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Assessment of the haematological profile of children with malaria parasitaemia treated with three different artemisinin-based combination therapies

Chukwuocha Uchechukwu Madukaku[1*], Onyirioha Misherk Chimezie[1,2], Nwakwuo Geoffrey Chima[1], Okorie Hope[3], Dozie Ikechukwu Nosike Simplicius[1]

[1]*Department of Public Health Technology, School of Health Technology, Federal University of Technology, Owerri, Imo State, Nigeria*

[2]*Unity Hospital and Maternity, Irette, Imo State, Nigeria*

[3]*School of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria*

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ABSTRACT

Objective: To assess the haematological profile of children with malaria, treated with three different artemisinin-based combination therapies in South Eastern Nigeria.
Methods: Using a multistage sampling technique, blood samples were collected from 105 randomly selected malaria positive primary school children aged 6-13 years. Pre- and post-assessment of their haematological profiles were respectively done on intervention of three different artemisinin-based combination therapies.
Results: Result showed a strong difference [(0.38 ± 0.31) g/dL] in haemoglobin levels with the artesunate-amodiaquine (t = 7.30, P < 0.05). Dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine showed haemoglobin (t = 4.49, P < 0.05) with mean difference [(0.64 ± 0.85) g/dL] and (t = 6.09, P < 0.05) with mean difference [(0.80 ± 0.78) g/dL] respectively. The mean difference of white blood cell was found to be negative but significant with artesunate-amodiaquine (-1.07 ± 3.12) at 95% confidence interval (*CI*) (-2.14, 0.00) and artemether-lumefantrine (-0.36 ± 0.28) at 95% *CI* (-0.45, -0.26) interventions respectively. Significant mean difference of neutrophils was only found for the DP interventions (4.54 ± 8.30) at 95% *CI* (1.69, 7.40) while lymphocytes indicated a significant mean difference between the pre/post-interventions (-3.60 ± 9.34) at 95% *CI* (-6.81, -0.39) with DP only.
Conclusions: Even though these findings do not indicate any life threatening events, they may have some useful implications for investigating future non-infectious diseases of blood origin. Further studies to determine the extent of involvement of malaria parasite as well as drug interactions in haematological alterations vis-a-vis its implication for noncommunicable disease are important.

1. Introduction

Malaria infection is common in Sub-Saharan Africa, but death directly attributed to the parasite is comparatively rare, largely because of acquired functional immunity[1]. There is a recent increase in the prevalence of noninfectious diseases in areas where infectious diseases have been highly endemic for several years[2-4]. The co-endemicity of infectious and noninfectious diseases is an emerging trend in Sub-Saharan Africa and should be a major cause for concern.

Chronic, subclinical infections cause anaemia or may encourage undernutrition, which in turn may increase susceptibility to severe clinical outcomes of subsequent malarial or other pathogenetic infections[1]. In the reverse, the aetiologies of malaria in the predisposing of some of these noninfectious diseases are unknown. Since some drugs also have some adverse

*Corresponding author: Chukwuocha Uchechukwu Madukaku, Department of Public Health Technology, School of Health Technology, Federal University of Technology, Owerri, Imo State, Nigeria.
Tel: +2348034712957
E-mail: uchukwuocha@gmail.com

effects on the cells, tissues and organs, their presence in the body together with the infectious disease agents may have some implications for clinical outcomes associated with noninfectious diseases[5]. Malaria infection is one that is very endemic for which drugs are frequently taken over long periods. The question is "can these interactions between drugs and infectious disease agents be the underlying cause of certain noninfectious diseases of unknown aetiology?"

The blood forms a medium for malaria parasite multiplication as well as site for most antimalarial drug activities[6-9]. Changes in haematological parameters are likely to be influenced by any disease condition which affects the hematopoietic physiology at any level. This is likely to happen with an endemic disease such as malaria that affects the host homeostasis at various fronts resulting in myriad of clinical presentations[10].

The extent of hematological alterations varies with level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity[11-13]. But the involvement of antimalarial drugs especially the artemisinin-based combination therapies (ACTs) is largely unknown. Studies using animal models indicated toxic effects of artemether to include embryotoxicity, gonadotoxicity, genotoxicity, cardiotoxicity, immunotoxicity, nephrotoxicity and allergic reaction[14-26]. However, any involvement of ACTs in haematological alterations as a result of malaria will pose a very dangerous threat especially if such alterations can be implicated in future noninfectious disease manifestations. Therefore, to ascertain any possible changes in the hematological profile of children with malaria after being treated with ACTs and any implicated factor, we assessed the haematological profile of children with malaria (parasitaemia) treated with three different ACTs.

2. Materials and methods

The study employed a quasi-experimental method without control exposing the children to interventions of three different ACTs after prior assessment of their blood and blood profiles. These included 105 primary school children of both sexes within the age range of 6-13 years confirmed to be positive for malaria parasitaemia.

These children were drawn from selected primary schools in Okohia, South Eastern Nigeria between March and July 2013. They were separated into 3 intervention groups of artesunate-amodiaquine (AA), dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL) with 35 children in each group.

Using a sterile needle and syringe, 5 mL of fresh venous blood was drawn from each of the subjects by a clean venepuncture and put into clean ethylene diamine tetraacetic acid containers (pre-samples).

The subjects were administered with antimalarial drugs. The post-samples were also collected after 4-7 days into an ethylene diamine tetraacetic acid container. The pre and post blood samples were assessed in the laboratory for different haematological profiles.

After collecting and collating the data into a spread sheet, the data was analyzed using IBM SPSS statistic version 20.

To calculate the mean differences between the pre-test and post intervention tests, a paired sample *t*-test was done for the three different interventions *i.e.* AA, DP and AL. The validity of the data for analysis was confirmed by testing the differences between the paired values for normal distribution using both the normal probability (quantile-quantile) plot and the Kolmogorov-Smirnov test.

The study received ethical approval from the Ethical Committee of the Department of Public Health Technology and Institutional Review Board of the School of Health Technology, Federal University of Technology Owerri, Nigeria. Informed written and oral consent were sought for an obtaining from the head teacher and parents/caregivers of children of the selected primary schools.

3. Results

The ratio of males to females in this study was 19:16 for AA, DP (13:22) and AL (3:4) interventions respectively. The mean age of children in the respective interventions include 10.97 for AA, 8.40 for DP and 7.00 for AL (Table 1).

Table 1

Some characteristics of children and antimalarial drug given.

Variables	AA	DP	AL
Male	19.00	13.00	15.00
Female	16.00	22.00	20.00
Male/female	19:16	13:22	3:4
Mean age (95% *CI*)	10.97 (10.48, 11.47)	8.40 (7.99, 8.81)	7.00 (6.61, 7.39)

CI: Confidence interval.

The nutritional status of the children in terms of mean weight and arm circumference was highest for the AA intervention, 25.5 ± 6.3 and 17.9 ± 2.1 respectively. AL intervention showed the lowest values of 23.5 ± 4.2 and 17.1 ± 1.1 respectively (Table 2).

Table 2

Nutritional components of children.

Interventions	Mean value		Mean range	
	Weight (kg)	Mid-arm circumference (cm)	Weight (kg)	Mid-arm circumference (cm)
AA	25.5 ± 6.3	17.9 ± 2.1	20.0	7.0
DP	23.7 ± 4.9	17.2 ± 1.4	26.0	7.0
AL	23.5 ± 4.2	17.1 ± 1.1	17.0	5.0

Table 3 indicates the haematological profile of children in the AA intervention. After pre- and post-intervention tests, haemoglobin of the children showed a strong difference (t = 7.30, P = 0.00) of approximately (0.38 ± 0.31) g/dL at 95% *CI*. The white blood cell (WBC) showed a slight significant change (t = -2.03, P = 0.05) with a mean difference (-1.07 ± 3.12).

Table 3

Haematological profile for AA intervention.

Variables	Pre/post-intervention (95% *CI*)	*t*	*P* value
Male/Female	1.19		
Mean age (years)	10.97 ± 1.54		
Haemoglobin (g/dL)	0.38 ± 0.31 (0.28, 0.49)	7.30	0.00
WBC 10^2 mm^3	-1.07 ± 3.12 (-2.14, 0.00)	-2.03	0.05
Lymphocyte (%)	1.29 ± 9.63 (-2.02, 4.50)	0.79	0.44
Neutrophils (%)	-0.23 ± 9.13 (-3.37, 2.91)	-0.15	0.88
Eosinophils (%)	0.29 ± 1.23 (-0.10, 0.67)	1.50	0.14
Monocytes (%)	0.11 ± 0.58 (0.09, 0.31)	1.16	0.25
Platelets (10^3)	-4.31 ± 40.85 (-18.35, 9.72)	-0.625	0.54

Lymphocytes, neutrophils, eosinophils, monocytes and platelets showed no statistically significant differences pre/post interventions (P = 0.44, 0.88, 0.14, 0.25 and 0.54 respectively) at 95% *CI*.

The haematological profile of children in the AA intervention is shown in Table 4 as follows: haemoglobin (t = 4.49, P = 0.000) with mean difference [(0.64 ± 0.85) g/dL], lymphocytes (t = -2.28, P = 0.029) with mean difference (-3.60 ± 9.34) and neutrophils (t = 3.24, P = 0.003) with mean difference (4.54 ± 8.30) at 95% *CI*. The platelets showed a slight significance (t = -2.04, P = 0.049) with a mean difference (-30.37 ± 87.96). The WBC, eosinophils, monocytes and platelets showed no statistically significant differences pre/post interventions (P = 0.213, 0.638, and 0.058 respectively) at 95% *CI*.

Table 4

Haematological profile for DP intervention.

Variables	Pre/post intervention (95% *CI*)	t	P value
Male/Female	0.59		
Mean age (yrs)	8.40 ± 1.19		
Haemoglobin (g/dL)	0.64 ± 0.85 (0.35, 0.93)	4.49	0.000
WBC 10^2 mm^3	0.45 ± 2.12 (-0.27, 1.18)	1.27	0.213
Lymphocyte (%)	-3.60 ± 9.34 (-6.81, -0.39)	-2.28	0.029
Neutrophils (%)	4.54 ± 8.30 (1.69, 7.40)	3.24	0.003
Eosinophils (%)	0.09 ± 1.07 (-0.28, 0.45)	0.48	0.638
Monocytes (%)	0.14 ± 0.43 (-0.01, 0.29)	1.97	0.058
Platelets (10^3)	-30.37 ± 87.96 (-60.59, -0.16)	-2.04	0.049

Table 5 indicates that only haemoglobin (t = 6.09, P = 0.000) with mean difference [(0.80 ± 0.78) g/dL] and WBC (t = -7.60, P = 0.000) with mean difference (-0.36 ± 0.28) showed any significant differences pre/post interventions in the AL intervention group. All other haematological parameters *i.e.* lymphocytes, neutophils, eosinophils, monocytes and platelets showed no statistically significant differences (P = 0.750, 0.834, 0.757, 0.422, and 0.192 respectively).

Table 5

Haematological profile for AL intervention.

Variables	Pre/post intervention (95% *CI*)	t	P value
Male/Female	0.75		
Mean age (years)	7.00 ± 1.14		
Haemoglobin (g/dL)	0.80 ± 0.78 (0.54, 1.07)	6.09	0.000
WBC 10^2 mm^3	-0.36 ± 0.28 (-0.45, -0.26)	-7.60	0.000
Lymphocyte (%)	0.54 ± 10.01 (-2.90, 3.98)	0.32	0.750
Neutrophils (%)	-0.37 ± 10.41 (-3.95, 3.20)	-0.21	0.834
Eosinophils (%)	-0.06 ± 1.08 (-0.43, 0.31)	-0.31	0.757
Monocytes (%)	0.57 ± 0.42 (-0.09, 0.20)	0.81	0.422
Platelets (10^3)	-9.20 ± 40.88 (-23.24, 4.84)	-1.33	0.192

Table 6 shows a comparison of the mean haematological parameters for all the interventions. Mean haemoglobin level was low compared to the normal range for both male and female and in both pre- and post-antimalarial interventions. Lymphocytes count was moderate within normal range and mean values for neurophils were high, whereas eosinophils and monocytes were low and platelets were normal for all interventions.

Table 6

Comparison of mean haematological parameters for all interventions.

Parameters	Normal value	AA		DP		AL	
		Pre-mean value	Post-mean value	Pre-mean value	Post-mean value	Pre-mean value	Post-mean value
Haemoglobin (g/dL)	12.0-18.0	9.89	9.51	10.58	9.94	10.87	10.07
WBC 10^2 mm^3	3.5-9.0	5.91	6.98	5.24	4.79	4.38	4.73
Lymphocyte (%)	16.0-33.0	55.29	54.00	53.74	57.34	57.83	57.29
Neutrophils (%)	45.0-62.0	43.29	43.51	45.71	41.17	41.49	41.86
Eosinophils (%)	1.0-3.0	1.17	0.89	0.97	0.89	0.86	0.91
Monocytes (%)	3.0-7.0	0.26	0.14	0.17	0.03	0.11	0.06
Platelets (10^3)	150.0-350.0	289.70	294.00	267.14	297.51	297.89	307.09

4. Discussion

The control of malaria has been challenged by increasing resistance of *Plasmodium falciparum* (*P. falciparum*) to antimalarial drugs, particularly chloroquine and sulfadoxine-pyrimethamine, and reduced efficacy of chloroquine in the treatment of uncomplicated malaria in the late 1990s and the first half of this decade[17,18], leading to sweeping changes in antimalarial treatment recommendations[19]. The National Malaria Control Programme recommended the use of ACTs for the treatment of uncomplicated malaria in 2005.

Changes in haematological parameters are likely to be influenced by any disease condition which affects the hematopoietic physiology at any level. Haematological abnormalities considered hallmark of malaria infection are common and more pronounced in *P. falciparum* malaria, infection, probably due to the higher levels of parasitaemia found in these patients[20]. The abnormalities previously reported include changes in haemoglobin, leucocyte count and platelet abnormalities resulting in defective thromboplastin, and disseminated intravascular coagulation[12,21,22]. But the implication of these abnormalities for the development of chronic noncommunicable disease is not well understood.

We applied three different interventions of ACT on children with parasitaemia to assess if there is any change in the haematological parameters assessed and the results showed that

a greater percentage of the pupils [19 (54.29%)] was exposed to AA compared to more females exposed to both DP [22 (62.86%)] and AL [20 (57.14%)] with a mean age of 10.97 for AA (95% *CI*, 10.48-11.47), DP (8.40), 95% *CI* (7.99-8.81) and AL (7.00), 95% *CI* (6.61-7.39).

Our results showed the highest mean weight and mean arm circumference values for the AA intervention (25.5 ± 6.3 and 17.9 ± 2.1 respectively), with AL intervention showing the lowest values of 23.5 ± 4.2 and 17.1 ± 1.1 respectively.

According to a study, if neither weight nor height can be measured, arm circumference can also be used to provide a broad estimate of body mass index (BMI). If the measured mid-arm circumference is less than 23.5 cm, BMI is likely to be less than 20 kg/m^2 and the person is likely to be underweight. Conversely, if arm circumference is more than 32.0 cm, BMI is likely to be more than 30 kg/m^2, and the person is likely to be obese[23]. From this study, the mean arm circumference was less than 23.5 cm indicating that a BMI is likely to be less than 20 kg/m^2 and underweight in the children studied. Although the effect of nutrition on the use of ACTs is not well understood, malnutrition predicted death in children with severe malaria and exposure to drugs that have effect on blood among malnourished children could be dangerous[24]. Limited data available suggests that malnutrition in fact increases the risk of dying from severe malaria[25-27].

The study intervention with AA showed a strong mean difference in haemoglobin of the children (t = 7.30, P < 0.05) of approximately (0.38 ± 0.31) g/dL at 95% *CI*. DP showed haemoglobin (t = 4.49, P < 0.05) with mean difference [(0.64 ± 0.85) g/dL] and AL (t = 6.09, P < 0.05) with mean difference [(0.80 ± 0.78) g/dL]. There was decrease in the haemoglobin level after the different intervention.

A previous study showed no significant difference in WBC and neutrophil counts before and after antimalarial treatment[28]. This corroborates our result for the DP intervention (0.45 ± 2.12) at 95% *CI* (-0.27, 1.18) which showed no significant difference in WBC as well. Our result on WBC however showed a significant mean difference (-1.07 ± 3.12) at 95% *CI* (-2.14, 0.00) for AA intervention and (-0.36 ± 0.28) at 95% *CI* (-0.45, -0.26) for AL intervention. The later interventions (AA and AL) increase and although there was decrease in the DP intervention, the difference was approximately insignificant. WBC counts during malaria are generally characterized as being low to normal. A phenomenon which is widely thought to reflect localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis[29]. But a study has documented a rise in WBC counts with artemether administration[15,30].

Although the reason for different WBC counts between different interventions in this study is not well understood, toxicity studies in dogs and rats revealed dose-dependent and potentially fatal neurotoxic effects after intramuscular injection of artemether at higher and multiple doses[31].

About 77.9% of the children in another study had normal WBC counts, and children with patent parasitaemia had lower WBC counts than those with none patent and those with malaria negative. According to McKenzie and Prudhomme *et al*., the increase in mean WBC count observed in the AA group following treatment, which lends support to the finding that *P. falciparum* infection contributes to the localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis[28,32].

This study showed a significant mean difference in neutophils with only DP intervention (4.54 ± 8.30) at 95% *CI* (1.69, 7.40). There was a high decrease in the mean neutrophil value in the DP intervention compared to others that show only slight and insignificant increase. This is contrary to a non-serious decline observed in the neutrophil counts in 12.5% of volunteers in another study[33].

However, the values were only marginally below the normal range. In granulocytosis, neutrophil count should be below 0.5 × 10^9/L [normal range (2.5-7.7) × 10^9/L], while other cell counts are usually normal[34]. The absolute number of neutrophils diminished slightly more in the amodiaquine group compared with chloroquine groups, but variation was mild suggesting that the differences between the groups were not of clinical significance[33]. Although the risks of serious toxic effects are considered to be much lower in therapeutic dosage, careful monitoring is important as the possibilities of toxicity in clinical practices cannot be excluded.

According to Imoru *et al*., the mean lymphocyte percentage in his study was normal and the mid-cell count in malaria infected and non-infected children showed no statistically significant differences (P > 0.05)[35]. However, other reports showed lymphopenia in some cases of acute malaria which is probably due to a redistribution of lymphocytes with sequestration in the spleen[36,37]. Our study indicated a significant mean difference between the pre- and post-interventions (-3.60 ± 9.34) at 95% *CI* (-6.81, -0.39) with DP. There was an increase in the mean lymphocyte in the post-intervention compared to other intervention. But despite this value, the post-intervention mean values were above normal in all interventions.

Platelets have been implicated in malaria pathogenesis and some previous studies have linked children who died of malaria to the presence of platelet clumps in their erythrocytes. It may play a role in the pathogenesis of human cerebral malaria[38-40].

It therefore remains a paradox that although platelets have been implicated in the pathogenesis of severe infection, most studies suggest that low count of platelets is not associated with an adverse outcome[41]. Children with low platelet counts were also

likely to have anaemia ($r = 0.2$, $P = 0.000\,3$) as previously reported from a Nigerian study[42]. Result from this study has indicated a normal mean value of platelet counts for all ACTs interventions although DP showed a significant difference [-30.37 ± 87.96 (-60.59, -0.16)] in the pre- and post-value across interventions higher than the increase in the value of platelets shown in other interventions.

Although the study samples were not followed over time to check for long-term implication of the administration of the interventions, changes in haematological profile were used to infer possible changes or clinical outcomes that are not yet well understood but believed to have implication for occurrence of noncommunicable disease.

The haematological aspects of malaria infection constitute a very interesting area in various reports. Results from our study demonstrate that in chemotherapeutic dosage, ACTs did not induce life-threatening haematological adverse effects on the children as against the effects of *P. falciparum*.

Although the effect of different antimalarial drugs especially the combination therapy on human is not well established, changes in biological profiles could be associated with vascular motor and auditory functions as well as other toxic effects including embryotoxicity, gonadotoxicity, genotoxicity, cardiotoxicity, immunotoxicity, nephrotoxicity and allergic reactions[14-16]. And this might posit a link with noncommunicable diseases development in later life.

This in fact needs to be investigated properly comparing the different results of the haematological findings in both immune and semi-immune individuals living in endemic areas and those with different forms of *Plasmodium*. This is particularly important to ascertain the short and long term effects of parasite/drug interactions in the blood.

Conflict of interest statement

We declare that we have no conflict of interest.

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