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Effect of intestinal helminth infection on haemoglobin levels and frequency of vaso-occlusive crises in children with sickle cell anaemia (SCA) attending Federal Teaching Hospital, Abakaliki, Nigeria

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Abstract

Introduction: *sickle cell disease is a genetic disorder of haemoglobin characterized by chronic anaemia due to ongoing haemolysis and is frequently associated with recurrent vaso-occlusive crisis (VOC). The affected patients often have impaired immunity and are predisposed to infections. It is known that infections and infestations are important trigger factors towards development of vaso-occlusive crises (VOC) in SCA patients. Aims and objectives: to demonstrate any significant difference in the Haemoglobin levels and frequency of vaso-occlusive crises among children with SCA harbouring intestinal helminthes compared with SCA patients without Intestinal Helminths.* ***Methods:*** *a hospital based cross sectional study was conducted between August and October 2018 in 120 children aged 2 to 18 years with sickle cell anaemia. Frequency of vaso-occulsive crisis in children with or without intestinal helminths was assessed using a structured questionnaire. Stool and Blood samples were collected and analysed using standard methods.* ***Results:*** *the ages of the children ranged between 2 - 18 years, 55.8% were males with a mean age of 8.6 (±4.6SD) years. Females had a mean age of 9.1 (±3.9SD) years. Eleven stool samples contained intestinal helminths. The median haemoglobin of the helminth-infected subjects was 6.5g/dl. There was no significant statistical difference in episodes of vaso-occlusive crises in those with or without helminthic infections (p = 0.822) though statistical difference in the haemoglobin levels appeared significant (p-value 0.010).* ***Conclusion:*** *haemoglobin levels appeared lower in children with intestinal helminthiasis while the frequency of vaso-occlusive crises did not differ.*

Introduction

Sickle cell disease (SCD) is the generic name that refers to a group of inherited haemoglobin disorders characterized by the presence of at least one abnormal HbS in the Hb genotype [1]. Sickle haemoglobin (HbS) results from a substitution of one amino acid (Valine) for another amino acid (Glutamic acid) at position six of the β-globin polypeptide chain [2]. About 100 million people worldwide are affected by the burden of sickle cell disease and it is directly responsible for over 50% of deaths in those with the most severe form of the disease [3]. According to a recent estimate, over 300,000 children are born every year with SCD and over 70% of these births occur in sub-Saharan Africa where majority of them die before the age of 5 years as a result of limited access to good management [3]. In Nigeria, sickle cell disease is one of the ten priority non-communicable diseases (NCDs) and it significantly contributes to morbidity and mortality in children and adults [1]. Because of its population, Nigeria stands as the most sickle cell endemic country in Africa with an annual infant death of 100,000 representing 8% of infant mortality in the country [1]. It is also estimated that about 24% of the Nigerian population have the sickle cell trait [1]. Under deoxygenating conditions, HbSS undergoes marked decrease in solubility, increased viscosity, and polymerization, thereby distorting the cell membrane giving it a sickle shaped appearance [2,4]. With recurrent episodes of sickling, membrane damage occurs and RBCs are no longer able to resume biconcave shape upon reoxygenation [4]. Also with repeated sickling, RBCs become more rigid due to gain of sodium and calcium, and loss of potassium [4]. These rigid nondeformable red blood cells are engulfed by phagocytic cells of the reticuloendothelial system especially the spleen and are subsequently haemolysed.

Vaso-Occlusive Crisis (VOC): this is the commonest manifestation of sickle cell anaemia [5]. It presents with pain and affects almost every part of the body [5]. The pain is a result of ischaemic tissue injury due to vascular occlusion by sickled red cells following chronic haemolysis [6]. This chronic haemolysis is usually accompanied by reticulocytosis which abundantly expresses the alpha-4 beta-1 integrin complex, which binds endothelial VCAM-1 receptors (endothelial adhesion) [2,4,7]. In the pathophysiology of VOC, sickle reticulocytes appear to initiate vascular

occlusion through the process of endothelial adhesion. This effect is heightened by continued red cell sickling and deposition of irreversibly sickled cells due to hypoxia and other factors that cause sickling [8]. Red cell sickling is more prominent during crisis and at even steady state though the rate is lower. Hence, for any factor to initiate VOC, then it must be able to significantly increase the rate of sickling or reduce the rate of un-sickling to such a level that would change the steady state of a patient into a state of crisis [9]. Certain risk factors have been associated with precipitation of vaso-occlusive crisis in SCA patients, these include dehydration, infections/infestations, physical exertions and exposure to extremes of temperature [6]. Research has shown that infections and infestations are important trigger factors towards development of VOC in adult SCA patients. In addition, helminth infected patients who have lower PCV values are also at increased risk of vaso-oclussive crises. These Researchers hypothesized that effects of intestinal helminthic infection may actually be worse in children with SCA not only because of associated ongoing haemolysis but because the worm burden is housed in smaller bodies when compared to adults [10].

The burden of intestinal parasites has been shown to be enormous among children living in the developing world [11]. Coincidentally, majority of the children with sickle cell anaemia reside in sub-Saharan Africa and other developing countries where intestinal helminthic infections are common [12]. Studies in the general population have shown that intestinal helminthic infection is associated with low haemoglobin level and impaired nutritional status of infected patients due intestinal blood and interference with nutrient absorption [13,14]. Helminthiasis is also associated with eosinophilia [15] and this may increase the risk of vaso occlusive crises (VOC) in these individuals. Eosinophils isolated from SCA subjects appear to have significantly greater affinity to fibronectin than eosinophils isolated from non-SCA subjects [16]. Such alteration may increase the role of eosinophils in vaso-occlusive crises especially when eosinophil count is increased. Since eosinophils in subjects with SCA show high cytoadherance, and intestinal helminthic infection is often associated with eosinophilia, it is likely that the presence of intestinal helminth infection will be associated with higher frequency of vaso-occlusive crises in patients. In 1993, Sodipo *et al*. [17] in Lagos tried to demonstrate the frequency of parasitic infections among 150 sickle cell anaemia patients on admission from various SCA crises. They obtained a parasitic infection prevalence rate of 53.1% which they concluded could have served as the possible cause of the various SCA crises especially VOC observed. There is paucity of published data on the frequency of VOCs among sickle cell anaemia patients with intestinal parasites infestation and the role of eosinophils in the pathogenesis of VOCs, hence the need to carry out this study. It is hoped that the findings made out would help in mapping out additional treatment such as routine de-worming sessions for paediatric SCA patients even in their steady state in our environment.

Methods

Study location and site: this was a hospital based cross sectional study conducted at the Federal University Teaching Hospital Abakaliki (FETHA), the major tertiary hospital in Ebonyi State South-Eastern Nigeria located in Abakaliki, the State Capital. The town has a population of 141,438 which is about 6.5% of the total population of the State according to the 2006 census. The inhabitants of the area are mostly of the Igbo ethnic group consisting of mainly traders, civil servants and farmers. The hospital is a 502-bedded facility takes care of the health needs of the Ebonyi people and other persons from neighbouring states. It is made up of so many departments including the sickle cell centre where the study was conducted and where about 130 SCA children come for regular clinic. The study was carried out from August 2018 to October, 2018 involving children aged 2-18 years with sickle cell anaemia recruited consecutively from the Sickle Cell Centre who met the inclusion criteria

during the study period. Folders of selected subjects were tagged to avoid selecting same subject twice. The sample size that was used for this study was determined using the sample size formula for population less than 10,000 [18].

$$S = \frac{N}{1 + \frac{n}{N}}$$

; S- desired sample size; N- number of sickle cell anaemia children in FETHA =130; n- Sample size if number of sickle cell anaemia children in FETHA is > 10,000. To find the value of n, therefore

$$n = \frac{Z^2 qp}{d^2}$$

; Z -standard normal deviate set at 1.96 corresponding to 95% confidence interval; p - prevalence helminthiasis in sickle cell patients 53%(0.53), [15]; q is 1 - p = 0.47; d -degree of accuracy desired, set at 0.5%= 0.05; Substituting accordingly; thus n =382. Therefore, since the minimum sample size (S) is

$$S = \frac{N}{1 + \frac{n}{N}}$$

; n=382, N=130. Substituting accordingly, thus S = 97

The minimum sample size for sickle cell anaemia group will be a minimum of 97 subjects, however to accommodate possible attrition, a response rate of 90.0 percent is anticipated (i.e. attrition rate of 10.0 percent). Thus the calculated sample size Ns [18] was:

$$N_s = n_f + (n_f x 0.1)$$

= 97 + (91 x 0.1) = 97 + 9 = 106. Thus, the calculated minimum sample size was 106. At recruitment, a pretested structured questionnaire was used to obtain information on the child´s bio-data, socio-economic class, use of anti-helminthic drugs and risk factors for intestinal helminths such as; source of drinking water, type of toilet facility, method of hand washing, level of education of subject and their use of foot-wears. Frequency of vaso-occlusive crises in the past 6months was ascertained by direct interviewing of patient and parents/guardians and review of patient´s folders. Blood Samples were collected from SCA subjects in Etylenediaminetetraacetate containers. The samples were then sent to the laboratory for haematological analysis using Blood auto-analyser (Mindray BC-5300 five-part auto-analyser manufactured by Guangzhou Medsinglong Medical Equipment Co. Ltd China) for haematocrit, haemoglobin level, leukocyte count (with differentials) and platelet count. Blood films of each subject were examined microscopically and the leukocyte counts were corrected for the presence of nucleated red cells. The stool samples were collected in clean glass bottles containing 5 mL of 10% formalin-saline solution and sent to the microbiology laboratory. They were subjected to microscopic examination for ova of intestinal helminths using WHO recommended method for stool analysis for identification of parasites, segments, ova, larvae orcysts of helminths (the Kato-Katz technique) [19].

Ethical considerations: ethical approval for the study was obtained from the Research and Ethics Committee of the Federal Teaching Hospital Abakaliki. Written informed consent duly signed were obtained from the parents/guardians. Subjects that had intestinal helminthic infection were given antihelminthic drug.

Data management: the data collated in the questionnaire were entered into Statistical Package for the Social Sciences (SPSS) version 21 for analysis. The results were presented using frequency tables and bar chart. The qualitative data (sex, sociodemographic data, and risk factors for intestinal helminthic infection) were summarized using percentages. Fisher´s exact test (for categorical variables with frequency <5) was used to test the non-association between the categorical variables and presence of intestinal helminths infection [20]. Variables that showed statistically

significant association with presence of intestinal helminths were further analysed using multivariate logistic regression to determine the adjusted odd ratio and confidence intervals. Independent T-test was used to determine if there is any significant difference in mean of the quantitative variable (VOCs) between group A and group B [20]. The level of significance was set at 0.05 (95% confidence interval).

Limitations of study: the small number of subjects with intestinal helminthiasis seen in the study may have actually influenced the result obtained and the conclusions drawn. We thus recommend that further studies be conducted with a larger study population. Other triggers for vaso-occlusive crises were also not considered in the study.

Results

A total of 129 children with SCA were screened for eligibility for the study, stool and blood samples were collected from 120 of them who were eligible and gave consent for the study.

Socio-demographic characteristics of subjects: the males accounted for 55.5% of the total study population while the subjects´ age ranged from 2-18years with the mean of 8.6(±4.6SD) years for the boys and 9.1 (±3.9SD) for the girls. Majority of the subjects (40%) were within 2-6 years of age as shown in Table 1.

Parasite burden and intensity of parasite infection: among those infected, *Ascaris lumbricoides* accounted for 72.7% and hookworms 27.3% of the total parasite burden as they were the only parasites identified. Seventy five percent and 66.7% of the subjects infected with *Ascaris lumbricoides* and hookworms respectively had light infection according to WHO classification [21]. There was no case of mixed infection. (Table 2) *Ascaris lumbricoides*: light infection (1-4999 epg), Moderate infection (5000-49,999 epg), Heavy infection (≥50,000 epg) Hookworms: light infection (1-1,999 epg) Moderate infection (2000-3,999 epg) Heavy infection (≥4,000 epg) epg= egg per gram of faeces. Table 3 below compares the haematological and WBC profiles of infected and non-infected SCA subjects. The median haemoglobin level in SCA subjects with intestinal helminthic infection was 6.5g/dl (range 5.0g/dl -12.4g/dl) while it was 7.9g/dl (range 3.2g/dl- 15.8) in those not infected. This finding is statistically significant (p-value 0.010). The comparison of the median total WBC and differentials between SCA subjects with intestinal helminthic infection and those not infected showed no statistically significant difference.

However, the range of values in all the haematological profiles were wider in the non-infected subjects compared to infected subjects. Table 4 shows analysis of haemoglobin level and WBC count by age group in subjects infected with intestinal helminths and those not infected. Subjects, 2-6years of age who had intestinal helminthic infection had significantly lower median haemoglobin level compared to non-infected subjects in same age group (U=50; p value-0.018). There was no significant difference in the median haemoglobin level between infected and non-infected SCA subjects above 6years of age. Table 4 shows the total white blood cell count in the various age groups (2-6 years, 7-12 years, and 13-18 years). The total WBC was observed to be higher in younger children. There was no significant difference in the median white blood cell counts across the various age groups between those who had intestinal helminthic infection and those who were not infected. There was minimal variation in the range of total white blood cell count in all the subjects (both infected and non-infected subject) across the age groups.

Vaso-occlusive crises in study subjects: Table 5 shows comparison of the mean number of episodes of vaso-occlusive crises between children with SCA who had intestinal helminthic infection and those not infected. There was no statistically significant difference in the mean number of episodes of vaso-occlusive crises between sickle cell anaemia subjects with intestinal helminthic infection compared to those that were not infected (p value

0.822). Episodes of vaso-occlusive crises did not vary so much across the age groups. There was no statistically significant difference in mean episodes of VOCs across the age groups between subjects with intestinal helminthic infection and those who were not infected.

Discussion

Intestinal helminthic infection is known to cause anaemia in affected children [13]. This may worsen the state of children with SCA who in addition, have decreased haemoglobin level due to ongoing haemolysis. Ohiolei *et al*. [20] demonstrated that haemoglobin level in subjects infected with intestinal helminths was significantly lower than the haemoglobin level of non-infected subjects with SCA. The lower haemoglobin level could be attributed to intestinal blood and nutrient loss due to the activities of the helminths in the host intestines [22]. This is similar to the finding of Ahmed and Uraka [10] who also found a significantly low haemoglobin level in SCA subjects infected with intestinal helminths compared to non-infected subjects. The difference in median total white blood cell count in SCA subjects with intestinal helminthic infection compared to non-infected subjects, was not statistically significant. This is also similar to the findings by Ahmed and Uraka [10]. This may due to the fact that leucocytosis in "general" is not the usual response to intestinal helminthic infection, rather eosinophilia may be seen [23]. However, the study result showed no significant difference in eosinophil count in SCA subjects with intestinal helminthic infection compared to non-infected subjects. This may be due to the small number of infected subjects. There was no statistically significant difference in median platelet count between SCA subjects who had intestinal helminthic infection and non-infected SCA subjects. This is also similar to the finding by Ahmed and Uraka [10]. Intestinal helminthic infections are not known to affect the platelet count. The wide range of values of haematological profiles in non-infected SCA subjects compared to infected subjects may be due to the small number of subjects with intestinal helminthic infection.

Definition of vaso-occlusive crisis and contributing factors: elevated leucocytes count (especially neutrophils) is associated with increased adherence of both WBCs and RBCs to the endothelium, and increased blood viscosity, which can lead to stasis, increased production of deoxygenated haemoglobin S and consequent sickling, thus promoting vascular occlusion [24-26]. However from the study, there was no evidence showing any statistical significance between the total/differential WBC count and episodes of VOCs in both the infected and the non-infected SCA subjects. Hence, there was no statistically significant difference in the mean episodes of vaso-occlusive crises (even across the age groups) between infected and non-infected subjects. This is may be due to the small number of subjects with intestinal helminthic infection in this study.

Conclusion

Though there was no significant difference in the mean episodes of vaso-occlusive crises or in the Total/Differential White blood Cell count (precisely Eosinophil count) between sickle cell anaemia subjects with intestinal helminthic infection and those without infection, the mean haemoglobin levels appeared to be affected with the infection particularly in the under-five patients. It is may be worthwhile to consider including de-worming prescriptions in the routine treatment of children with Sickle cell anaemia.

What is known about this topic

- *That infections and infestations are important trigger factors towards development of vaso-occlusive crises (VOC) in SCA patients;*

- *That helminth infected adult SCAs with lower PCV values and high White Blood cells count (particularly eosinophils which have been shown to play a role in triggering VOCs) appear to possess an increased risk of developing VOCs;*
- *That this risk may be worse with children with SCA and helminthiasis because of associated ongoing haemolysis and their higher worm burden.*

What this study adds

- *That there may not be a significant difference in the mean episodes of vaso-occlusive crises between sickle cell anaemia subjects with intestinal helminthic infection and those without helminth infection;*
- *That there might not be any significant difference in the contribution of total/differential white blood cell count precisely eosinophils in the development of vaso-occlusive crises;*
- *However, that mean haemoglobin levels appear to be significantly reduced in SCA paediatric patients under 5 years of age with intestinal helminthiasis.*

Competing interests

The authors declare no competing interests.

Authors' contributions

All the authors have read and agreed to the final manuscript.

Tables

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Table 3: comparison of haematological and WBC profiles between SCA children infected with helminths and those that are not infected

Table 4: comparison haemoglobin level (Hb) across the various age groups between infected and non-infected SCA subjects

Table 5: comparison of means of episodes of vaso-occlusive crises between SCA children infected with helminths and those that are not infected

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Table 1: socio-demographic characteristics		
Socio-demographic variable	**Frequency**	**%**
Age group (in years)		
2-6	48	40.0
7-12	40	33.3
13-18	32	26.7
Gender		
Male	67	55.8
Female	53	44.2
Place of Residence		
Urban	66	55.0
Semi-Urban	14	11.7
Rural	40	33.3
Number of children in the household		
<4	51	42.5
≥4	69	57.5
Position of child in the family		
<4	92	76.7
≥4	28	23.3
Educational level of Child		
Pre-Nursery	13	10.8
Nursery	37	30.8
Primary	30	25.0
Secondary	40	33.3
Educational level of Father		
No formal education	12	10.0
Primary/Junior Secondary	28	23.3
School cert/Grade II	27	22.5
NCE/OND	6	5.0
University Education	47	39.5
Educational Level of mother		
No formal education	6	5.0
Primary/Junior Secondary	31	25.8
School cert/Grade II	45	37.5
NCE/OND	4	3.3
University education	34	28.3
Socio-economic class		
High socio-economic class	30	25.0
Middle socio-economic class	44	36.7
Low socio-economic class	46	38.3

Table 2: species of intestinal helminths		
Type of helminth	**Frequency**	**%**
Ascaris lumbricoides	8	72.7
Hookworm	3	27.3
Total	11	100
Intensity of Infection		
Light		
lumbricoides	6	75.0
Hookworms	2	66.7
Moderate		
lumbricoides	2	25.0
Hookworms	1	33.3
Total	11.0	100.0

Table 3: comparison of Haematological and WBC profiles between SCA children infected with helminths and those that are not infected

Haematological profile	**SCA patients without helminthic infection**		**SCA patients with helminthic infection**		**Man Whitney U-test P-value**
	Median	**Range**	**Median**	**Range**	
Haemoglobin (g/dl)	7.9	3.2-15.8	6.5	5.0-12.4	0.010*
Total WBC (cells/mm^3)	22,000	5,410-54,000	28,000	20,500-34,000	0.075
Platelet count (x103cells/mm^3)	236	82-1,245	318	209-396	0.079
Neutrophil count (cells/mm^3)	9,360	1,927-35,880	10,080	7480-19170	0.471
Lymphocyte count (cells/mm^3)	10,750	1,970-27,540	17,360	7830-26180	0.170
Eosinophil count (cells/mm^3)	330	0-1,130	280	120-500	0.675
Monocyte count (cells/mm^3)	0	0-1,400	0	0-800	0.370
Basophil count (cells/mm^3)	0	0-600	239	0-280	0.056
*statistically significant					

Table 4: comparison haemoglobin level (Hb) across the various age groups between infected and non-infected SCA subjects					
Age group (Years)	**SCA patients without helminthic infection PCV (%)**		**SCA patients with helminthic infection**		**Man Whitney U-test (P-value)**
	Median Hb (g/dl)	**Range (g/dl)**	**Median Hb (g/dl)**	**Range (g/dl)**	
2-6years	7.2	4.3-11.1	6.3	5-6.5	50 (0.018*)
7-12 years	8.9	5.3-11.7	6.5	5.0-12.4	42 (0.487)
13-18 years	8.3	3.2-15.8	9.1	6.0-12.2	23 (0.708)
Age group (Years)	Comparison of total white blood cell count across the various age groups (Infected and Non-infected P-value)				
	Median	Range	Median	Range	
2-6	24500	8460-54000	28000	27000-28000	0.061
7-12	25500	5400-54000	20500	20000-21000	0.247
13-18	13600	7000-54000	33000	32000-34000	0.086
Hb-Haemoglobin * statistically significant					

Table 5: comparison of means of episodes of vaso-occlusive crises between SCA children infected with helminths and those that are not infected.					
Age (Years)	**Mean episodes of VOCs all subjects**	**Helminth Present**		**t-test**	**p-value**
		Yes (Mean±SD)	**No (Mean±SD)**		
All the subjects	2.6 ± 2.1 (N=120)	2.7 ± 0.65 (n=11)	2.6 ± 2.2 (n=109)	0.226	0.822
2-6	2.5 ± 2.4 (n=48)	3.2 ± 0.4 (n=6)	2.4 ± 2.5 (n=42)	0.72	0.478
7-12	2.5 ± 1.8(n=40)	2.0 ± 0.0(n=3)	2.6 ±1.9(n=37)	-0.538	0.590
13-18	2.7 ± 1.8(n=32)	2.5 ± 0.7(n=2)	2.8 ±1.9(n=30)	-0.217	0.830