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LEVELS OF EOSINOPHIL AND MICROPHAGE-SECRETED CYTOKINES IN PLASMODIA- INFECTED CHILDREN IN PORT HARCOURT, NIGERIA

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ABSTRACT
Infection with plasmodia and the attendant complications, including hyper-secretion of pro-inflammatory cytokines, remain a leading cause of high morbidities and mortalities among children in African countries and moreso in Nigeria. This study was aimed at assessing the levels of some eosinophil and macrophage-secreted cytokines (IL-1beta, IL-6, IL-10 and TNF – alpha) in children between 2-12 years of age infected with malaria parasite in Port Harcourt, Nigeria. Blood samples were collected from 352 children (215 test subjects and 137 controls) who were visiting the Rivers State University Teaching Hospital, New Mile One Hospital and Rivers State College of Health Science and Technology Health Centre, Port Harcourt. Thick blood films were stained with Giemsa method and parasitemia were determined. The cytokines levels were estimated using the ELISA machine (Stat fax-2100) and result reported according to the manufacturers manual. Out of 352 samples that were examined, 215 were infected with various degrees of parasitemia, while the remaining 137 were not infected. There was no case of complication or severe anaemia in malaria as hemoglobin (Hb) of < 5g/dl or packed cell volume (PCV) of < 15% with parasitemia of > 250,000 parasites/ul. On the cytokines, regression analysis demonstrated a negative relationship between parasite density and IL-10 (p=0.09), IL-10 and IL-6 (p= 0.017) while positive relationship were seen between IL-6 parasite density with a p-value of 0.000, TNF-a and parasite density with a p-value of 0.1248, IL-10 and IL-1beta with p-value of 0.4582. The cytokines also showed mean values such as 139.33 ± 7.7, 17.74 ± 1.36, 466.17 ± 21.59 and 66.57 ± 4.03 for IL-10, IL-1beta, IL-6 and TNF-alpha respectively. The raised plasma level of cytokine, IL-10 among the subjects in this investigation could be an advantage as it inhibits the production of pro-inflammatory cytokines that are triggered by malaria parasites or a disadvantage as it down regulates the response of the immune system.

KEYWORDS: Cytokines, Plasmodia, Malaria.

INTRODUCTION
Malaria is very common in the tropics and ranks amongst the first three killer diseases in Africa. Children below the age of five and pregnant women are the groups with the highest risk of the disease that present as anaemia and cerebral malaria.[1,2] It has been a major public health problem, causing more than 781,000 deaths/year inspite of various interventions aimed at eliminating or reducing the scourge. These measures, among others are said to have saved an estimated 735,000 lives and in 34 African countries in the last decade.[3] Nonetheless, in most African countries, malaria is still the leading cause of illness and deaths, accounting for 25–40% of all outpatient visits at health care facilities, 20% of hospital admissions, and 15% of inpatient deaths.[4] Malaria is caused by the protozoan parasite *Plasmodium. P. falciparum, P. ovale, P. vivax, P. knowlesi and P. malariae* are the most common species, with *P. falciparum* being the most virulent. The World Health Organization recommends that persons in epidemiological settings with suspected malaria should receive a parasitological confirmation of diagnosis,[3] Microscopic detection and identification of plasmodium species in Giemsa-stained thick blood films (for screening) and thin blood films (for species confirmation) is the accepted worldwide "gold standard" used for the routine diagnosis of malaria.[3,5]

Eosinophil (acidophil) is one of the immune system components responsible in fighting parasitic infections. It can be stimulated either directly by malaria parasites or by cytokines or by other mediators produced during the malaria infection. They play a protective role against malaria infection by killing the malaria parasites but may also exert a negative effect if not regulated, by releasing granule proteins like eosinophil cationic protein(ECP)

and eosinophil protein X/eosinophil-derived neurotoxin.[6]

Macrophages are phagocytic cells derived from monocytes at its late stage. Macrophages together with pituitary organ and numerous cell types, including dendritic cells and White blood cells produce movement inhibitory defensive or damaging role in the resistant reaction to various pathogens.[7] Macrophages are found in every tissue wondering about in a non-directional movement (Amoeboid Movement) and waiting for a potential pathogen. Macrophages are of different forms and are called different names in different parts of the body: kuffer cells in the liver, histocytes in the bone marrow, microglia (neuroglia) in the brain amongst others, but they all belong to the mononuclear phagocyte system.[8] Macrophages are classified into M1 and M2 macrophages, M1 being those that support inflammation while M2 macrophages are those that are against and decrease inflammation but encourage tissue repair. Their difference is seen in their metabolism as M1 macrophages possess the ability to stimulate arginine to the killer molecule known as nitric oxide, while M2 macrophages have the ability to stimulate arginine to the repair molecule ornithine.[9]

Cytokines such as IL-10, IL-1beta, IL-6, IL-12, TNF-alpha, IFN-gamma amongst others are secreted during malaria infection. They are immune cells and mediate the innate immune response of the host against pathogens. The pro-inflammatory cytokines (IL-1, IL-6, IL-12, TNF-alpha, IFN-gamma amongst others) function in killing and clearance of parasites while IL-10 (anti-inflammatory cytokine) tend to moderate the secretion of the pro-inflammatory cytokines to prevent hyper-secretion that could be detrimental to the host. Data on the level of eosinophil and macrophage-secreted cytokines in children infected with malaria parasite in Port Harcourt, Nigeria, have not been captured previously, hence the reason for this study in the area.

MATERIALS AND METHODS

Study Area

Study was carried out at the Rivers State University Teaching Hospital (formerly known as BraithWaite Memorial Specialist Hospital (BMSH), New Mile One Hospital and Rivers State College of Health Science and Technology Health Centre, all located in Port Harcourt, Nigeria.

Sample Collection and Processing

Following ethical approval obtained from Rivers State Hospital Management Board and consent from Parents/Guardians, Two milliliters (2ml) of whole un-coagulated blood were randomly collected from Three hundred and fifty-two (352) children, aged between 2-12 years, who were visiting the aforementioned Health facilities from February to July, 2018.

Inclusion Criteria

These included children within 2-12 years, who were infected with malaria parasites. Furthermore, children within 2-12years who were not infected with malaria parasites and had not also been treated for malaria for about one month, served as control.

Exclusion Criteria

Children below the age of 2years and those above the age of 12, children that have been treated of malaria within the space of 2-3 weeks prior to the day of sample collection were excluded from this research.

Procedure

Thick blood films were made on glass slide, dried and stained with giemsa and examined under the microscope using 100X objective for parasite determination. Complete blood counts were estimated using Sysmex XP-300 Haematology Auto-Analyzer according to the manufacturer's manual. The cytokines IL-10, IL-6, IL-1beta and TNF-a were estimated according to the reagent manufacturer's manual on ELISA machine (Stat fax-2100).

RESULTS

Out of 352 samples that were examined, 215 were infected with various degrees of parasitemia, while the remaining 137 were not infected. Table 1 shows a comparative result of haematological variables (PCV, HB, WBC, NEUT and LYMP) between the test group (children infected with malaria parasite) and the control group (children that are not infected with malaria parasite). There was no case of complication or severe anaemia in malaria as hemoglobin (Hb) of < 5g/dl or packed cell volume (PCV) of < 15% with parasitemia of > 250,000 parasites/ul. Table 2 shows a comparative result of cytokine variables (IL-10, IL-1beta, IL-6 and TNF-a) of the test group (children infected with malaria parasites) against that of the control group (children that are not infected with malaria parasite). Fig.1 is a graphic negative representation of the relationship between parasite density and the plasma level of IL-10 of the children infected with malaria parasite. Fig. 2 is a positive graphic representation of the relationship between parasite density and plasma level of IL-6 of the children infected with malaria parasite. Fig. 3 is a positive graphic representation of a relationship between parasite density and plasma level of TNF-a of the children infected with malaria parasite. Regression analysis demonstrated a negative relationship between parasite density and IL-10 (p=0.09), IL-10 and IL-6 (p= 0.017) while positive relationship were seen between IL-6 parasite density with a p-value of 0.000, TNF-a and parasite density with a p-value of 0.1248, IL-10 and IL-1beta with p-value of 0.4582. The cytokines also showed mean values such as 139.33 ± 7.7, 17.74 ± 1.36, 466.17 ± 21.59 and 66.57 ± 4.03 for IL-10, IL-1beta, IL-6 and TNF-alpha respectively.

Table 1: Comparison of haematological variables of malaria-infected children and control.

	PCV	*HB*	*WBC*	*NEUT*	*LYMP*
Test n= 215	34.83 ± 2.76	11.58 ± 0.92	8.96 ± 4.56	57.25 ± 14.00	41.66 ± 13.57
Control n= 137	36.06 ± 1.41	11.98 ± 0.46	7.33 ± 1.39	56.54 ± 8.36	42.95 ± 8.36
p-values	0.001	0.001	0.001	0.549	0.27
t Stat	5.511	5.506	4.904	0.598	1.1

Legend
PCV – Packed Cell Volume
HB - Haemoglobin
WBC –White Blood Cells
NEUT - Neutrophils
LYMP -Lymphocytes

Table 2: Comparison of cytokine variables of malaria-infected children and control.

	IL-10	*IL-1BETA*	*IL-6*	*TNF-ALPHA*
Test n= 215	139.33 ± 7.7	17.74 ± 1.36	466.17 ± 21.59	66.57 ± 4.03
Control n= 137	17.91 ± 2.03	6.46 ± 1.92	13.01 ± 1.54	7.92 ± 1.56
p-values	0.0001	0.0001	0.001	0.0001
t Stat	15.23	8.2	20.98	14.51

Legend
IL – Interleukin
TNF- Tumor Necrosis Factor

$R^2 = 0.0132$
p-value = 0.09

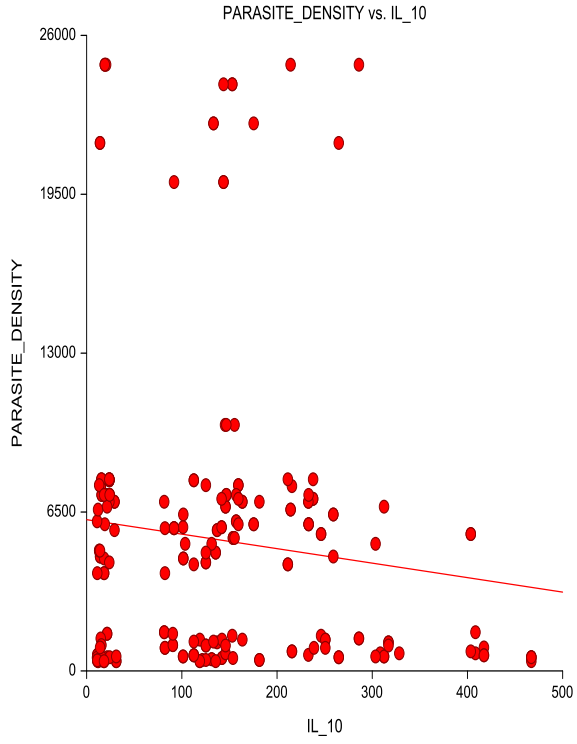

Fig.1. Regression analysis of parasite density and IL-10 of malaria infected children.

$R^2 = 0.0857$
p-value = 0.000

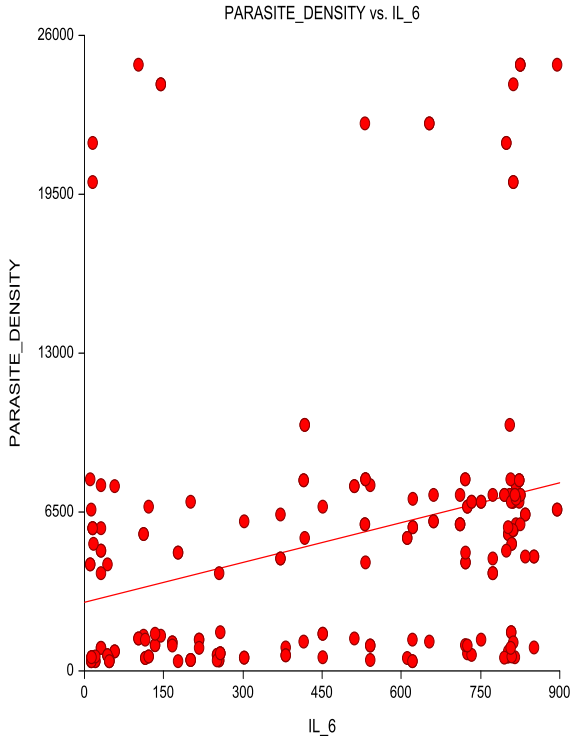

Fig. 2. Regression analysis of parasite density and IL-6 of malaria infected children.

$R^2 = 0.0110$
p-value = 0.1248

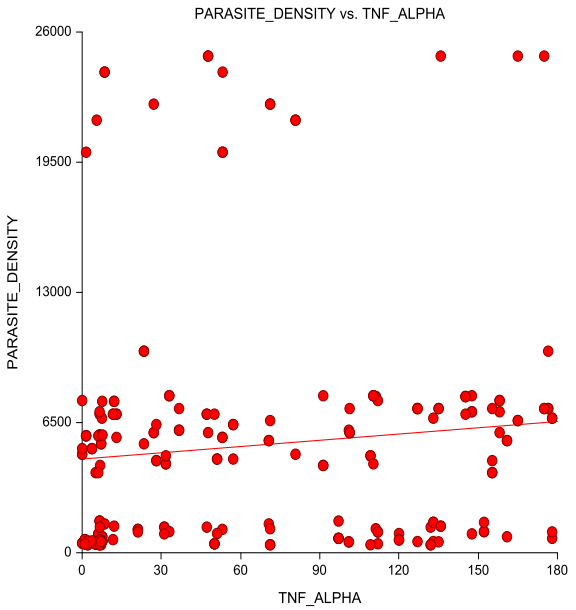

Fig. 3. Regression analysis of parasite density and TNF-alpha of malaria infected children.

DISCUSSION

This investigation was conducted among children between the ages of 2-12year old living in Port-Harcourt, who were infected with malaria parasites to assess the level of their eosinophil and macrophage-secreted cytokines. The eosinophil and macrophage-secreted cytokines of these children infected with malaria parasites were contrasted to those of children without malaria infections (negative controls) and derivations were made dependent on level of these eosinophil and macrophage-secreted cytokines on the two groups.

The level of anti-inflammatory cytokine, interleukin 10 (IL-10) in this examination was found to be significantly higher in children with malaria parasites than with the control group. The test group had a mean value of 139.33 ± 7.7 as against that of the control group with mean value of 17.91 ± 2.03 and this is significant (p=0.0001) as shown in table 2. This finding is in agreement with previous investigations[10], that demonstrated high level of IL-10 in uncomplicated malaria parasitic infections but at variance with other investigations done in other parts of Africa that showed low levels of IL-10[11,12] The disparity may be as a result of the endemicity of malaria infection in our study area and the level of immunity of the children examined in the other parts of Africa.

Regression analysis demonstrated a negative relationship between IL-10 and parasite density (R^2 =0.0132 and p= 0.09) as seen in Fig. 1. Interleukin 10 (IL-10) is an anti-inflammatory cytokine and will in general counter or balance the effects of pro-inflammatory cytokines. Repeated malaria infection can bring about compromise in host immune system as a result of raised IL-10 level in blood plasma, thereby interrupting the response of Th1 and encouraging persistence of parasites.[13] Interleukin 10 (L-10) performs a vital role in regulating the immune system (immunoregulation) inhibiting (limiting or diminishing) the production of pro-inflammatory cytokines especially Tumor Necrosis Factor Alpha (TNF-a) and Interleukin 6 (IL-6), moderating Th1 function and improving or facilitating the activities of Natural Killer cells (NK-Cells)[14]

The level of the pro-inflammatory cytokine, interleukin 6 (IL-6) in this study was also seen to be significantly higher (p=0.0001) in the test group when compared to the control group with mean values of 466.17 ± 21.59 and 13.01 ± 1.54 respectively as shown in table 2. This agrees with some previous investigations done[15,16,17,18] that observed high level of IL-6 in Plasmodia-infected children when compared to the control group but disagrees with other reports[19,20] that showed low level of IL-6 when compared to the negative control group. The disparity may also be as a result of the endemic nature of our study area that may have led to higher level of parasitemia and have stimulated the production of high level of IL-10 that brought down the level of IL-6. Interleukin 6 (IL-6) is a very vital pro-inflammatory cytokine and its production is enhanced by Tumor Necrosis Factor Alpha (TNF-a). It functions together with other inflammatory cytokines in managing parasitemia [21]. In this study, it is observed that IL-6 has a positive relationship with parasite density (R^2=0.0857 and p=0.000) as shown in Fig. 2. It shows that higher parasite density will trigger higher IL-6 production and this therefore suggests that IL-6 level in plasma increases with increase in parasitemia.

Tumor necrosis factor alpha (TNF-a) was also seen to be significantly high in the blood plasma of the subjects when compared with the control group with mean values of 66.57 ± 4.03 and 7.92 ± 1.56 respectively, with p-value=0.0001 as shown in table 2. It also has a positive relationship with parasite density with R^2 = 0.0110 and p=0.1248 as shown in Fig. 3, which suggest that the level of TNF-a in plasma increases with increase in parasite density. This is in similarity with a previous examination done by[22] that also observed high level of TNF-a in children with malaria parasite when compared with the control group and this is suggestive of a widespread response by Th1 in the brief stage of infection with malaria parasites.

Tumor necrosis factor alpha (TNF-a) has been identified to support the killing and destruction of malaria parasites but this action if not checked properly could lead to complicated malaria disease. The main mechanical pattern of the molecules that orchestrate the various manifestation in patients with malaria infection are unclear but it is believed that virulence factor, drug resistant, age amongst others are implicated.[23,24]

Among the pro-inflammatory cytokines that were significantly high in blood plasma of children infected with malaria parasite was IL-1b with mean values of

17.74 ± 1.36 and 6.46 ± 1.92 for the test and control groups respectively (p = 0.0001) as shown in table 2. This is in agreement with a previous study that showed high level of IL-1b in children infected with malaria parasites when compared with adults and control group. Children with malaria infection are said to have high plasma level of interleukin 1b (IL-1b).[22]

Children infected with malaria, within our locality have elevated levels of the plasma cytokines. Effective treatment of malaria will inhibit production of these pro-inflammatory cytokines and therefore obviate the detrimental effects.

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