was found in systolic blood pressure under low zinc level stratification while body mass index had a statistical significance under increased zinc level stratification (Table 1).

Table 1: General study characteristics stratified by zinc levels

	Zn levels <1.8µg/ml							Zn levels >1.8µg/ml						
	Controls		**Steady state**		**VOC**			**Controls**		**Steady state**		**VOC**		
	$AA_{(37)}$	**$AS_{(9)}$**	**$SS_{(16)}$**	**$SC_{(9)}$**	**$SS_{(8)}$**	**$SC_{(2)}$**	**p-value**	**$AA_{(18)}$**	**$AS_{(8)}$**	**$SS_{(63)}$**	**$SC_{(22)}$**	**$SS_{(23)}$**	**$SC_{(11)}$**	**p-value**
Age	28.70±11.10	27.67±9.25	32.75±12.07	31.22±15.06	23.50±7.03	20.5±0.71	0.384	22.44±1.54	22.38±1.41	27.76±10.93	33.32±16.29	29.64±9.50	36.18±15.36	0.005
Education status (tertiary)	28 (76%)	7 (8%)	7 (44%)	2 (22%)	5 (63%)	1 (50%)	*Frequencies <5 made chi test and Yate's correction inappropriate	18 (100%)	8 (100%)	30 (48%)	15 (68%)	15 (65%)	3 (27%)	*Frequencies <5 made chi-suare test and Yate's correction inappropriate
Marital status (married)	8 (22%)	2 (22%)	15 (94%)	3 (33%)	0	0		18 (100%)	8 (100%)	12 (19%)	5 (23%)	2 (9%)	5 (45%)	
Alcohol	8 (22%)	0	3 (19%)	0	5 (63%)	0		4 (22%)	0	7 (11%)	5 (23%)	5 (22%)	2 (18%)	
Religion (Christian)	35 (95%)	9 (100%)	15 (94%)	8 (89%)	8 (100%)	2 (100%)		18 (100%)	8 (100%)	54 (86%)	18 (82%)	22 (96%)	9 (82%)	
BMI (kg/m^2)	25.84±3.94	25.29±5.85	22.81±3.63	21.76±3.93	16.95±7.65	21.92±63.50	0.162	25.28±5.14	26.29±6.26	20.68±4.74	23.06±3.13	22.16±3.30	23.04±6.02	0.001
SBP	81.57±4	89.07±	115.6	116.7	110.1	110.5	0.00	115.50	112.38±	113.35±	117.68±1	114.71±1	121.18±	0.331

(mmHg)	4.07	40.43	9±10.31	8±16.87	3±13.58	±17.68	8	±9.76	7.80	11.96	4.04	2.94	8.21	
DBP (mmHg)	93.30±23.01	94.44±26.333	71.38±11.59	69.78±15.64	65.25±8.68	63.5±21.92	0.000	75.89±5.74	70.38±4.57	70.03±11.38	72.22±12.49	71.50±13.42	74.82±12.25	0.000
HR (bpm)	74.30±8.02	73.78±11.42	82.13±7.44	79.67±9.45	88.00±12.24	86.00±5.66	0.001	78.61±6.65	74.38±7.01	84.74±8.80	81.50±10.18	88.29±9.93	87.18±11.80	0.000
Temp (ºC)	33.73±7.81	36.78±0.42	36.54±0.31	36.5±0.37	36.43±0.30	36.95±0.21	0.358	36.52±0.26	36.45±0.20	36.40±0.29	36.44±0.28	36.48±0.31`	36.6±0.49	0.353
Pain rating	0.35±1.53	0.56±1.67	1.19±2.64	2.78±2.86	5.38±2.92	5.25±0.35	0.000	0.28±0.96	0.00±0.00	0.97±1.74	1.00±1.63	6.18±2.07	6.09±2.07	0.000
Hb (g/dl)	13.40±2.82	12.60±2.14	8.64±1.61	10.77±1.42	8.13±0.80	11.75±0.07	0.000	12.70±1.14	11.83±1.81	7.79±1.21	10.39±1.32	8.08±1.54	10.02±0.59	0.000
Rbc (10^6/mm^3)	5.06±1.09	4.87±0.65	3.17±0.84	4.09±0.64	2.84±0.57	4.55±0.60	0.000	4.86±0.56	4.76±0.54	2.82±0.47	3.98±0.80	2.72±0.64	3.78±0.35	0.000
Wbc (10^3/mm^3)	4.89±2.05	4.21±1.85	9.17±3.30	6.42±1.60	14.15±5.47	5.90±0.57	0.000	3.35±2.19	2.83±1.35	9.91±2.92	7.54±1.97	14.97±16.29	8.26±2.56	0.000
Plt (10^3/mm^3)	192.00±147.98	198.93±140.68	349.27±105.46	266.78±81.57	515.63±290.62	225.50±72.83	0.000	217.28±74.74	251.29±52.86	417.35±141.19	284.76±78.87	443.30±134.50	273.90±126.09	0.000
MCV (µm^3)	80.45±8.92	77.00±7.79	82.13±10.15	77.89±10.75	86.25±10.98	79.5±10.61	0.390	76.83±4.71	73.29±6.85	81.90±10.89	78.71±7.54	88.74±10.12	79.30±7.12	0.000

Legend: *mean ± sd SBP = systolic blood pressure DBP= diastolic blood pressure HR= Heart Rate Hb= Haemoglobin wbc= White blood cell Plt= Platelet MCV= Mean corpuscular volume rbc= red blood cell VOC= vaso-occlussive crisis

Supplementary Table 1 **Gender – matched characteristics in steady state and VOC**

	Males								Females							
	Steady state				**VOC**				**Steady state**				**VOC**			
	$SS_{(30)}$	**$SC_{(11)}$**		**p-value**	**$SS_{(12)}$**	**$SC_{(5)}$**	**$SF_{(2)}$**	**p-value**	**$SS_{(49)}$**	**$SC_{(20)}$**		**p-value**	**$SS_{(24)}$**	**$SC_{(8)}$**		**p-value**
Age	29.34±12.29	37.91±17.29		0.085	29.08±8.75	23.40±6.60	28.55±14.85	0.488	28.41±10.72	29.85±14.45		0.650	27.88±9.70	40.95±15.70		0.021
BMI (kg/m^2)	19.89±4.70	22.38±3.83		0.124	20.20±1.79	19.82±3.39	20.30±0.51	0.950	21.41±5.36	22.85±3.18		0.266	21.60±5.83	24.56±6.30		0.255
SBP (mmHg)	116.93±4.70	122.72±17.27		0.187	116.75±13.04	114.20±12.07	119.00±15.56	0.891	112.00±12.35	114.50±12.48		0.378	112.17±13.03	122.88±7.22		0.041
DBP (mmHg)	70.51±13.73	74.82±16.23		0.401	71.83±11.98	67.60±18.09	83.00±7.07	0.423	70.18±9.85	69.70±11.67		0.862	69.25±13.03	76.50±9.36		0.175
HR (bpm)	85.24±7.43	79.00±16.22		0.097	84.72±9.53	91.20±10.13	76.00±8.49	0.183	88.59±9.17	82.05±8.08		0.011	89.96±10.41	84.38±11.16		0.229
Temp (ºC)	36.44±0.34	36.55±0.33		0.361	36.42±0.32	36.96±0.54	37.02±0.57	0.034	36.42±0.27	36.40±0.29		0.892	36.50±0.31	36.42±0.32		0.553
Hb (g/dl)	8.19±1.65$_{(21)}$	11.61±0.79		0.000	8.90±1.42	11.14±0.60		0.004	7.89±1.18$_{(43)}$	9.95±1.06$_{(20)}$		0.000	7.70±1.34$_{(15)}$	9.7±0.35$_{(7)}$		0.001
Rbc (10^6/mm^3)	2.87±0.67	4.26±0.79		0.000	3.10±0.50	4.23±0.44		0.001	2.92±0.55	3.88±0.70		0.000	2.59±0.61	3.67±0.38		0.000
Wbc (10^3/mm^3)	9.32±3.20	7.84±1.63		0.091	11.38±3.00	7.70±2.67		0.032	9.94±2.91	6.89±2.00		0.000	16.37±17.03	7.99±2.58		0.216
Plt (10^3/mm^3)	383.67±154.86	260.10±76.2		0.019	394.60±135.82	273.40±72.74		0.087	410.04±126.81	289.00±80.10		0.000	494.05±196.40	258.71±167.47		0.006
MCV (µm^3)	83.86±8.79	82.5±8.48		0.677	84.40±11.05	81.20±7.19		0.563	81.02±11.42	76.45±7.87		0.111	89.86±9.57	78.00±7.44		0.009
Zinc (µg/ml)	2.57±1.39$_{(20)}$	2.48±1.05$_{(10)}$		0.858	2.32±0.78$_{(9)}$	2.64±1.16		0.879	2.53±0.89$_{(41)}$	2.46±1.18$_{(12)}$		0.902	2.37±0.75$_{(19)}$	3.88±1.73$_{(5)}$		0.007

Legend: *mean ± sd SCD = Sickle Cell Disease HR= Heart Rate Hb= Haemoglobin wbc= White blood cell Plt= Platelet MCV= Mean corpuscular volume

Zinc, Alcohol Intake And Voc In Scd Subjects

Student's t-test analysis between SCD subjects who take in alcohol yielded a significant finding (Table 2) likewise zinc levels in patients experiencing VOC (Table 2).

Table 2: Zinc stratification, VOC and alcohol intake in SCD subjects

	Zinc(µg/ml)		p-value
	≤1.80µg/ml	**≥1.80µg/ml**	
[Zn] in patients experiencing VOC	$1.40\pm0.25_{(11)}$	$3.03\pm0.90_{(29)}$	0.000
[Zn] in those who take Alcohol	$147\pm5.8_{(10)}$	$293.5\pm74.8_{(17)}$	0.000

Supplementary Table 2 **Gender – matched characteristics in controls and VOC**

	Males					Females				
	Controls			**VOC SCD cases**		**Controls**			**VOC SCD cases**	
	$AA_{(38)}$	**$AS_{(9)}$**	**p-value**	**$SCD_{(19)}$**	**Control X VOC p-value**	**$AA_{(17)}$**	**$AS_{(8)}$**	**p-value**	**$SCD_{(32)}$**	**Control X VOCp-value**
Age	28.61±10.90	27.44±9.32	0.768	27.53± 8.68	0.908	22.29±2.39	22.63±1.85	0.726	30.98±12.45	0.006
BMI (kg/m^2)	25.66±3.90	25.49±5.47	0.914	20.11±2.14	0.000	25.65±5.38	24.36±4.09	0.554	22.36±5.99	0.145
SBP (mmHg)	88.62±44.44	118.80±6.53	0.050	116.32±12.33	0.007	103.31±22.11	108.12±8.17	0.560	114.84±12.64	0.052
DBP (mmHg)	92.11±21.37	93.56±27.23	0.863	71.89±13.49	0.002	77.53±15.62	71.38±4.34	0.290	71.06±12.65	0.239
HR (bpm)	74.66±7.51	71.44±11.01	0.298	85.53±10.09	0.000	78.06±8.20	77.00±6.41	0.751	88.56±10.71	0.000
Temp (°C)	34.10±7.43	36,70±0.43	0.304	36.64±0.49	0.205	36.41±0.17	36.5±0.26	0.309	36.49±0.31	0.576
Hb (g/dl)	13.24±2.18	13.23±1.21	0.990	9.47±1.57	0.000	12.99±2.91	11.19±2.06	0.131	8.21±1.47	0.000
Rbc ($10^6/mm^3$)	5.07±0.88	4.98±0.61	0.775	3.38±0.81	0.000	4.81±1.10	4.65±0.54	0.702	2.86±0.73	0.000
Wbc ($10^3/mm^3$)	4.44±2.12	4,43±1.44	0.989	10.16±3.21	0.000	4.81±1.10	2.60±1.52	0.000	14.27±15.17	0.006
Plt ($10^3/mm^3$)	177.27±136.18	184.93±132.66	0.879	345.75±130.25	0.000	254.50±86.73	265.29±53.08	0.750	435.21±211.56	0.001
MCV (μm^3)	78.89±8.77	79.71±2.13	0.748	84.00±9.82	0.109	79.98±5.52	70.57±7.89	0.002	86.89±10.37	0.000
Zinc (µg/ml)	0.87±1.06	1.65±1.85	0.096	2.38±0.89	0.000	2.12±1.37	2.13±1.40	0.987	2.69±1.17	0.252

Legend: *mean ± sd SBP = DBP= HR= Heart Rate Hb= Haemoglobin wbc= White blood cell Plt= Platelet MCV= Mean corpuscular volume

Determining Odds Ratio

Using the reference range for zinc (0.7-1.8 µg/ml), only one SCD subject was zinc deficient. Therefore, testing the hypothesis that if you are an SCD patient, then you are truly Zinc deficient was redefined to include zinc exposure using 1.8 µg/ml as the cut off. The odds ratio for zinc exposure (*See supplementary Tables 3a, b, c*) appeared to have protective effect on sickle cell disease either in VOC state or steady state

Supplementary Table 3a**: Establishing the odds when SCD and controls are compared**

	SCD	Controls	
≥1.80µg/ml zinc levels	92	26	Odds ratio = 4.522 CI = 2.441– 8.373 p-value =<0.0001
≤1.80 µg/ml zinc levels	36	46	

***Supplementary Table 3b*: Establishing the odds when VOC and Steady state are compared**

	VOC	Steady state	
≥1.80µg/ml zinc levels	28	66	Odds ratio = 0.964 CI= 0.418-2.224 p-value =0.932
≤1.80 µg/ml zinc levels	11	25	

***Supplementary Table 3c*: Establishing the odds when VOC and controls are compared**

	VOC	Controls	
≥1.80µg/ml zinc levels	28	26	Odds ratio = 4.504 CI= 1.930-10.507 p-value =0.0005
≤1.80 µg/ml zinc levels	11	46	

Discussion

The current study presented a novel insight in zinc level stratification and showed statistical significance among SCD subjects with VOC (p-value =0.000), among SCD patients who take in alcohol (p – value = 0.0001) and some selected hematological profile in SCD pain.

Despite the growing evidence of the role of zinc in pain modulation, mechanisms still remain unclear [16, 17]. For instance, in some painful conditions, zinc deficiency [13] has been suggested to decrease hemoglobin levels, increase white blood cells and increase platelets. However, we show for the first time that, this notion was rather observed in both increased and decreased zinc stratifications in the current study, implying a plausible different mechanism in SCD pain.

SCD pain reflects a mixed pain state with patients experiencing peripheral sensitization, neuropathy, central sensitization of the central nervous system, while others experience peripheral nociceptive [18] input (vaso-occlusion, sickling, and mediators of inflammation). Nociceptive pain has been indicated to increase following a zinc deficiency in animal studies [17].

As established previously that SCD patients were zinc deficient [9]; a record of zinc supplementation was noted in the current study for SCD subjects. Simply put, SCD subjects were already exposed to zinc before the commencement of the project. However, the calculated odds ratio for zinc exposure seemed protective in sickle cell disease.

The study seemed to refute Ugwu et al., [13] on the positive association between zinc and hemoglobin in SCD subjects alone; perhaps, because SCD patients were already exposed to zinc supplementation. Increased zinc requirement due to SCD hemolysis [9] seem to be the basis for zinc deficiency in SCD. Zinc deficiency affects T-helper1 (TH1) role [19], IL-2 (interleukin - 2) production [20] and cell mediated immunity [7].

There seem to be a high proportion of apparently normal zinc levels and some outliers in the study population with females experiencing VOC and having statistical significance in zinc levels (p = 0.007). The interplay between zinc deficiency and gender seemed quite controversial; since possible factors such as alcohol consumption, malnutrition and zinc supplementation status [21] could be confounders that needed to be controlled. However, in this study, all SCD subjects were on zinc supplements while few SCD subjects take in alcohol and analysis stratified to control this confounder (alcohol) yielded statistical significance (p = 0.0001).

Aside the fact that alcohol consumption enhances oxidative stress, SCD itself creates a high oxidative burden. SCD oxidative stress affects caspase 3 activation and RBC membrane [22]. The elevated free Hemoglobin levels in the Fenton reaction [23], higher autoxidation of Hb S [24], and recurrent ischemia-reperfusion injury [25], do generate free radicals. Also, pro - inflammatory response induced by constant monocytes and neutrophils recruitment likewise cause complications [26]. Neutrophils are part of granulocytes; and monocytes are part of non-granulocytes. Both granulocytes and non-granulocytes make up

white blood cells (WBC) [27]. In this study, WBC was elevated in SCD subjects, an indication of an inflammatory [28] process occurring.

Vast experimental studies have suggested impeccable evidence of zinc as an anti-oxidative stress agent targeting NF-κB [29], PPARs [30] and Nrf2 [31] in the body. NF-κB is induced by lipid peroxidation [32]. Yet, lipid peroxidation in liver and red blood cells normally results in the production of reactive oxidant species. Zinc thereby inhibits lipid peroxidation [33] in order to stabilize bio-membranes [34] and it is by these effects reduces vaso-occlusive crisis [5].

The study population's characteristics and gender-matched data conformed to the existing literature that SCD subjects were relatively lean [35] and had unique hematological profile. Leanness may be related to retarded growth brought about by perhaps, zinc deficiency (Table 1; [5].

Limitations

- Hormones and gut microbiota are a necessary information when assessing zinc status, additional study is needed.

Conclusion And Recommendation

Average [Zn] in SS individuals experiencing VOC was relatively low with a p-value of 0.000 among the entire SCD subjects experiencing VOC, as well as a p – value of 0.0001 among SCD patients who take in alcohol. The odds ratio for the exposure of zinc appeared protective in sickle cell disease either in VOC or steady state.

Recommendation

- A more diverse cohort on zinc status, taking into consideration, hormones and microbiota may be helpful.

Acknowledgement

The authors duly acknowledge the staff of the Sickle Cell Clinic, Korle-Bu, Accra, Ghana and Medical Biochemistry, Univ. of Ghana Medical School.

Funding disclosure: This work was financed by AGK and EVA.

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The authors declare that there is no conflict of interest.

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