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The value of C–reactive protein in the diagnosis of septicaemia in children with malaria

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Malaria and septicaemia, both major causes of infant and early childhood morbidity and mortality in Nigeria, often co-exist and are difficult to differentiate. This study was designed to test the hypothesis that C-reactive protein (CRP) levels could differentiate between malaria and malaria coexisting with septicaemia. One hundred and fifty-one children aged 6 to 60 months with fever without localising signs and 141 aged/sex-matched controls were studied. C-reactive protein levels in all the children were determined while the febrile children had their blood cultures done. ANOVA and students't' test were used to determine the difference between groups. Sensitivity, negative predictive and positive predictive values for malaria coexisting unit septicaemia were calculated for various levels of CRP. One hundred and thirty (86.1%) of the subjects had malaria alone while 21 (13.9%) had malaria coexisting with septicaemia. Organisms isolated were mainly *Enterobacteriaceae* (7), *Staphylococcus aureus* (9), *Salmonella* spp. (4) and *Streptococcus pneumoniae* (1). The mean serum CRP levels in subjects with malaria alone and malaria coexisting with septicaemia were 82.16 ± 44.94 mg/l and 108.44 ± 55.65 mg/l respectively (P=0.0176). At the diagnostic level of 90 mg/l (value just greater than the mean for malaria alone), CRP was highly sensitive (sensitivity 76.2%) in detecting septicaemia in 21 subjects with co-morbidity while specificity (36.9%) was low. It is concluded that CRP can differentiate between malaria and malaria with septicaemia. In children with malaria, antibiotics should be started at the CRP level of ≥ 90 mg/l.

Key words: Malaria, septicaemia, C-reactive protein, diagnosis.

INTRODUCTION

Malaria infection, and septicaemia defined as the presence of infection (in the blood) together with systemic

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manifestations of infection (Levy, 2003; Dellinger, 2013), are major causes of infant and early childhood morbidity and mortality in Nigeria (Adepoju et al., 2017; Akpede et al., 1993). Both often co-exist (Akpede et al., 1993; Tupchong et al., 2015). While each has different modality of treatment, clinical differentiation can be difficult. This causes delays in treatment and worsens outcome. In addition, attempt to use laboratory parameters like white blood cell count, absolute neutrophil count and erythrocyte sedimentation rate to differentiate malaria from malaria coexisting with septicaemia have not been sufficiently reliable (Rasmussen and Rasmussen, 1982; Enyuma et al., 2015). However, C-reactive protein CRP, an acute phase reactant, has been found useful in differentiating bacterial from non-bacterial infections in various circumstances (Liu et al., 2013; Sabel et al., 1974). Though malaria infection is known to stimulate CRP production, we hypothesized that malaria co-existing with septicaemia would stimulate even more CRP production. This work was designed to determine the value of C-reactive protein levels in differentiating between malaria alone and malaria with septicaemia in children.

MATERIALS AND METHODS

Consecutive studies of 292 (151 febrile subjects without localizing sign/signs and 141 age/sex matched healthy controls) children, aged 6-60 months were carried out. These were attending the Children Emergency Room (CHER) and the Child Welfare Clinic (CWC) of the University of Calabar Teaching Hospital (UCTH) Nigeria. All had their blood films (thick and thin) examined for malaria parasite after staining with Giemsa stain and read under $\times$100-power microscope (WHO, 1991). In addition, blood culture (not done on controls), was done and antibiotic sensitivity of isolated organisms was determined by the Disk Diffusion Method (Tendencia, 2004). Erythrocyte sedimentation rate (ESR) (Westergren method) was also done. Total white blood cell count (WBC) absolute neutrophil count (ABNC) were also done within 2 h of collection of sample by an Automated Haematology System Cell Counter (ADVIA(g) 60 Closed Tube (CT) manufactured by Bayer Cooperation, New York, United States of America. Serum CRP was estimated for both subjects and controls using High Sensitivity Enzyme Immunoassay (EIA) kit – Kalon Biological Ltd., U.K. Data were analysed in groups using EPI info version 6; 2002. ANOVA and Students t-test were used to determine the significance of the difference between three and two groups respectively. A p-value of < 0.05 was regarded as significant. Sensitivity, specificity, positive predictive value and negative predictive value for malaria coexisting with septicaemia were calculated for various levels of CRP. Ethical clearance was obtained from the Reasearch Ethics Committee of the University of Calabar Teaching Hospital, Calabar, Nigeria.

RESULTS

One hundred and fifty one subjects were recruited. Eighty-six (57.0%) were males while 65 were females (43.0%) giving a male to female ratio of 1.3: 1. One hundred and thirty (86.1%) subjects had malaria alone while 21 (13.9%) had malaria coexisting with septicaemia. Organisms isolated were *Staphylococcus aureus* (9), *Enterobacteraeciae* (7), *Salmonella* spp. (4) and *Streptococcus pneumoniae* (1) most of which were sensitive to gentamycin and ceftriaxone (Table 1). The mean age of those who had malaria with coexisting septicaemia was 20.28 $\pm$ 9.27 months with a range of 6 to 38 months. The means of WBC count, absolute neutrophil count, and ESR could not differentiate subjects with malaria alone from those with malaria coexisting with septicaemia (Table 2a and b). The range of CRP levels for children with malaria alone was 0.00 – 210.70 mg/L and for children with malaria and septicaemia 7.30-202.80 mg/dL. The respective means were 82.16 $\pm$ 44.94 mg/l and 108.44 $\pm$ 55.65 mg/L respectively. This difference was statistically significant at $p<0.0176$ (Table 3). At a diagnostic level of 90 mg/l (value greater than the mean for malaria alone), CRP was highly sensitive (sensitivity=76.2%) in detecting septicaemia in 21 subjects with co-morbidity while the specificity was low (36.9%) (Table 4). Out of 141 aparasitaemic controls, 37 had serum CRP values above normal (0.2 to 6.0 mg/l).

DISCUSSION

This study has confirmed a high prevalence (13.9%) of septicaemia coexisting with malaria in young children. The main isolates were *Enterobacteriaceae, S. aureus* and *Salmonella* spp. Most (88.8%) of these isolates were sensitive to gentamycin and ceftriaxone. The high prevalence of septicaemia in children with malaria has been attributed to possible impairment of the capacity of monocytes that have ingested erythrocytes to phagocytose and kill bacteria that invade the blood stream. Also, the immune system of children with *Plasmodium falciparium* malaria is thought to be further impaired by a transient loss of B-cell function and reduction in T-lymphocytes (Weinstein and Swartz, 1974; Whittle et al., 1984). In addition, cytoadherence of infected erythrocytes to vascular endothelium occurs in *P. falciparum* malaria (Warrell et al., 1990). This may lead to obstruction of blood flow and capillary damage with resultant vascular leakage of protein and fluid, and oedema and tissue anoxia in the brain, heart, lungs, intestines, and kidneys. Damage to the intestinal mucosa due to tissue anoxia makes it possible for enteric organisms to escape into the blood stream (Warrell et al., 1990; John, 2016). This may explain why enteric organisms were the main isolates in this study.

The means of WBC count, absolute neutrophil count, and ESR could not differentiate subjects with malaria alone from those with malaria coexisting with septicaemia. Several factors including haemolysis

Table 1. Antimicrobial sensitivity pattern of isolates from children with septicaemia.

Sensitivity	Organisms Isolated			
	Entero (7)	Staph (9)	Salmon (4)	Strep (1)
Gentamycin	7(100.0%)	8(88.8%)	4(100.0%)	1(100.0%)
Erythromycin	-	8(88.8%)	-	1(100.0%)
Penicillin	-	0(0.0%)	-	-
Chloramphenicol	2(28.6%)	4(44.4%)	0(0.0%)	0(0.0%)
Ampicillin	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Cotrimoxazole	2(28.6%)	1(11.1%)	0(0.0%)	0(0.0%)
Streptomycin	4(57.1%)	5(55.5%)	-	-
Ceftazidime	6(85.7%)	7(77.7%)	4(100.0%)	-

Entero=*Enterobacteraeceae*; Staph=*Staphylococcus aureus*; Salmon=*Salmonella spp*; Strep=*Streptococcus pneumonia*. Figures in parentheses represent the total number of each bacterial isolate. - = not tested

Table 2a. The means, standard deviations and ranges of WBCS, ABNCS and ESR in children with malaria alone, malaria with septicamia and in controls.

Status	WBC/mm^3				ABNCs/mm^3			ESR/ mm in 1st h			P-value
	Number of Subjects	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
Malaria	130	7.39	4.25	1.20 - 23.50	4166.83	2774.32	247.00 - 14775.00	46.31	32.18	3.0 - 145.00	0.1532
Malaria with septicaemia	21	6.72	4.37	3.20 - 19.70	3527.95	2747.82	779.00 - 1152.00	35.57	29.17	1.0 -100.00	
Control	141	7.17	2.93	0.00 - 21.00	2825.64	1917.21	430.00 -16758.00	18.82	19.65	0.00 - 130.00	

Table 2b. Comparison of all groups using T-stat.

Status	Number of subjects	Comparison	WBC	ABNC	ESR	CRP
Malaria(a)	130	a versus b	*1.9021 (0.4082)	0.9804(0.3285)	1.435 (0.1532)	2.4015 **(0.0176)**
Malaria with Septicaemia(b)	21	b versus c	*3.2106 (0.3013)	1.4700 (0.1436)	3.395 (0.0009)	20.710 (0.0000)
Controls(c)	141	a versus c	*0.3398 (0.7062)	4.6400 (0.0000)	8.555 (0.0000)	19.85 (0.0000)

*=T-stat. Figures in parenthesis are p-values. WBC = White blood count; ABNC =Absolute neutrophil count; CSR = Eythrocyte sedimentation rate; CRP = C- reactive protein.

Table 3. Mean CRP levels and p-values in subjects with malaria, malaria with bacteraemia, and in controls.

Status	Number of subjects	CRP mg/l			Comparison	T-stat	p-value
		Mean	SD	Range			
Malaria (a)	130	82.16	44.94	0.00 – 210.70	a versus b	2.40	0.02
Malaria with septicaemia (b)	21	108.44	55.65	7.30 – 202.50	b versus c	20.710	0.00
Controls (c)	141	5.58	8.52	0.00 – 38.00	a versus c	19.85	0.00

Comparison of all groups:F-stat= 209.32; p-value=0.00.

Table 4. sensitivity, specificity, and positive and negative predictive values of CRP 40 to 100 mg/L in subjects with malaria coexisting with septicaemia.

CRP (MG/L)	Sensitivity	Specificity	Positive predictive values	Negative predictive values
50	81.0	27.6	15.3	90.0
60	81.0	30.0	15.7	90.6
70	81.0	33.1	16.3	91.5
80	76.2	36.9	16.3	90.5
90	76.2	36.9	16.3	90.5
100	38.1	83.1	27.0	89.2

(common in malaria), endogenous steroid, and catecholamines lead to increase in WBC and absolute neutrophil count, while female sex, anaemia, dilutional problem, increased temperature of specimen especially in the tropics, and a tilted measuring tube lead to increase in ESR. These factors might come into play during acute illnesses thereby rendering WBC, absolute neutrophil count, and ESR less appropriate for use in the detection of septicaemia in children with malaria (Philips et al., 1986; McLellan and Giebink 1986; Bouree et al., 2000; Enyuma et al., 2015).

While malaraia alone stimulated CRP production in this study, those with malaria coexisting with septicaemia had higher mean serum CRP levels (108.44 mg/l) than those with malaria alone (82.16 mg/l). This difference was highly significant ($p<0.0176$). Whether this high mean level in subjects with co-morbidity was as a result of a synergistic or summation effect is unclear. It is probable that their combined activation of mononuclear cells resulted in the production of higher levels of inflammatory cytokines (tumour necrosis factor, interleukins 1, 2 and 6) that stimulated hepatic synthesis of higher inflammatory proteins including CRP (Bouree et al., 2000). It has been observed that patients with bacterial infections tend to have higher peaks of CRP than those with other conditions (Landry et al., 2017). Thus, serum CRP could distinguish between malaria and malaria co-existing with septicaemia. When this was done at CRP value of 90 mg/l, it gave a sufficiently high sensitivity of 76.2% and a negative predictive value (NPV) of 90.5% but a low specificity of 36.9% and positive predictive value (PPV) of 16.3%. Despite the low specificity and low positive predictive value, CRP has several advantages over ESR, WBC`, and absolute neutrophil count. It is known to rise 6-12 hafter onset of illness (Kohli et al., 1993) and not affected by immunological status of the child, age, sex, or anaemia (Black et al., 2004). It returns to normal within a week of successful treatment while it takes three to six weeks for ESR to do so (McLellan and Giebink, 1986; Kohli et al., 1993). It is cost effective and is adapted for easy technique of measurement (Kohli et al., 1993). In India, Chhatriwala et al. (2014) demonstrated the prognostic value of CRP in children with malaria, though they did not explore the role of septicaemia in this response.

Septicaemia is rapidly fatal in young children if not treated promptly. Therefore, it is recommended that at CRP levels of ≥ 90 mg/l antibiotics should be started. We recommend in our environment that gentamycin and ceftriaxone be used. These can be withdrawn if preliminary blood culture result is negative.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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