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The burden of anaemia in *Plasmodium falciparum* parasitized and non-parasitized children, Minna, north central Nigeria

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Abstract

Plasmodium falciparum infection may cause severe anaemia, particularly in children. There are several kinds of anaemia, produced by a variety of underlying causes. This study however, was conducted for a period of 3 months between February and May, 2018, to assess the frequency and types of anaemia in malarial infection. A total of 301 children below 17 years were recruited from the community and selected healthcare facilities. Thick and thin films of the blood samples were prepared for parasite identification and a complete blood count was conducted to determine the presence of anaemia. Multinomial logistic regression was used to predict relationships between anaemia and *P. falciparum*. Children with anaemia as a result of low levels of hemoglobin and hematocrit were observed as 65% and 61% respectively. Meanwhile, in parasitized subjects, prevalent rates of anaemia decreases with age and the overall prevalence was recorded as 49%(150) and 47%(142) in children with low levels of hemoglobin and hematocrit respectively. It was also observed that *P. falciparum* parasite was not a significant factor in the anaemia transmission ($p>0.05$). Similarly, anaemia prevalence decreases as the severity intensified (from severe, to moderate and mild), with most prevalence observed in mild anaemia 31%(96). The attributable risk of anaemia by malaria in this study was observed in low hemoglobin anaemia as 7.12% which was lower than what was observed in low hematocrit anaemia 8.11%. This study provides a significant relationship between anaemia sub-types and malaria infection and proves anaemia to be a major public health problem in this community as over 60% of the study population had anaemia.

Keywords: Anaemia; hematocrit; hemoglobin.

Introduction

About 90% of the global burden of malaria occurs in Africa, south of Sahara and each year an estimated 300 to 650 million clinical cases of malaria occur, making it one of the most common infectious diseases worldwide. Anaemia is a frequent feature in *plasmodium* infection which can occur as a result of the reduction in circulating red-cell mass below normal levels [1]; it is also characterized by a low level of hemoglobin in the blood [2]. Anaemia is a widespread public health problem and its severity is a significant cause of childhood mortality [3]. Its high prevalence and consequences on children's health, especially for their growth and development, have made it an important public health concern, given the difficulty in implementing effective measures for controlling the anaemia [4].

In most countries of sub-Saharan Africa, anaemia is a moderate or severe public health problem causing significant morbidity and mortality [5] and malaria is one of the factors that contributes to the anaemia burden in children. The mechanism of anaemia infection is characterized by a reduction in hemoglobin levels causing a reduced oxygen demands of the body. In African children, this hematological state is determined by combinations of nutritional inadequacy, infectious diseases (malaria, hookworm infections, and human immunodeficiency virus infections), and the genetic constitution of red cell hemoglobin [6-9].

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Malarial anaemia is a multifactorial disease for which its complex origin is not fully defined. Severe malarial anaemia is one of the main clinical presentations of severe malaria caused by *P. falciparum* [10]. The aetiology of severe malarial anaemia in malaria endemic areas may include a number of distinct as well as overlapping features, such as lysis of *plasmodium*-infected RBCs due to malaria infection [11], splenic sequestration of RBCs [12], dyserythropoiesis and bone marrow suppression [13], infectious diseases, and chronic transmission of malaria.

The importance of anaemia as a cause of death may well be under-estimated because of difficulty in diagnosis, especially where parasitemia may be low and the clinical picture may be confused with other causes of anaemia [14]. However, considering that specific hematological changes associated with malaria infection may vary with the level of malaria endemicity [15], and the parasite species, this research tries to study and characterize the hematological indices involved in malarial anaemia in endemic regions of Minna, north central, Nigeria.

Methods

Description of the study-area

A cross sectional study was carried out in Minna, the capital city of Niger State, Nigeria, of which samples were collected for a period of 3 months between February and May, 2018 from selected healthcare facilities and from apparently healthy children from the community. Minna is located at Latitude 9.62^0 and Longitude 6.55^0 and it is situated at elevation 243 meters above sea level, covering a land area of 88 km^2. It has an estimated population of 1.2 million. Minna has a tropical climate with two seasons, the rainy and the dry seasons, which start from May-October and December-March respectively. However, there is a transitional period of April and November. It has an average annual temperature of 27.5^0C in Minna, with rainfall average of 1229 mm and relative humidity of 61.00%. The temperatures are highest on average in March, at around 30.5°C. August is the coldest month, with temperatures averaging 25.3^0C.

Study population

The study-subjects included children of both sexes between 6 months to 5 years, 6 to11 years and 12 to 17 years. A total of 301 samples were drawn randomly from healthy children from the community; and also from children who visited the outpatient departments of selected healthcare facilities with signs of fever within the period of the study. Meanwhile, socio-demographic data was collected by a face to face interview. Samples collected from each child were used for determination of malaria parasite status and full blood count evaluation.

Parasitological examination

About 1 ml of venous blood samples were taken using disposable syringes from children into a tube containing Ethylene Diamine Tetra Acetate (EDTA). Safety procedures was adopted by swabbing the area to be pricked with alcohol swab and then allowed to dry before collection of blood. The tubes were properly labeled and transported to the laboratory in ice packs at temperature between 4-8°C for further analysis as recommended by WHO. Afterwards, thick and thin films of the blood samples were prepared using standard procedures with 3% Giemsa stain pH 7.0 for 45 minutes. The blood films were then examined microscopically using 100x (oil immersion) objectives as described elsewhere [16]. Parasite density per microliter of blood (parasitaemia) was estimated from the thick film according to the method of counting parasites as described elsewhere [17], based on the number of parasites per 200 leukocytes on thick blood film with reference to participants white blood cell count, this was then multiplied by an assumed WBC count of 8000. Parasitaemia was categorized as low (<1000 parasite/ìl), moderate (1001-10,000 parasite/ìl), high (>10,001/ìl) and hyperparasitemia ($\geq$ 100,000) [18-19].

Hematological examination

A complete blood count including values for white blood cell (WBC), red blood cell (RBC) and platelet counts, hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) was obtained using an autohaematology analyzer, the Beckman Coulter counter, following the manufacturer's instructions. Anaemia was defined when Hb <12.0 g/dL and <13.0 g/dL in female and male, respectively. The degree of the anaemia was further classified as described elsewhere [20], with little modification, as normal (12/13 g/dL -16/17 g/dL for female and male, respectively), high (>16 g/dL or >17 g/dL for female and male, respectively), severe (Hb < 7.0 g/dL), moderate (Hb 7.0 to 10.0 g/dL), and mild (Hb >10 g/dL <12 or 13 g/dL for females and males, respectively).

Ethical permission

The study was approved by the Niger State Ministry of Health, administrative clearance was obtained from the coordinator, Primary Health Care, Chanchaga Local Government. Furthermore, informed consent was sought from the parents or guardians of children captured in this study.

Data analysis

Data analysis was performed using SPSS version 23.0 statistical software programs. Prevalence of anaemia and comparisons between the anaemia sub-types were

done by using bivariate correlations. Differences in proportions of age, sex and parasite densities were evaluated using Pearman's *chi*-square. Multinomial logistic regression was used to predict relationships between anaemia and *P. falciparum*. The attributable risk of anaemia due to malaria was determined using $[(n1\ m0 - n0\ m1)/\ n(n0 + m0)] \times 100$, where $n0$ = anaemic children without malaria and n1 = anaemic children with malaria, whereby $n0 + n1 = n$, $m0$ = non-anaemic children without malaria, and m1 = non-anaemic children with malaria, whereby $m0 + m1 = m$. The *P* value less than 0.05 was considered statistically significant.

Results

Characteristics of the study-population

Out of the 301 children recruited for this study, about 46.20% (139) children were between the ages of 6 months to 5 years. Meanwhile, children between the age-groups 6 to 11 years and 12 to 17 years observed a population frequency of 37.20%(112) and 16.00% (50), respectively. More than half of the study populations were males 57.80%(174) most of which were within the age group of 6 months to 5 years. Furthermore, more than 75%(226) of the samples collected from children were infected with *P. falciparum*, while the remaining 25%(75) were negative. Children with anaemia as a result of low levels of hemoglobin, hematocrit and red blood cells where observed to be 65. 10%(196), 6..80%(186) and 8.00%(24), respectively.

Prevalence of anaemia in P. falciparum parasitized and non-parasitized blood samples from children with respect to age

As seen in Table 1, the prevalence of anaemia due to low levels of hemoglobin and hematocrit for *P. falciparum* negative and infected children from different cohorts were highlighted. Out of the 75 non-parasitized (negative) cases, 21(6.97%), 20(6.64%) and 5(1.66%) participants were observed with low levels of hemoglobin within the different age groups of 6 months to 5years, 6 to 11 years and 12 to 17 years, respectively. Meanwhile, same figures were also observed in children with low levels of hematocrit, except the age group 12 to 17 years which observed only 3(0.99%) cases. A total of 29(9.63%) and 31(10.29%) participants had normal hemoglobin and hematocrit levels with no *P. falciparum* infection, respectively. In addition, data showed that about half of the population had malarial anaemia with 150(49.83%) and 142(47.17%) cases for both low levels of hemoglobin and hematocrit, respectively. More importantly, prevalent rates of both low levels of hemoglobin and hematocrit, decreases as the age increases. Children infected with *P. falciparum* with normal hemoglobin and hematocrit levels were 76(25.24%) and 84(28.23%), respectively. Thus, for both *P. falciparum* parasitized and non-parasitized blood samples, there was no statistical significant differences between age of child and anaemia; and no significant correlations exists between them, at $p > 0.05$.

Table 1. Prevalence of anaemia in *P. falciparum* parasitized and non-parasitized blood samples from children with respect to age.

		Hemoglobin			Hematocrit		
Parasite	Age grps	Normal %(n)	Hb %(n)	Total %(N)	Normal %(n)	Hc %(n)	Total %(N)
Neg.	6 mnths-5 yrs	5.31(16)	6.97(21)[a]	12.29(37)	5.31(16)	6.97(21)[b]	12.29(37)
	6 yrs-11 yrs	3.32(10)	6.64(20)[a]	9.96(30)	3.32(10)	6.64(20)[b]	9.96(30)
	12 yrs-17 yrs	0.99(3)	1.66(5)[a]	2.65(8)	1.66(5)	0.99(3)[b]	2.65(8)
	Total	**9.63(29)**	**15.28(46)**	**24.91(75)**	**10.29(31)**	**14.61(44)**	**24.91(75)**
Pos.	6 mnths-5 yrs	11.96(36)	21.92(66)[c]	33.88(102)	12.29(37)	21.59(65)[d]	33.88(102)
	6 yrs-11 yrs	8.63(26)	18.60(56)[c]	27.24(82)	9.96(30)	17.27(52)[d]	27.24(82)
	12 yrs-17 yrs	4.65(14)	9.30(28)[c]	13.95(42)	5.64(17)	8.30(25)[d]	13.95(42)
	Total	**25.24(76)**	**49.83(150)**	**75.08(226)**	**28.23(84)**	**47.17(142)**	**75.08(226)**
	Total	**34.88(105)**	**65.11(196)**	**100(301)**	**38.20(115)**	**61.79(186)**	**100(301)**

[a] significant difference and correlation was not observed between age and low levels of hemoglobin in non-parasitized subjects ($x^2 = 0.691$, $p = 0.708$; $r = 0.069$, $p = 0.556$).
[b] significant difference and correlation was not observed between age and low levels of hematocrit in non-parasitized subjects ($x^2 = 2.326$, $p = 0.313$; $r = -0.050$, $p = 0.670$).
[c] significant difference and correlation was not observed between age and low levels of hemoglobin in parasitized subjects ($x^2 = 0.264$, $p = 0.707$; $r = 0.021$, $p = 0.751$).
[d] significant difference and correlation was not observed between age and low levels of hematocrit in parasitized subjects ($x^2 = 0.244$, $p = 0.885$; $r = -0.029$, $p = 0.665$).

Degree of anaemia infection in P. falciparum *parasitized and non-parasitized blood samples from children*

As highlighted in Table 2, only 2 cases (0.66%) of severe anaemia (as a result of low levels of hemoglobin) was observed in *P. falciparum* non-parasitized samples. Meanwhile, moderate and mild anaemia were observed in 10(3.32%) and 33(10.96%) cases, respectively. On the contrary, *P. falciparum* infected samples showed the highest degree of infections with the highest prevalence observed in mild anaemia 96 cases (31.89%), followed by moderate anaemia with 42 cases (13.95%) and the least was observed in severe anaemia with 11 cases (3.65%). Furthermore, more than 34% (105) of the entire study population had normal hemoglobin levels with 76 cases (22.25%) of *P. falciparum* parasitized and 29 cases (9.63%) of non-parasitized children. Interestingly, blood samples with high hemoglobin levels was detected in only one case each 0.33% for both parasitized and non-parasitized samples. There was no statistical significant difference and significant correlations observed ($x^2 = 2.643$, $p = 0.619$; $r = 0.015$, $p = 0.799$) between *P. falciparum* infection and the degree of anaemia, at $p > 0.05$.

Table 2. Degree of anaemia infection in plasmodium parasitized and non-parasitized blood samples from children.

	Malaria parasite		
Anaemia severity	Negative %(*n*)	Positive %(*n*)	Total %(*n*)
Normal *(12/13 g/dL -16/17 g/dL)	9.63(29)	22.25(76)	34.88(105)
Mild anaemia *(>10? g/dL <12/13g/dL)	10.96(33)	31.89(96)[a,b]	42.85(129)
Moderate anaemia (7.0 - 10.0? g/dL)	3.32(10)	13.95(42)[a,b]	17.25(52)
Severe anaemia (< 7.0? g/dL)	0.66(2)	3.65(11) [a,b]	4.31(13)
High anaemia *(>16 g/dL or >17 g/dL)	0.33(1)	0.33(1) [a,b]	0.66(2)
Total	**25.00(75)**	**75.00(226)**	**100(301)**

* for female and male, respectively.
[a] Significant difference not observed between *P. falciparum* infection and the degree of anaemia, at $p > 0.05$.
[b] No significant correlations between *P. falciparum* infection and the degree of anaemia, at $p > 0.05$.

Multinomial logistic regression analysis examining anaemia with P. falciparum *parasite density*

Regression analysis to determine and quantify the association between anaemia and parasite density is seen in Table 3. The reference category was high parasite density of >10,001 category. OR (95% CI) for anaemia among participants with low parasite density were 0.543(0.145-39.506) and 0.323(0.250-67.438) for hemoglobin and hematocrit anaemia, respectively, compared to high parasite density; while the OR (95%) among the moderate parasite density were 0.646(0.122 29.746) and 0.370(0.226-54.468) for hemoglobin and hematocrit anaemia, respectively, compared to high parasite density. The multinomial logistic regression model demonstrated that anaemia is not a significant predictor to *P. falciparum* parasite density.

Table 3. Multinomial logistic regression analysis examining anaemia with parasite density.

Factoral	Sig.	95% (Confidence Interval)
Parasite density		
Negative	Intercept	.000
Hemoglobin	0.599[a]	(0.130 34.252)
Hematocrit	0.257 [a]	(0.310 80.101)
<1,000 low parasitemia	Intercept	.000
Hemoglobin	0.543 [a]	(0.145 39.506)
Hematocrit	0.323 [a]	(0.250 67.438)
501-10,000 moderate parasitemia		
Intercept		.000
Hemoglobin	0.646 [a]	(0.122 29.746)
Hematocrit	0.370 [a]	(0.226 54.468)

The reference category is: >10,001 high parasitemia.
[a]Parasitemia is not a significant predictor of anaemia at 95% CI.

Attributable risk of anaemia due to malaria

The attributable risk of anaemia by malaria in the study population was observed in low hemoglobin anaemia as 7.12% which was lower than the anaemia as a result of low levels of hematocrit with 8.11%. However, the attributable risk of anaemia in relation to the moderate parasite density were 8.26% and 8.33% for both low hemoglobin and low hematocrit anaemia, respectively.

Discussion

This study proves that anaemia is a common hematological condition in this community with over 60% of the study participants with this condition, and the peak was observed in children between 1-5 years.

This trend was also observed in a similar study conducted by Sumbele, *et al.,* (2016) which also observed an anaemia prevalence of 62% with the peak in children below 5 years of age. Though, statistical significant difference was not observed between anaemia and age, but however, anaemia decreases as the age increases. In addition, the prevalence of anaemia observed among Plasmodium parasitized children was much higher in all age groups compared to non-parasitized children; this findings were observed for both hemoglobin and hematocrit anaemia. Furthermore, it was also observed that *P. falciparum* parasite was not a significant factor in the development or onset of anaemia and this was in discordance with a study conducted by Mockenhaupt, *et al* (1999) which observed the influence of *P. falciparum* infection on hemoglobin levels differed significantly between *P. falciparum* positive and negative samples.

Mild anaemia is more pronounced in this population for both *Plasmodium* parasitized and non-parasitized children with 31.89% and 10.96%, respectively. Interestingly, blood samples with high hemoglobin levels (more than normal) was observed in one case each 0.33% in both Plasmodium parasitized and non-parasitized children. Significant relationship does not exist between *P. falciparum* infection and the severity of anaemia infection.

Parasitemia is not a significant predictor of anaemia at 95% confidence interval. Moreover, the attributable risk of anaemia by malaria in this study was observed in hemoglobin deficiency anaemia as 7.12% which was lower than the low hematocrit anaemia with 8.11%. This was similar to the AR obtained by Sumbele, *et al* (2016) which observed 7.6% AR in anaemia caused by malaria in the studied population.

Conclusion

Anaemia was observed to be a major public health problem in this community as over 60% of the study-population had anaemia. The World Health Organization (WHO), considers anaemia prevalence over 40% as a major public health problem, between 20 and 40% as a medium-level public health problem, and between 5% and 20% as a mild public health problem. More significantly, this study provides a significant relationship between malaria and *P. falciparum* infection and it proves that *P. falciparum* parasite was not a significant factor in the development or onset of anaemia. Detailed knowledge of this relationship will aid our physicians to be more effective in dealing with this public health menace. More importantly, there is an urgent need to avoid the effect of anaemia in children, most especially children below 5 years particularly in developing countries by integrated preventive strategies of malaria control combined with dietary modification to improve iron intake, which can save the disease from going into chronic stage and to some extent release the burden of *P. falciparum* and anaemia from our society.

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