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Responses of selected haematological and biochemical parameters to artesunate/artemether-lumefantrin combination therapy in children with severe malaria

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Abstract

Background: Severe malaria affects several haematological and biochemical parameters with vast clinical manifestations which may lead to death. However, the rate at which these respond to artesunate/artemether-lumefantrin combination therapy as monitoring tools for therapeutic response and recovery in children with severe malaria is not documented.

Objective: The aim of the study was to determine the responses of selected haematological and biochemical parameters to artesunate/artemether-lumefantrin combination therapy in children with severe malaria with the goal of identifying parameters with very fast response for early monitoring of therapeutic response and recovery.

Materials and methods: The level of selected haematological parameters [haemoglobin (Hb), packed cell volume (PCV), total leucocytes (TWBC), neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E), basophils (B) and platelets (P)] and serum level or activity of some biochemical parameters [malondialdehyde (MDA), protein carbonyls (PCO), nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), vitamins A, C, and E, C-reactive proteins (CRP), bicarbonate (HCO_3^-) and glucose] were measured using standard methods in 100 children (1-10 years) with severe malaria before treatment (day 0), 48 hours of treatment (day 2) and 48 hours after treatment (day 7) according to WHO recommended dosage of artesunate/ artemether-lumefantrin combination therapy, using 200 clinically healthy children as control.

Results: Eosinophils, monocytes, basophils, HCO_3^-, PCO/SOD, CRP/SOD and HCO_3^-/glucose in case children were normalised during treatment (day 2) indicating fastest response while the level or activity of TWBC, N, L, SOD, GR, GPx and glucose was normalised after treatment (day 7) depicting fast response. The level or activity of PCV, Hb, P, MDA, PCO, NO, CAT, GSH, vitamins A, C, E and CRP in case children were significantly different ($p<0.05$) compared to control after treatment (day 7) indicating slow response.

Conclusion: This study showed that eosinophils, monocytes, basophils, HCO_3^-, PCO/SOD, CRP/SOD and HCO_3^-/glucose have the fastest responses to treatment in severe malaria, and may be used as additional parameters for early monitoring of therapeutic response in children with the disease.

Keywords: *Severe malaria, haematological and biochemical parameters, monitoring, children, Artesunate-lumefantrine combination*

Résumé

Contexte: Le paludisme sévère affecte plusieurs paramètres hématologiques et biochimiques avec de nombreuses manifestations cliniques pouvant entraîner à la mort. Cependant, le taux auquel ils répondent à la thérapie combinée par l'artésunate/ artéméther-lumefantrine comme outils de surveillance pour la réponse thérapeutique et le rétablissement chez les enfants atteints du paludisme grave n'est pas documenté.

Objectif: Le but de l'étude était de déterminer les réponses de certains paramètres hématologiques et biochimiques à la thérapie combinée d'artésunate/ artéméther-lumefantrine chez les enfants atteints du paludisme sévère dans le but d'identifier des paramètres avec une réponse très rapide pour un suivi hâtif de la réponse thérapeutique et de la récupération.

Matériaux et méthodes: Le niveau de paramètres hématologiques sélectionnés [hémoglobine (Hb), volume cellulaire emballé (PCV), leucocytes totaux (TWBC), neutrophiles (N), lymphocytes (L), monocytes (M), éosinophiles (E), basophiles (B) et les plaquettes (P)] et le niveausérique ou l'activité de certains paramètres biochimiques [malondialdéhyde (MDA), protéines carbonylées (PCO), oxyde nitrique (NO), glutathion (GSH), super-oxyde dismutase (SOD), catalase (CAT), La glutathion réductase (GR), la glutathion peroxydase (GPx), les vitamines A, C et E, les protéines C-réactives (CRP), le bicarbonate (HCO_3^-) et le

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glucose] ont été mesurées en utilisant des méthodes standard chez 100 enfants (1-10 ans) avec un paludisme grave avant traitement (jour 0), 48 heures de traitement (jour 2) et 48 heures après traitement (jour 7) selon la posologie recommandée par l'OMS pour la thérapie combinée avec l'artésunate/ artéméther-lumefantrine, en utilisant 200 enfants cliniquement sains comme témoins .

Résultats: Eosinophiles, monocytes, basophiles, HCO_3-, PCO/SOD, CRP/SOD et HCO_3-/glucose dans le cas où les enfants étaient normalisés pendant le traitement (jour 2) indiquant la réponse la plus rapide alors que le niveau ou l'activité de TWBC, N, L, SOD , GR, GPx et glucose ont été normalisés après traitement (jour 7) représentant une réponse rapide. Le niveau ou l'activité de PCV, Hb, P, MDA, PCO, NO, CAT, GSH, vitamines A, C, E et CRP dans le cas où les enfants étaient significativement différents ($p < 0,05$) par rapport au contrôle après traitement (jour 7) indiquant réponse lente.

Conclusion: Cette étude a montré que les éosinophiles, les monocytes, les basophiles, le HCO_3- , PCO/SOD, CRP/SOD et HCO3-/glucose ont les réponses les plus rapides au traitement contre le paludisme sévère et peuvent être utilisés comme paramètres supplémentaires pour le suivi hâtif de la réponse thérapeutique chez les enfants atteints de la maladie.

Mots-clés: *Paludisme sévère, paramètres hématologiques et biochimiques, surveillance, enfants, combinaison Artesunate-Lumefantrine*

Introduction

Malaria, caused by the bite of Plasmodium carrying female anopheles mosquito, remains a major cause of morbidity and mortality worldwide. Globally, malaria accounts for 350 to 500 million cases with 100 to 300 million deaths, the majority of whom are young children in sub-Sahara Africa particularly Nigeria, Congo, Ethiopia and Uganda [1]. *P. falciparum* is the predominant Plasmodium specie in sub-Sahara Africa, and the major cause of malaria cases and death [1].

Due to increase in resistance of malaria parasite to conventional anti-malaria drugs, the World Health Organization (WHO) developed a treatment approach recommending intramuscular or intravenous artesunate as the first line of treatment in severe malaria [2]. Artesunate is a semi-synthetic derivative of artemisinin and the mechanism of action is based on its hydrolysis to dihydroartemisinin with the release of carbon-centered reactive species which attack and destroy the sarcoplasmic endoplasmic reticulum ATPase gene of the malaria parasite leading to the parasite death [3]. However, due to their short half-life (2-3 hours), and to prevent development of resistance, artemisinin compounds are combined with one or two long-acting antimalarial drugs such as lumefantrine (half-life: 4-6 days in falciparum malaria patients), amodiaquine, mefloquine or sulfadoxine/pyrimethamine as artemisinin-based combination therapy (ACT) [4,5]. Artesunate/ artemether-lumefantrin combination therapy is one of the most common combination therapy used in the treatment of severe malaria [6]. Lumefantrine is a synthetic aryl-amino alcohol antimalarial and functions by interfering with the haem polymerization process; a critical detoxifying pathway for the malaria parasite [7].

Uncomplicated malaria, when not promptly and properly diagnosed and treated, may progress to severe malaria which is characterised by hyperparasitaemia, anaemia, jaundice, respiratory distress, renal insufficiency, convulsion, unconsciousness, coma and a host of other symptoms and could result in death [2]. Once diagnosed accurately, effective treatment and management of severe malaria especially in children require precise and accurate monitoring of therapeutic response. The use of parasite density in monitoring therapeutic response has been a common practice. This however, is limited by the effect of sequestration especially in *falciparum* malaria and inter-microscopists variation in reporting the parasite density [8,9]. Likewise, the use of clinical presentations for this purpose is flawed by inter-clinician variation in interpreting clinical presentations [10]. With these noted shortcomings, coupled with the high fatality rate of falciparum malaria in children, there is a need to explore more accurate, objective and fast-response methods for monitoring therapeutic response in children with severe malaria.

Severe malaria affects several haematological and biochemical parameters with vast clinical manifestations which may lead to death [11]. However, information on the rate of responses of these parameters to treatment is not documented. Therefore, this study was carried out to determine the rate of responses of selected haematological and serum biochemical parameters to artesunate/ artemether-lumefantrin combination therapy in children with severe malaria.

Materials and methods

Study population

One hundred children with severe malaria (case children) aged 1 to 10 years, treated at the paediatrics' wards of Jos University Teaching Hospital; a

reference tertiary hospital in Jos, and 200 clinically healthy children (1-10 years) without malaria attending the hospital for medical check- ups and routine immunization (serving as control) were recruited for this study from 28th April 2014 to 15th February, 2016. Jos is the capital city of Plateau state in Nigeria, located between latitude 80° 24'N and longitude 80°32' and 100°38' E.

Children who met the study's inclusion criteria were recruited consecutively for the study. The inclusion criteria for the case children were: (i) assent of the child and of the parent/caregiver's consent; (ii) children aged 1 to 10 years clinically presenting with severe malaria without any other ailment as diagnosed by the paediatrician; (iii) children with one or more symptoms of malaria complications such as fever, anaemia, respiratory distress and jaundice; (iv) children microscopically confirmed of hyperparasitaemia; (v) children confirmed by laboratory tests as presenting with only severe malaria after excluding other disease conditions such as septicaemia, helminthiasis, typhoid, shigellosis, glucose-6-phosphate dehydrogenase deficiency (G6PDD), sickle cell disease, human immune-deficiency virus (HIV) and hepatitis B; (vi) children on admission in the hospital using mosquito bed-net; (vii) children on artesunate/artemether-lumefantrine combination therapy; and (viii) children that recovered and were discharged by the 7th day of admission.

The inclusion criteria for the control children were: (i) children aged 1-10 years diagnosed as clinically healthy by the paediatrician; (ii) assent of the child and parent/caregiver; (iii) children confirmed by laboratory tests as clinically healthy; and (iv) all children enrolled as control were negative for malaria parasite thick-smear examination (for malaria). They were without febrile episodes in the past 6 months and were not on antimalarial drugs for the past 2 weeks or on paracetamol in the past 24 hours and without any sign of anaemia or neurological involvement.

Study design

This was a prospective longitudinal hospital-based case-control study.

Sample size determination

In determining the minimum sample size, the prevalence rate of 57.7% reported by Angyo *et al.* [12] among children with severe malaria attending JUTH was used as the reference.

Using a prevalence of 57.7%, the sample size needed to achieve a precision of 1% at 95% confidence level was obtained from the equation below (Falade *et al.*) [13]:

$$n = \frac{p(1-p)}{(d/Z\alpha/2)^2}$$

Where n= sample size; d=0.01; Zα=1.96; p-value=0.57

$$n = \frac{0.57(1-0.57)}{(0.01/1.96/2)^2}$$

n =100 (i.e 100 cases and 100 controls)

Ethical statement

This study was carried out in line with the ethics guiding research undertakings on human subjects as approved by the ethical committees of University of Ilorin (reference No. UERC/ASN/2014/013) and Jos University Teaching Hospital (reference No. JUTH/DCS/ADM/127/XIX/5933). Informed consents of the children's parent (s) or caregivers were obtained before enrolment, after due explanation of the aims and procedures of the project.

Malaria diagnosis

Malaria parasite test was determined by microscopy using duplicate slides of Giemsa stained thick and thin blood films [14]. Malaria parasite density was determined by the number of parasites/µl of blood (thick film) method using respective patient's total white blood cell count [13]. Hyperparasitaemia in children was defined as parasite count >200 x 10^3 parasites/µl [15].

Administration of drugs

Case children were given 2.4 mg/kg of artesunate intravenously at 0 hours, then 1.2 mg/kg at 12, 24 and 48 hours (if the patient was able to swallow, the daily dose was given orally). This was followed by oral administration of artemether-lumefantrin as 5 to 24mg/kg of artemether and 29 to 144mg/kg of lumefantrin as fixed dose over 3 days [2].

Samples collection and methods of laboratory analysis

After clinical assessment, stool samples were collected once into transparent stool containers from both case and control children. This was used for exclusion of helminthiasis and pathological enteric bacterial infection, using stool microscopy and culture tests. Normal saline method was used for microscopy and Selenite-F and dextrose-citrate agar for culture as was described by Cheesbrough [16].

Five millilitres (5ml) of blood was aseptically collected from both case and control subjects using needle and syringe. In the control, this was done once while in the case children this was done before initiation of treatment on the day of admission (day 0), then 48 hour after initiation of treatment (day 2) [17]. Another sample was collected 48 hours after the last dose of the combination therapy i.e. 7th day of initiation of treatment [18].

Two millilitres (2ml) of the blood was dispensed into EDTA tube for screening tests for exclusion of other abnormalities, malaria parasite and haematological tests. Screening tests for exclusion of other abnormalities carried out include: Haemoglobin genotype for exclusion of sickle cell disease using electrophoresis as described by Roberts and Williams [19]; glucose-6 phosphate dehydrogenase (G6PD test for exclusion of G6PD deficiency using the meth-haemoglobin qualitative method described by Brewer *et al.*,[20]; hepatitis B surface antigen test for exclusion of viral hepatitis using rapid diagnostic test (RDT) kit from Standard Diagnostics, Korea; and blood culture for exclusion of septicaemia by direct aseptic injection of the blood into brain heart infusion broth and thioglycollate broth (at 1:20 dilution) as was described by Cheesbrough [16].

The remaining 3ml of the blood was dispensed into screw-caped plain sample tube for biochemical assays. It was allowed to clot and retract at room temperature (22-27 °C) for about 20 minutes. The serum was separated after centrifuging at 3000 revolutions per minute (RPM) for 5 minutes in a clinical bench top centrifuge (MSE minor England) using Pasteur pipette and divided into three different aliquots into pre-cleaned, dried, metal and steroid free cryo-vials for immediate HIV screening using the immunochromatographic technique (RDT; Standard Diagnostics, Korea) and then stored at -20 °C for analysis of biochemical parameters.

Unless otherwise stated, all the reagents used for this study were of analytical grade and were prepared in distilled-deionized water. Tests were carried out in duplicate tubes.

Haematological analysis

Packed cell volume (PCV) was determined using Hawskley haematocrit centrifuge by centrifuging the sealed blood filled capillary tube at 3000 revolutions per minute for 5 minutes [21]. Haemoglobin level was determined colorimetrically using Drabkins solution as stated by Facer [22]. Total differential leucocytes and platelets counts were done manually [21].

Biochemical assays

Malondialdehyde (MDA), protein carbonyl (PCO) and nitric oxide (NO) concentrations were determined by the methods of Satoh [23], Reznick and Packer [24], and Griess [25] respectively. Superoxide dismutase (SOD) activity was determined by measuring the level of inhibition of epinephrine according to the method of Hara and Irwin [26]. Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide to water and oxygen according to the method of Sinha [27]. Glutathione peroxidase (GPx) activity was determined by measuring the rate of oxidation of glutathione according to the method of Paglia and Valentine [28]. Glutathione reductase (GR) was determined by monitoring the reduction of oxidized glutathione to glutathione in the presence of β-nicotinamide adenine dinucleotide phosphate (NADPH) which is oxidised to $NADP^+$ following the procedures of Goldberg and Spooner [29]. Glutathione (GSH) concentration was determined using the method of Beutler *et al.* [30]. Vitamins A, C and E concentrations were determined by the method of Hasan *et al.* [31]. The serum levels of bicarbonate, glucose and C-reactive protein (CRP) were determined following the methods of Tietz [32], Barham and Trinder [33] and Black *et al.* [34] respectively.

Data analysis

Results were expressed as mean ± standard error of the mean (S.E.M.) for case and control children. Data were analysed using One-way analysis of variance (ANOVA) followed by post hoc Duncan multiple range test and paired t-test, differences were considered signiûcant at $P<0.05$ when compared with control. The procedures were pérformed using SPSS software (version 19.0, SPSS Inc., Chicago, IL).

The responses of the parameters were categorised as: Fastest response (those that their mean levels increased or decreased steadily such that there was no significant difference between their day 2 through day 7 levels when compared with the mean control values, fast response (those that increased or decreased steadily and their mean levels by day 7 were not significantly different with the control), slow response (those that increased or decreased steadily and their mean levels by day 7 were significantly different with control).

Results

Age and gender distribution of the case and control children

Out of the 100 case and 200 control children recruited for this study, 72 (72.0%) and 123 (61.5%) were aged

1 to 5 years respectively and, following the same order, 43(43.0%) and 90 (45.0%) were males. There were no significant differences in age (p=.181) and sex (p =.880) distribution of the children with or without malaria (Table 1).

Table 1: Age and gender distribution of the case and control children

	Control N (%)	Case N (%)	p-value
Age			
1 to 5 years	123(61.5)	72 (72.0)	0.181
5.1 to 10 years	77 (38.5)	28 (28.0)	
Total	200 (100.0)	100 (100.0)	
Gender			
Male	90 (45.0%)	43 (43.0)	0.880
Female	110 (55.0)	57 (57.0)	
Total	200 (100.0)	100 (100.0)	

presented three or more signs of severe malaria before treatment which resolved progressively with treatment. Fever, anaemia, jaundice, respiratory distress and prostration were the most common signs before treatment. None had cerebral malaria. One hundred percent (100%) of the children recovered and were discharged by the 7th day of admission (Table 2).

Responses of selected haematological parameters to artesunate/artemether-lumefantrin combination therapy in children with severe malaria

The levels of packed cell volume (PCV), haemoglobin and platelet count were significantly lower ($p<0.05$) in case children before treatment compared to control but were significantly increased ($p<0.05$) during and after treatment (Table 3). However, the blood counts of total leucocytes, neutrophils, lymphocytes, eosinophils, monocytes and basophils counts were significantly higher ($p<0.05$) in case children before treatment compared to control. Total leucocytes, neutrophils count and lymphocytes count levels were significantly reduced ($p<0.05$) in case children during treatment and normalised after treatment (Table 3). The eosinophils, monocytes and basophils counts were

Table 2: Clinical presentations in children with severe malaria; before, during and after treatment

Clinical presentations	Day 0 N (%)	Day 2 N (%)	Day 7 N (%)
Fever + vomiting + jaundice + hepatomegaly + anaemia	13(100.0)	0 (0.0)	0 (0.0)
Fever + anaemia + prostration + jaundice + respiratory distress	29(29.0)	0 (0.0)	0 (0.0)
Fever + prostration + jaundice	21 (21.0)	0(0.0)	0 (0.0)
Fever + prostration + anaemia + respiratory distress + diarrhoea + vomiting	12 (12.0)	0 (0.0)	0 (0.0)
Fever + unconsciousness + respiratory distress + diarrhoea + bleeding + anaemia	6 (6.0)	0 (0.0)	0 (0.0)
Fever + anaemia + jaundice	10(10.0)	0 (0.0)	0 (0.0)
Fever + convulsion + diarrhoea+ anaemia	5 (5.0)	0 (0.0)	0(0.0)
Fever + jaundice	0(0.0)	8 (8.0)	0 (0.0)
Fever + anaemia	0 (0.0)	5 (5.0)	0 (0.0)
Fever + anaemia + hepatomegaly + jaundice	0 (0.0)	4 (4.0)	0 (0.0)
Fever	0 (0.0)	34 (34.0)	0 (0.0)
Anaemia	0 (0.0)	5(5.0)	2 (2.0)
Jaundice	0 (0.0)	5 (5.0)	0 (0.0)
Anaemia + hepatomegaly	0 (0.0)	0 (5.0)	2(2.0)
Improving	0 (0.0)	11(11.0)	0 (0.0)
Stable	0 (0.0)	28(28.0)	96 (96.0)
Others (uncommon signs presented by few of the children include: cyanosis, dysuria and oliguria)	4 (4.0)	0 (0.0)	0 (0.0)
Total	100(100.0)	100(100.0)	100 (100.0)

Day 0= before treatment; Day 2= 48 hours of treatment; Day 7= 48 hours after treatment.

Clinical presentations in children with severe malaria before, during and after treatment

We documented the clinical presentations of children with severe *P. falciparum* malaria; before treatment, 48 hours of treatment and 48 hours after treatment. One hundred percent (100%) of the children

normalised in case children during treatment and after treatment (Table 3).

Table 3: Haematological parameters of children with severe malaria treated with artesunate/artemether-lumefantrin combination therapy

Haematological indices	Control	Cases		
		Day 0	Day 2	Day 7
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
PCV (%)	36.68 ± 0.27[a]	26.59 ±0.50[d]	31.11± 0.16[c]	34.16 ± 0.22[b]
Haemoglobin (g/dl)	12.15 ± 0.09[a]	8.86 ±0.17[d]	10.36 ± 0.05[c]	11.28 ± 0.08[b]
Total leucocytes (x10⁹/L)	6.14 ± 0.10[a]	9.50 ± 0.30[c]	8.23 ± 0.23[b]	6.63 ± 0.18[a]
Neutrophils count (x10⁹/L)	2.25 ± 0.06[a]	4.91 ±0.23[c]	3.76 ± 0.18[b]	2.54 ± 0.12[a]
Lymphocytes count (x10⁹/L)	3.85 ± 0.09[a]	4.49 ±0.17[b]	4.44 ± 0.11[b]	4.03 ± 0.09[a]
Eosinophils count (x10⁹/l)	0.0003 ± 0.00006[a]	0.056 ±0.010[b]	0.008 ± 0.002[a]	0.006 ± 0.003[a]
Monocytes count (x10⁹/L)	0.006 ± 0.001[a]	0.15 ± 0.03[b]	0.02 ± 0.004[a]	0.000 ± 0.000[a]
Basophils count (x10⁹/L)	0.00 ± 0.00[a]	0.0001 ± 0.00006[b]	0.00 ± 0.000[a]	0.00 ± 0.000[a]
Platelets count (x10⁹/L)	283.17 ± 1.71[a]	178.64 ± 3.95[d]	215.62 ± 1.11[c]	240.74 ±1.40[b]

Each value is a mean of n determinations ± S.E.M. (n is 100 for case children and 200 for control children). Values carrying different superscripts along the same row are significantly different ($p<0.05$).
Day 0 = before treatment; Day 2 = 48 hours of treatment; Day 7 = 48 hours after treatment.

Table 4: Serum levels of selected oxidative biomarkers of children with severe malaria treated with artesunate/artemether-lumefantrin combination therapy

Oxidative Biomarkers	Control	Cases		
		Day 0	Day 2	Day 7
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
MDA (µmol/ml)	7.46 ± 0.25[a]	23.50 ± 0.55[c]	10.34 ± 0.42[b]	10.97 ± 0.40[b]
PCO (nmol/ml)	35.24 ± 0.48[a]	223.22 ± 6.55[d]	141.22 ± 3.16[c]	50.22 ± 2.24[b]
NO(µmol/L)	222.74 ± 1.77[a]	183.49 ± 5.36[c]	203.78 ± 2.25[b]	239.71 ± 4.60[d]

Each value is a mean of n determinations ± S.E.M. (n is 100 for case children and 200 for control children). Values carrying different superscripts along the same row are significantly different ($p<0.05$).
Day 0 = before treatment; Day 2 = 48 hours of treatment; Day 7 = 48 hours after treatment.
MDA = malondialdehyde; PCO = protein carbonyls; NO = nitric oxide

Table 5: Serum levels of selected enzymatic antioxidants of children with severe malaria treated with artesunate/artemether-lumefantrin combination therapy

Enzymatic Antioxidants	Control	Cases		
		Day 0	Day 2	Day 7
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
SOD (U/ml)	2.88 ±0.14[a]	0.76 ± 0.08[b]	1.04 ± 0.08[b]	3.01 ± 0.20[a]
CAT (U/ml)	44.41 ± 1.05[a]	9.71 ± 0.46[d]	14.73 ± 0.81[c]	20.13 ± 0.62[b]
GPx (U/l)	19.49 ±0.46[a]	392.62 ± 32.43[c]	164.41 ± 2.54[b]	29.84 ± 1.00[a]
GR (U/l)	63.04 ± 0.80[a]	183.94 ± 5.81[c]	106.74 ± 1.55[b]	68.18 ± 1.12[a]

Each value is a mean of n determinations ± S.E.M. (n is 100 for case children and 200 for control children). Values carrying different superscripts along the same row are significantly different ($p<0.05$).
Day 0 = before treatment; Day 2 = 48 hours of treatment; Day 7 = 48 hours after treatment.
SOD = superoxide dismutase; CAT= catalase; GPx = glutathione peroxidase; GR= glutathione reductase

Responses of selected serum oxidative biomarkers to artesunate/artemether-lumefantrin combination therapy in children with severe malaria

The responses of serum levels of malondialdehyde (MDA), protein carbonyls (PCO) and nitric oxide (NO) in case children before treatment (day 0), 48 hours of treatment (day 2) and 48 hours after treatment (day7) showed that MDA and PCO levels were significantly higher ($p<0.05$) in case children before, during and after treatment compared to control (Table 4). However, treatment with artesunate/artemether-lumefantrin combination therapy significantly reduced ($p<0.05$) the levels of MDA and PCO (Table 4). NO level was significantly lower ($p<0.05$) before and during treatment, but was significantly increased ($p<0.05$) after treatment in case children compared to control. Treatment with artesunate/artemether-lumefantrin combination therapy significantly increased ($p<0.05$) NO level (Table 4).

Responses of selected serum antioxidants to artesunate/artemether-lumefantrin combination therapy in children with severe malaria .

The responses of some serum enzymatic antioxidants to artesunate/artemether-lumefantrin combination therapy in children showed that the activity of SOD was significantly lower ($p<0.05$) in case children before and during treatment with artesunate/artemether-lumefantrin combination therapy compared to control but was normalised after treatment (Table 5). Catalase activity was significantly lower ($p<0.05$) in case children before, during and after treatment with artesunate/artemether-lumefantrin combination therapy compared to control but the activity was significantly increased ($p<0.05$) by treatment. The activities of GPx and GR were significantly higher ($p<0.05$) in case children before and during treatment with artesunate/artemether-lumefantrin combination compared to control but were normalised after treatment (Table 5). The levels of GSH, vitamins A, C and E were significantly lower ($p<0.05$) in case children before, during and after treatment with artesunate/artemether-lumefantrin combination therapy compared to control but their levels were significantly increased ($p<0.05$) by treatment (Table 6).

Responses of serum bicarbonate, glucose and C-reactive protein to artesunate/artemether-lumefantrin combination therapy in children with severe malaria

The responses of serum bicarbonate, glucose and C-reactive protein (CRP) concentrations to artesunate/artemether-lumefantrin combination therapy in children with severe malaria showed that the level of bicarbonate was significantly lower ($p<0.05$) in case children before treatment compared to control but was normalised during and after treatment (Table 7). Also, glucose concentration was significantly increased ($p<0.05$) in case children before treatment compared to control, but was normalised after treatment (Table 7). The level of CRP was significantly higher ($p<0.05$) in case children before treatment compared to control but was reduced significantly ($p<0.05$) by treatment (Table 7).

Table 6: Serum levels of selected non-enzymatic antioxidants of children with severe malaria treated with artesunate/artemether-lumefantrin combination therapy

Non-enzymatic Antioxidants	Control	Cases		
		Day 0	Day 2	Day 7
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
Glutathione (mg/l)	395.72 ± 9.55^{a}	171.94 ± 6.18^{d}	248.34 ± 5.82^{c}	344.92 ± 3.37^{b}
Vitamin A (µg/dl)	45.50 ± 0.48^{a}	21.13 ± 0.51^{d}	26.60 ± 0.59^{c}	34.80 ± 0.39^{b}
Vitamin C (mg/dl)	7.52 ± 0.31^{a}	2.51 ± 0.13^{c}	3.93 ± 0.11^{b}	3.78 ± 0.06^{b}
Vitamin E (mg/dl)	5.63 ± 0.43^{a}	2.92 ± 0.11^{c}	3.04 ± 0.05^{c}	4.61 ± 0.07^{b}

Each value is a mean of n determinations ± S.E.M. (n is 100 for case children and 200 for control children). Values carrying different superscripts along the same row are significantly different ($p<0.05$).
Day 0 = before treatment; Day 2 = 48 hours of treatment; Day 7 = 48 hours after treatment.

Responses of selected combined serum parameters to artesunate/artemether-lumefantrin combination therapy in children with severe malaria

The levels of some combined biochemical parameters to artesunate/artemether-lumefantrin combination therapy in children with severe malaria were investigated in this study. The levels of HCO_3/Glucose, GSH/NO and GSH/PCO ratios were significantly lower ($p<0.05$)in case children before treatment compared to control while the levels of

PCO/SOD and CRP/SOD ratios were significantly higher ($p<0.05$) in case children before treatment compared to control (Table 8). During treatment, the levels of HCO_3/glucose, PCO/SOD and CRP/SOD ratios were normalised and sustained after treatment. The levels of GSH/NO and GSH/PCO ratios in case children were significantly increased ($p<0.05$) during and after treatment (Table 8).

redistribution of these leucocytes from peripheral circulation to their primary resident tissues following the introduction of effective antimalarial drug, destruction of the parasites and subsequent fall in inflammatory reactions [35-38]. This finding corroborates the report of Ayodele [39] that these haematological parameters (eosinophils, monocytes and basophils) have direct response to the malaria

Table 7: Serum levels of bicarbonate, glucose and C-reactive protein of children with severe malaria treated with artesunate/artemether-lumefantrin combination therapy

Parameters	Control	Cases		
		Day 0	Day 2	Day 7
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
Bicarbonate (mmol/l)	24.55 ± 0.25[a]	22.28 ± 0.51[b]	25.13 ± 0.61[a]	24.15 ± 0.40[a]
Glucose (mmol/l)	3.85 ± 0.05[a]	4.40 ± 0.13[b]	4.36 ± 0.11[b]	4.02 ± 0.11[a]
C-reactive protein (mg/l)	6.05 ± 0.24[a]	120.54 ± 4.65[d]	80.69 ± 4.65[c]	20.21 ± 8.47[b]

Each value is a mean of n determinations ± S.E.M. (n is 100 for case children and 200 for control children). Values carrying different superscripts along the same row are significantly different ($p<0.05$).
Day 0 = before treatment; Day 2 = 48 hours of treatment; Day 7 = 48 hours after treatment

Table 8: Serum levels of selected combined biochemical parameters of children with severe malaria treated with artesunate/artemether-lumefantrin combination therapy

Combined Parameters	Control	Cases		
		Day 0	Day 2	Day 7
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
HCO_3^-/Glucose	6.05 ±.12[a]	4.85 ±.20[b]	5.60 ± 0.21[a]	6.07 ± 0.24[a]
GSH/NO	1.30 ± .05[a]	0.41 ± 0.06[d]	0.80 ± 0.04[c]	1.12 ± 0.04[b]
GSH/PCO	11.17 ± .32[a]	0.35 ± 0.06[d]	1.37 ± 0.07[c]	7.30 ± 0.24[b]
PCO/SOD	65.59± 13.40[a]	2880.66 ± 413.04[b]	255.39 ± 19.96[a]	26.71 ± 2.73[a]
CRP/SOD	4.63 ± .55[a]	1705.21 ± 249.43[b]	163.99 ±17.34[a]	11.37 ± 1.40[a]

Each value is a mean of n determinations ± S.E.M. (n is 100 for case children and 200 for control children). Values carrying different superscripts along the same row are significantly different ($p<0.05$).
Day 0 = before treatment; Day 2 = 48 hours of treatment; Day 7 = 48 hours after treatment.
GSH = glutathione, NO = nitric oxide; PCO = protein carbonyl; SOD = superoxide dismutase; CRP = C-reactive protein

Discussion

In this study, we demonstrate that the blood count of eosinophils, monocyte, basophils and serum level of HCO_3^-, PCO/SOD, CRP/SOD and HCO_3^-/Glucose respond quickly to artesunate/artemether-lumefantrin combination therapy in children with severe malaria.

Severe malaria is associated with alterations in the normal ranges of haematological parameters [12]. The fastest response of the blood count of eosinophils, monocytes and basophils to artesunate/artemether-lumefantrin combination therapy in children with severe malaria could be due to fast parasite and anti-malarial drug. The fast response of TWBC to treatment as observed in this study could have been influenced by the fast responses of N and L which are the predominant leucocytes in peripheral circulation even in the control subjects [37]. The rates of responses of TWBC, N and L could be related to increased splenic sequestration of leucocytes following antimalarial therapy [39] and haemoglobin are markers of erythropoietic function [40]. The slow responses of PCV and haemoglobin as observed in this study could be explained on the premise that artemisinins act by lysing parasite infected erythrocytes and clearance of the parasites

from circulation elicits the red blood cells to divide gradually and replenish the host blood [40] thus, causing the slow response of these indices to treatment. This observation is in agreement with the report of Camacho *et al.* [41] which showed that anaemia was resolved on day 28.

Severe malaria induces oxidative stress which is associated with alterations in the normal ranges of oxidative biomarkers in the serum [42, 43]. In this study, the slow response of serum levels of the oxidative parameters (malondialdehyde, protein carbonyls and nitric oxide) to artesunate/artemether-lumefantrin combination therapy in children with severe malaria makes them unfit for early monitoring of therapeutic response and recovery in children with severe malaria. Artemisinin drugs are oxidative drugs which cause increase in reactive species and oxidation of lipids and proteins [45, 46]. Thus, the effect of artemisinin could account for the slow responses of the oxidative parameters to treatment. Fabbri *et al.* [46] reported that oxidative stress biomarkers resolved and were not significantly different from control by day 14 in treated patients with *P. vivax* infection.

The normal serum levels or activities of both enzymatic and non-enzymatic antioxidants were altered in patients with severe malaria [47-50]. The fast response of SOD to the treatment in this study may be due to the enzyme's high catalytic efficiency. It has been reported that SOD has a very large catalytic efficiency (kcat/K_M) of ~7 x10^9 M^1S^1 [50]. The fast responses of serum GR and GPx activities to the treatment in this study could be related to the fast oxidation of GSH to GSSG and reduction of GSSG to GSH by these enzymes due to rise in reactive species as a result of the oxidative action of artemisinins [44]. Serum catalase activity unlike the other antioxidant enzymes showed a slow response to treatment in this study despite its reported high catalytic efficiency of 4.0x$10^8$$M^{-1}s^{-1}$ [51]. This could be due to the binding of artemisinin to the enzyme's heme protein [52], thereby retarding the activity of the enzyme [53] and hence response to treatment and patient's recovery. The slow responses of all the non-enzymatic antioxidants (GSH, vitamins A, C and E) to treatment could be related to the increase in reactive species due to the oxidative artemisinin drug [44, 45].

Severe malaria also alters the normal level of bicarbonate [54], glucose [54] and C-reactive protein [55] in the serum. Serum bicarbonate is a marker of respiratory and renal functions [56]. The fastest response of serum bicarbonate to artesunate/artemether-lumefantrin combination therapy in children with severe malaria observed in this study may be attributed to the fast rise in pH due to decreased lactic acid production because of parasite destruction and clearance [57]. The fastest response of serum bicarbonate could also be due to the very fast response of carbonic anhydrase; the enzyme responsible for interconversion of carbondioxide and bicarbonate with catalytic efficiency (kcat/K_m) of 10^8 $M^{-1}S^{-1}$ [58]. The fast response of serum glucose to treatment observed in this study could be related to the hyperinsulinaemic effect of artemisinin drugs and the short half-life of these drugs [59]. CRP is one of the most widely used acute phase protein because of its fast rise and rapid kinetics [60,61]. From this study, the slow response of serum CRP to treatment could suggest that some inflammatory reactions were still on in the children even though they had recovered [62].

The serum levels of the selected combined serum parameters that are associated with severe malaria can be helpful for monitoring therapeutic response in children with the disease [63]. The fastest responses of combinations of some of the parameters such as PCO/SOD, CRP/SOD and HCO_3^-/glucose to treatment as obtained in this study were in consonance with the finding that combining parameters from distinct pathological pathways improve predictive accuracy over individual biomarkers and each marker may independently contribute information regarding the nature of disease and therapeutic response [63]. This implies that the fastest response parameters are useful in early detection of treatment success or failure and recovery in children with severe malaria as well as early detection of uncommon adverse drug side effect that may be applicable to few individuals.

In conclusion, this study shows that the level of eosinophils, monocytes, basophils, HCO_3^-, PCO/SOD, CRP/SOD and HCO_3^-/glucose have the fastest responses to treatment in severe malaria and can therefore be employed for early monitoring of therapeutic response in children with the disease.

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