

EFFECTS OF CHILDHOOD MALARIA ON THE BIOCHEMICAL AND HAEMATOLOGICAL PROFILES OF INFECTED CHILDREN IN ANAMBRA STATE, NIGERIA

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Abstract

This study was carried out to establish data on the effects of childhood malaria on plasma levels of some haematological and biochemical parameters in the pathology of malaria in Anambra state children. The levels of Sodium ion (Na^+), Bilirubin, Alkaline Phosphatase (ALP), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Total iron concentration, Haemoglobin (Hb), Packed Cell Volume (PCV) and White Blood Cell (WBC), in blood samples of malaria parasitaemic children and control were evaluated using standard methods. Chi – square, Fisher – least significance difference, t – test, analysis of variance and multiple regressions were employed to test significant differences among the variables. For all determination, the significant difference was set at $p < 0.05$. The female children infected with malaria parasites had a significantly higher mean PCV than the male in both communities (31.73 ± 4.58 to 28.25 ± 3.75) and hospitals (31.12 ± 11.78 to 28.27 ± 5.56) surveyed respectively. Also the female infected children had a significantly higher WBC count than male. WBC count in malaria infection was gametocyte count dependant. Gametocyte count of 1 - 10 and 11 - 100 in both communities and hospitals surveyed respectively had significant different WBC count. In the age group comparison of malaria infected and uninfected, children 0 – 4.9 years had significant higher mean serum level of bilirubin (0.55 ± 0.25 to 0.35 ± 0.14). Children aged 10 – 14.9 years old had significantly higher mean level of Hb (10.30 ± 0.29 to 10.90 ± 0.26) than other age groups in the survey. Comparing the parameters with age showed that the PCV and Hb of the malaria infected children aged 5 – 9.9 years were significantly lower than the control. Sex and age may be a determining factor for low PCV since the male infected children had a significant lower PCV in both community and hospital surveys. Hypernatraemia was generally observed in malaria infection amongst children in Anambra state.

In the hospital infection there was significant elevation in the serum level of the liver enzyme SGOT in the *Plasmodium* infected male more than their female counterpart.

Introduction

Malaria is no longer the leading cause of death among children in sub-Saharan Africa. In 2000, globally, malaria accounted for 7% of deaths in children aged less than 5 years. As a result of the large decreased malaria mortality in children aged less than 5 years, malaria accounted for just 5% of under-five deaths globally in 2015, and 10% of under-five deaths in sub-Saharan Africa, where it is now the fourth highest cause of death (WHO, 2015). However, malaria still remains perhaps the heaviest public health burden in Nigeria. Seven million confirmed cases of malaria and 6,087 deaths were recorded in 2014, according to the World Malaria Report. Malaria parasites target red blood cells (RBCs), biochemical and haematological changes are one of the most common complications and thus play a major role in the outcomes of the disease. Malaria is caused by a protozoan parasite of the genus: *Plasmodium*. *P. falciparum*, *P. ovale*, *P. vivax*, and *P. malariae* are all the species that infects human, with *P. falciparum* being the most virulent. Haematologic changes that is associated with malaria infection include anaemia, thrombocytopenia, and disseminated intravascular coagulation (Chandra and Chandra, 2013). This may be related to the overt biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite. In Anambra state and other documented endemic sites in Nigeria, malaria is holoendemic and the number of asymptomatic cases in children and adults are on the increase (Nwaorgu, 2011 and Okeke *et al.*, 2016). It is a common knowledge that malaria infection provokes morphological, ultra structural and biochemical changes in erythrocyte membrane to enable compartmentalization, growth, cell differentiation, increased nutrient uptake and survival of the parasite (Sherman and Greenan, 1984; Gero and Kirk, 1994; Iwalokun, *et al.*, 2002). Malaria pathogenesis is based mainly on extensive changes in biochemical and haematological parameters (Bidaki and Dalimi, 2003; Adeosun *et al.*, 2007). WHO's criteria acknowledge that some biochemical and haematological features should raise the suspicion of severe malaria (WHO, 2000). Ogunledun *et al* (1990) reported that a clear diagnosis of malaria can be done based on identification of the parasite in blood film along with typical signs and symptoms associated with the disease.

Entry of *P. falciparum* into erythrocytes usually leads to a marked increase in secretion of inflammatory cytokines (TNF α , IL-1, IL-10, and IFN γ), endothelial cell activation (due to over expression of cell adhesion molecules; ICAM-1, VCAM-1), activation of the coagulation cascade (due to platelet consumption and endothelial damage), and sequestration of parasitized RBCs (due to over expression of cell adhesion molecules, pfEMP, and iNOS).

These along with other mechanisms set in motion events that ultimately result in morphological and numerical changes in the different blood cell lines (Haruna *et al.*, 2013). Significant alterations in both biochemical and haematological variables have been reported during malaria infection (Wareel *et al.*, 1990; WHO, 2000; Bidaki and Dalimi, 2003; Mishra and Mohanty, 2003). However, few studies have been conducted to explore the possible biochemical and haematological changes in children with malaria parasites living in malaria-endemic area of Anambra state, Nigeria. This study was carried out with the main objective of determining whether the possible biochemical and haematological changes in children suffering from *P. falciparum* in the state, within the specified period, showed a difference from the expected values according to Sood, 2006.

Materials and methods

Study Area

The study was carried out in Anambra state in south - eastern Nigeria. It lies in the tropical rainforest belt and has an estimated population of about 4 million made up of 2,117,984 males and 2,059,844 females (NPC, 2006; MLS, 2010). Anambra state is made up of twenty one local government areas. This study was conducted in thirteen communities and eleven general hospitals purposively selected from thirteen local government areas in the state.

Study population

Eighty-two suspected *falciparum* malarial infected children aged 0 - 14.9 years from the sampled households in the communities and 166 of their counterparts that attended the outpatient clinics of the selected General hospitals in Anambra state were involved in the study. The criteria for the purposive selection of these hospitals are the existence of a functional hospital with facilities on the ground as most of them were existing only on paper, number of patients that attends the hospitals and availability of health workers who will attend to the patients. The diagnosis of malaria was confirmed by thick and thin film with Giemsa staining of malaria parasite. Infected children were recruited into the study while malaria parasite free children served as control group.

Sampling Technique

Stratified random sampling was used. All hospitalized or non-hospitalized patients (children 0-14.9 years) with acute febrile illness were randomly sampled two times in a month from April 2012 to March 2013. Fifty homes were randomly sampled by balloting in each of the 13 communities and 11 general hospitals for 12 months.

Ethical Clearance and Exclusion Criteria

Ethical clearance was obtained from the University of Nigeria Teaching Hospital Ituku - Ozalla in Enugu state, with which permission was obtained from the management of the selected General Hospitals for the study. Informed consent was obtained from the mothers/caregivers before the collection of blood samples. Children who have sickle cell anaemia were excluded from the study because of the fact that malaria parasites do not grow well in sickle cells.

Collection of blood samples

A volume of 5 ml of the venous blood sample was drawn into tubes containing the anticoagulant potassium ethylenediamine-tetra acetic acid (EDTA) for malaria parasite species identification and quantification, haematological and biological analysis. Blood was sampled from all the children within the age bracket that their parents gave their consent.

Preparation of thick and thin films blood smears for microscopy

The diagnosis of malaria was confirmed by thick and thin film with Giemsa staining of malaria parasite. Thick film blood smears were prepared from 1ml blood sample according to Sood (2006). Large drop of blood samples were deposited at one end of the slide and were spread out evenly with the corner of another slide to a diameter of about 20mm. They were put in distilled water for 10 minutes for dehaemoglobinisation, dried in a flat position to ensure even distribution of blood and stained with Giemsa's stain for 20 minutes. The stain was washed out with buffered water of pH 6.8, stood upright to dry in the air and viewed under x 100 objective (oil immersion) lens. The thick smears were used to confirm the presence or absence of malaria parasite. Thin films were prepared to determine parasitaemia. The degree of parasitaemia were graded according to the number of parasite per micro litre thus, low 1-999 (+), moderate 1000-9999 (++) and severe >100000 (+++) (Cheesbrough, 2006).

Biochemical studies:

Four ml of blood samples from the 5ml obtained from the sampled children from the different communities and hospitals was used for the analysis. The blood samples were centrifuged in a Teco diagnostic cf_ 30 2005 model at rpm 1000 for 45 minutes and serum separated into plain clean glass tube for biochemical tests. Total ion, SGOT and SGPT were determined by colorimetric method (Optima model SPA – 300) as reported by Reitman S. and Frankel. S. (1957). They were read at 550nm. Total ion reagents were from Teco diagnostics, while SGOT and SGPT reagents were from Bronila diagnostics Enugu state, Nigeria. Total bilirubin was determined using Jendrassik and Grof's method, Total protein was determined

using Biuret method, ALP was measured using Kind and King's method and Na^+ ion was determined by the use of Flame photometer 129 as described by Sood (2006).

Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software version 20.0 (IBM Corporation, Armonk, New York). Differences in haematological and biochemical parameters and parasite density between *Plasmodium* infected and uninfected children were assessed using the independent t-test. The independent t-test was also used to compare gametocyte counts of infected children surveyed from the communities and the hospitals and to ascertain the endemicity of malaria in the area by comparing the different *Plasmodium* gametocyte counts of infected and uninfected children. Analysis of variance (ANOVA) was used to compare the effects of the different factors (sex, gametocyte count and age) on the haematology and biochemical parameters.

Results

Children (asymptomatic and symptomatic) between the ages of 0 -14.9 (4.12 ± 0.27) years were sampled from thirteen communities and eleven General Hospitals in Anambra State. A total of eighty-two children were sampled in the community which include 39 males (47%) and 43 females (53%). One hundred and sixty six (166) children who attended the out patient clinic of General Hospitals/Comprehensive Health Centres in Anambra state were sampled, comprising of 95 males (57.2%) and 71 females (42.8%). *Plasmodium falciparum* malaria parasite was identified in the infected children (100% prevalence). Community prevalence of malaria infection was 46.3% while hospital prevalence was 94.6%.

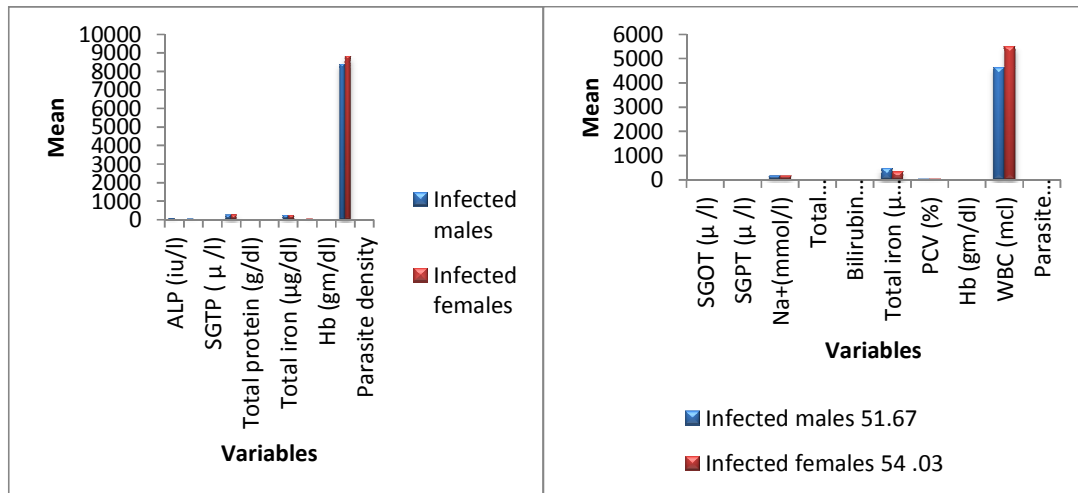
Serum levels of the biochemical and haematological indices of malaria infected and uninfected children in Anambra state, Nigeria, were determined based on sex, parasite count and age. Table 1, figure 1 on sex - related biochemical and haematological indices, showed that there were no significant differences in all the investigated biochemical parameters in the communities but the mean PCV and WBC differed significantly with females having higher values of PCV (31.73 ± 4.58 to 28.25 ± 3.73 and WBC (5495.45 ± 1197.81 to 4631.25 ± 821.15). Similarly, male infected children in the hospital surveyed had significant higher SGOT (33.89 ± 32.61 to 23.29 ± 19.71) but there were no significant differences in the six others measured. The female infected children had higher mean PCV, while the male had higher SGOT (33.89 ± 32.61 to 23.29 ± 19.71) even though all were within the normal ranges (Table 1).

The comparison of the effects of sex on the biochemical and haematological indices, and the parasite density of malaria infected male and female children in Anambra state, Nigeria is summarized in Table 2 figure 2. Female infected children in hospitals had higher WBC and parasite density more than their counterparts in the communities. Likewise, male infected children in the hospitals had higher parasite density and WBC more than the male in the communities.

Table 1: Biochemical and haematological indices, and parasite density of malaria infected and control subjects in relation to sex in Anambra State, Nigeria

Variables	Infected males	Infected females	Standard range	P – value
Communities				
Sampled size (n)	16	22	–	0.05*
ALP (iu/l)	51.67 ± 11.21	54.03 ± 13.61	20 - 140	0.574 ^{ns}
SGOT (µ l)	5.72 ± 4.21	7.36 ± 8.23	8 – 40	0.472 ^{ns}
SGPT (µ l)	4.84 ± 3.0	7.20 ± 12.09	5 – 35	0.452 ^{ns}
Na⁺ (mmol/l)	155.25 ± 45.63	152.75 ± 67.40	133 – 155	0.8886 ^{ns}
Total protein (g/dl)	6.49 ± 23.38	6.62 ± 0.22	6.0 – 8.0	0.696 ^{ns}
Bilirubin (mg/dl)	0.57 ± 33	0.47 ± 0.18	0.0 2 -10	0.239 ^{ns}
Total iron (µ g/dl)	475.12 ± 745.33	327.45 ± 80.46	6 – 150	0.360 ^{ns}
PCV (%)	28.25 ± 3.75	31.73 ± 4.58	25	0.018*
Hb (gm/dl)	9.88 ± 1.27	10.45 ± 1.79	10 – 13	0.28 ^{ns}
WBC (x10³/l)	4631.25 ± 821.15	5495.45 ± 1197.81	4,500 – 10,000	0.018*
Parasite density (µ l)	1.24 x 10 ⁴ ± 1.34 x 10 ³	1.54 x 10 ⁴ ± 1.38 x 10 ³		0.511 ^{ns}
Hospitals				
Sampled size (n)	90	67	–	0.05*
ALP (iu/l)	63.95 ± 66.77	1.028 ± 7822.51	20 - 140	0.244 ^{ns}
SGOT (µ l)	33.89 ± 32.61	23.29 ± 19.71	8 – 40	0.020*
SGTP (µ l)	21.15 ± 56.94	14.29 ± 25.19	5 – 35	0.359 ^{ns}
Na⁺ (mmol/l)	244.50 ± 78.87	256.82 ± 88.34	133 - 155	0.403 ^{ns}
Total protein (g/dl)	8.66 ± 11.10	9.26 ± 9.56	6.0 – 8.0	0.734 ^{ns}
Bilirubin (mg/dl)	2.27 ± 13.41	0.80 ± 0.35	0.02 – 10	0.371 ^{ns}
Total iron (µg/dl)	223.22 ± 148.99	217.69 147.30	6 – 150	0.817 ^{ns}
PCV (%)	28.27 ± 5.56	31.12 ± 11.78	25	0.046*
Hb (gm/dl)	10.52 ± 9.93	9.87 ± 2.04	10 – 13	0.595 ^{ns}
WBC (x10³/l)	8377.78 ± 2587.48	8812.68 ± 2356.14	4,500 – 10,000	0.281 ^{ns}
Parasite density (µ l)	7.21 x 10 ⁴ ± 1.94 x 10 ³	1.21 x 10 ⁵ ± 2.31 x 10 ³		0.078 ^{ns}

Statistical significance = (p > 0.05).



Communites

Hospitals

Figure 1: Biochemical and haematological indices, and parasite density of malaria infected and control subjects in relation to sex in Anambra State, Nigeria

Table 2: Comparison of sex - related biochemical and haematological indices, and the parasite density of malaria infected male and female children in Anambra State, Nigeria

Parameters	Community	Hospital	Community	Hospital
ALP (iu/l)	54.44 ± 3.83 ^a	63.95 ± 7.04 ^a	52.00 ± 2.29 ^a	1042.37 ± 970.15 ^a
SGOT (μ/ml)	4.38 ± 0.48 ^b	33.87 ± 3.44 ^a	8.34 ± 1.90 ^b	23.49 ± 2.44 ^a
SGPT (μ/ml)	7.36 ± 3.56 ^a	21.15 ± 6.04 ^a	5.41 ± 0.65 ^a	14.42 ± 3.12 ^a
Na+(mmol/l)	145.59 ± 15.37 ^b	244.50 ± 8.42 ^a	161.24 ± 9.87 ^b	255.15 ± 10.94 ^a
Total protein (g/dl)	6.61 ± 0.15 ^b	8.66 ± 1.2 ^b	38.60 ± 32.07 ^a	9.26 ± 1.19 ^b
Bilirubin (mg/dl)	0.49 ± 0.3 ^a	2.27 ± 1.41 ^a	0.54 ± 0.70 ^a	0.81 ± 0.4 ^a
Total iron (μg/dl)	515.50 ± 182.92 ^a	223.22 ± 15.71 ^b	292.38 ± 21.12 ^b	219.30 ± 18.20 ^b
PCV (%)	30.94 ± 1.15 ^a	28.27 ± 0.59 ^a	29.71 ± 1.02 ^a	31.20 ± 1.46 ^a
Hb (gm/dl)	10.72 ± 1.15 ^a	10.52 ± 1.05 ^a	9.82 ± 0.39 ^a	9.88 ± 0.25 ^a
WBC (x10 ³ /l)	5143.75 ± 330.27 ^b	8315.62 ± 286.59 ^a	5142.86 ± 222.49 ^b	8865.91 ± 287.20 ^a
Parasite density(μ/l)	11981.25 ± 1955.19 ^b	71989.11 ± 542.04 ^{ab}	15957.14 ± 3781.49 ^b	123140.91 ± 28706.33 ^a

N/B: Mean values in a row with different alphabets as superscript are significantly different (p < 0.05)

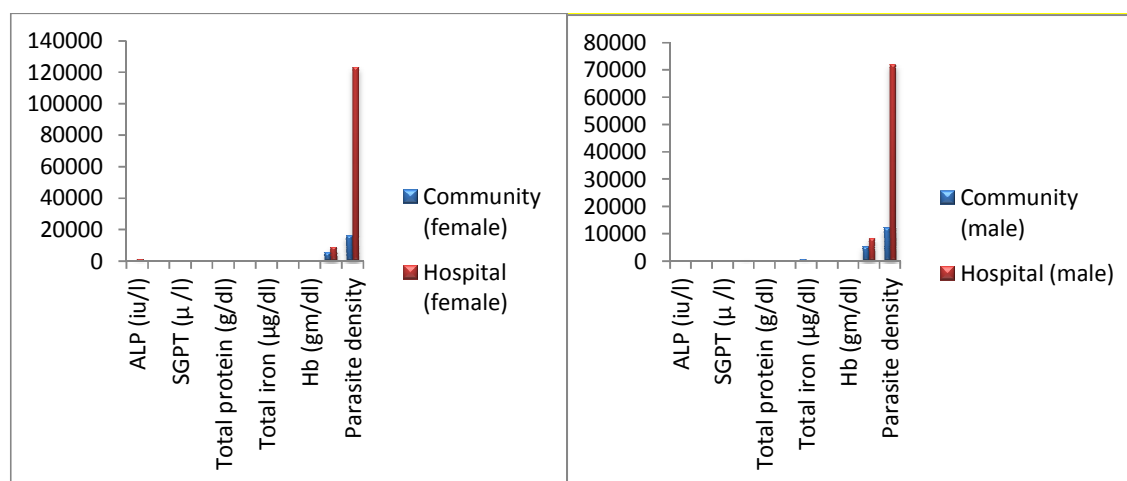


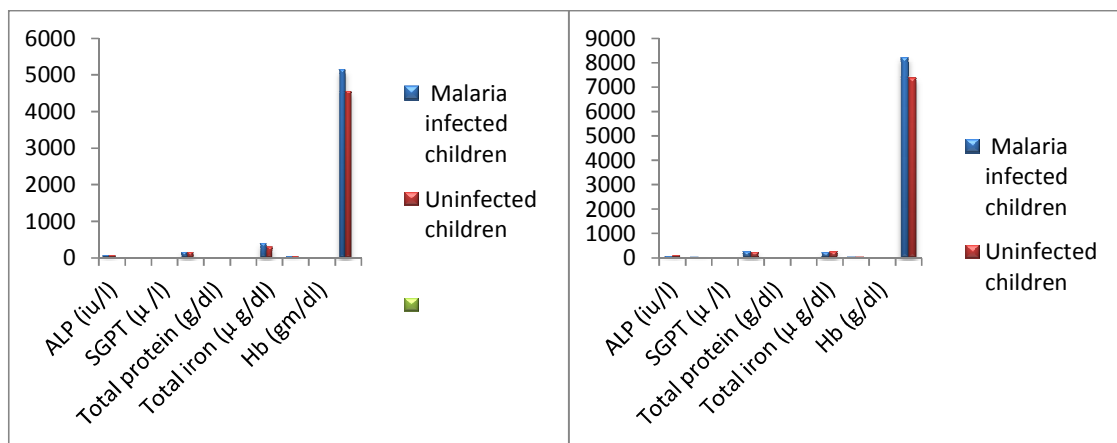
Figure 2: Comparison of sex - related biochemical and haematological indices, and the parasite density of malaria infected male and female children in Anambra State, Nigeria

Tables 3 and 4, figure 3 and 4 summarised the effects of malaria parasite count on the biological and haematological parameters. There were no significant differences in the parameters of children with 1 – 10 in the communities. The malaria infected children had a higher significant white blood cell count than their uninfected counterpart while the differences in their mean PCV (%) and Hb (g/dl) were not significant. Like wise there were no significant differences between the levels of the liver enzymes and haematological parameters of malaria infected and uninfected children with 1-10 and 11- 100 gametocytes from the hospitals but there was a significant difference in their WBC. Both malaria infected and uninfected children from hospital had the same mean PCV ($P = 29.33$). Over all comparison showed that children with 11 – 100 counts had higher parasite density more than those with 1- 10 from hospitals and communities, also infected children from hospital with 1-10 had higher Hb compared to their counterparts from the communities (Figure 4).

Table 3: Biochemical and haematological indices of malaria infected and uninfected children with different gametocytes count in Anambra State, Nigeria (Communities and hospitals)

Variables	Malaria infected children	Uninfected children Communities 1-10 gametocytes	Standard range	P – value
Sampled size (n)	38	38	-	1.00
ALP (iu/l)	53.03 ± 12.55	55.18 ± 12.312	20 - 140	0.437 ^{ns}
SGOT(μ/ml)	6.67 ± 6.18	6.28 ± 9.55	8 - 40	0.838 ^{ns}
SGPT(μ/ml)	6.21 ± 9.38	5.09 ± 2.31	5 – 35	0.447 ⁿ
Na ⁺ (mmol/l)	153.80 ± 52.12	145.41 ± 25.60	133 – 155	0.347 ^{ns}
Total protein (g/dl)	6.49 ± 109.25	6.23 ± 11.27	6.0 – 8.0	0.366 ⁿ
Bilirubin (mg/dl)	0.51 ± 0.25	0.448 ± 0.16	0.02 – 10	0.163 ^{ns}
Total iron (μ g/dl)	389.63 ± 484.09	292.43 ± 70.99	6 – 150	0.192 ^{ns}
PCV (%)	30.26 ± 454	30.14 ± 5.00	25	0.905 ^{ns}
Hb (gm/dl)	10.21 ± 1.60	10.33 ± 1.56	10 – 13	0.719 ^{ns}
WBC(x10 ³ /l)	5131.58±1129.01	4551.14±1005.66	4,500 – 10,000	0.016 [*]
		Hospitals 1 – 10 gametocytes		
Sampled size (n)	104	90	-	0.128 ^{ns}

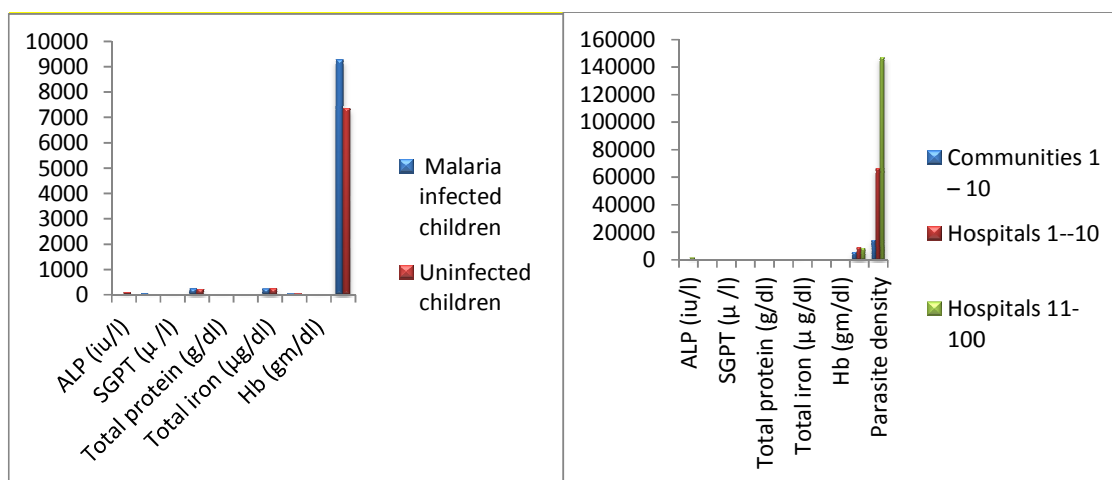
ALP (iu/l)	61.20 ± 60.65	75.78 ± 39.29	20 - 140	0.48 ^{ns}
SGOT (μ /l)	26.59 ± 29.32	23.44 ± 20.71	8 - 40	0.754 ^{ns}
SGPT (μ /l)	17.62 ± 50.17	17.93 ± 17.54	5 - 35	0.985 ^{ns}
Na⁺ (mmol/l)	255.91 ± 90.03	222.56 ± 90.34	133 - 155	0.289 ^{ns}
Total protein (g/dl)	7.87 ± 7.76	12.70 ± 17.49	6.0 - 8.0	0.118 ^{ns}
Bilirubin (mg/dl)	1.95 ± 12.49	1.14 ± 0.74	0.02 - 10	0.846 ^{ns}
Total iron (μ g/dl)	217.36 ± 152.66	248.76 ± 150.14	6 - 150	0.555 ^{ns}
PCV (%)	29.33 ± 10.186	29.33 ± 5.17	25	0.999 ^{ns}
Hb (g/dl)	10.35 ± 9.29	9.89 ± 1.84	10 - 13	0.909 ^{ns}
WBC(x10³/l)	8204.8 ± 2619.51	7355.56 ± 4578.51	4,500 - 10,000	0.386 ^{ns}
11-100 gametocytes				
Sampled size (n)	53	9	-	0.044*
ALP (iu/l)	1.29 ± 879.15	75.78 ± 39.29	20 140	0.683 ^{ns}
SGOT (μ /l)	34.78 ± 25.48	23.44 ± 20.71	8 - 40	0.211 ^{ns}
SGPT (μ /l)	19.01 ± 36.69	17.93 ± 17.54	5 - 35	0.932 ^{ns}
Na⁺ (mmol/l)	236.42 ± 67.87	222.56 ± 90.34	133 - 155	0.592 ^{ns}
Total protein (g/dl)	10.98 ± 15.38	12.70 ± 17.49	6.0 - 8.0	0.762 ^{ns}
Bilirubin (mg/dl)	1.04 ± 0.43	1.14 ± 0.74	0.02 - 10	0.590 ^{ns}
Total iron (μ g/dl)	227.72 ± 139.01	248.76 ± 150.14	6 - 150	0.680 ^{ns}
PCV (%)	29.81 ± 5.41	29.33 ± 5.17	25	0.808 ^{ns}
Hb (gm/dl)	10.04 ± 1.80	9.10 ± 1.84	10 - 13	0.938 ^{ns}
WBC (x10³/l)	9266.98 ± 2071.46	7355.56 ± 4578.51	4,500 - 10,000	0.042*



Communities: 1 – 10 gametocytes

Hospitals: 1 – 10 gametocytes

Figure 3: Biochemical and haematological indices of malaria infected and uninfected children with different gametocytes count in Anambra State, Nigeria (Communities and hospitals)



(a)

(b)

Figure 4: Hospitals infected and uninfected children with 11-100 gametocytes (a) and comparison of malaria gametocytes count in the communities and hospitals in Anambra State, Nigeria (b).

Table 4: Comparison of malaria gametocytes count - related biochemical and haematological indices, and the parasite density of malaria infected children from communities and hospitals in Anambra State, Nigeria

Variables	Communities		Hospitals	
	1 – 10	1 -10	11- 100	
Gametocytes count				
ALP (iu/l)	53.05 ± 2.09 ^a	65.07 ± 5.36 ^a	1280.18 ± 1208.13 ^a	
SGOT (µ /l)	6.63 ± 1.13 ^b	29.11± 30.34 ^a	30.19± 24.16 ^a	
SGPT (µ /l)	6.25± 1.56 ^a	18.65 ± 5.24 ^a	17.59± 4.09 ^a	
Na+(mmol/l)	154.47± 8.66 ^b	255.74± 8.81 ^a	230.88± 6.26 ^a	
Total protein (g/dl)	24. 77± 18.20 ^a	8.17± 0.99 ^a	10.36± 1.74 ^a	
Bilirubin (mg/dl)	0.52± 0.42 ^a	2.07± 1.24 ^a	0.84± 0.41 ^a	
Total iron (µ g/dl)	388.86± 80.76 ^a	211.56± 15.53 ^b	241.00± 17.37 ^b	
PCV (%)	30.24± 0.76 ^a	30.04± 1.04 ^a	28.49± 3.80 ^a	
Hb (gm/dl)	10.21± 0.27 ^a	9.70± 0.20 ^a	11.33± 1.76 ^a	
WBC (x10³/l)	5143.24 ± 187.79 ^b	8703.45 ± 273.81 ^a	8247.17 ± 287.85 ^a	
Parasite density(µ /l)	14237.84 ± 2303.59 ^b	66221.90 ± 4753.24 ^b	146896.23 ± 39208.35 ^a	

N/B: Mean values in a row with different alphabets as superscript are significantly different (p <0.05)

Table 5, figures 5 and 6 showed that there were no significant differences in the levels of ALP, SGOT, Na⁺ and total protein of the malaria infected children 0 – 4.9 years from the communities compared to the uninfected, but the serum level of bilirubin (0.55 ± 0.25 to 0.35 ± 0.14) differed significantly. Also there were no significant differences in PCV, Hb and WBC of the two groups. On the other hand there were no significant differences in all the measured biochemical and haematological parameters of malaria infected and uninfected children between 5 - 9.9 years. Likewise there were no significant differences in serum levels of all measured biochemical parameters in the age group 10 - 14.9 years. However, the mean Hb (10.30 ± 0.29 to 10.90 ± 0.26) of malaria uninfected children was significantly higher than the infected children.

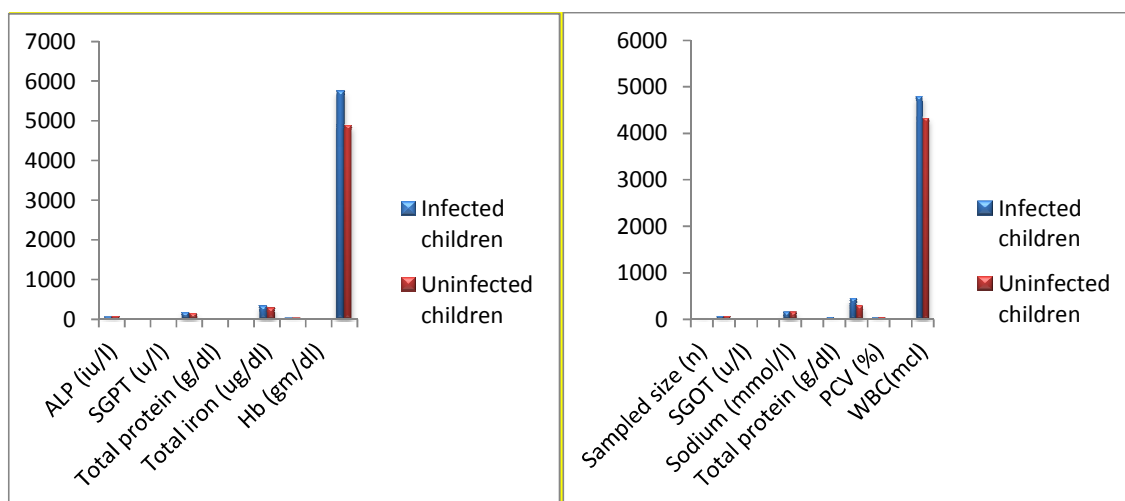
Similarly, there were no significant differences in the biochemical parameters of malaria infected and uninfected children of 0 – 4.9 and 5 – 9.9 years from the hospitals but the mean PCV and Hb differed significantly in 5 – 9.9 years age group.

Figure 6 (c) showed that hospital malaria infected children 0 – 4.9 and 10 – 14.9 years had higher WBC and parasite density compared to their community counterparts.

Table 5: Biochemical and haematological indices of malaria infected and uninfected children of different age groups from the communities and hospitals in Anambra State, Nigeria

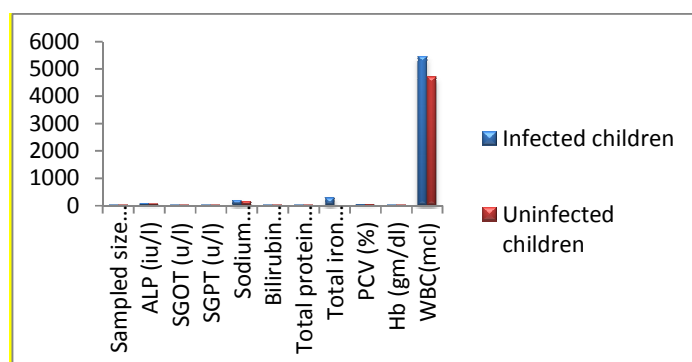
Communities				
Variables	Infected children	Uninfected children	Standard range	P – value
Communities 0 – 4.9 years				
Sampled size (n)	11	15	-	0.514 ^{ns}
ALP (iu/l)	57.03 ± 14.57	62.36 ± 11.87	20 – 140	0.314 ^{ns}
SGOT (u/l)	9.72 ± 11.29	4.05 ± 1.97	8 – 40	0.067 ^{ns}
SGPT (u/l)	4.65 ± 3.32	6.27 ± 2.19	3 – 35	0.571 ^{ns}
Sodium (mmol/l)	157.82 ± 47.11	144.67 ± 10.97	133 – 155	0.304 ^{ns}
Total protein (g/dl)	6.37 ± 0.60	6.46 ± 0.63	6.0 – 8.0	0.703 ^{ns}
Bilirubin (mg/dl)	0.55 ± 0.25	0.35 ± 0.14	0.02 – 10	0.016*
Total iron (ug/dl)	328.82 ± 80.55	283.07 ± 50.22	6 – 150	0.087 ^{ns}
PCV (%)	29.91 ± 4.70	29.07 ± 6.49	25	0.718 ^{ns}
Hb (gm/dl)	9.92 ± 1.83	9.93 ± 2.02	10 – 13	0.99 ^{ns}
WBC (x10³/l)	5745.45 ± 997.36	4880.00 ± 120 to 46	4,500 – 10,000	0.64 ^{ns}
5 – 9.9 years				
Sampled size (n)	23	24	–	0.982 ^{ns}
ALP (iu/l)	50.20 ± 9.97	52.53 ± 11.81	20 – 140	0.470 ^{ns}
SGOT (u/l)	5.00 ± 3.31	7.85 ± 12.72	8 – 40	0.305 ^{ns}
SGPT (u/l)	7.09 ± 11.85	5.08 ± 2.62	5 – 35	0.423 ^{ns}
Sodium (mmol/l)	146.98 ± 41.49	147.96 ± 33.47	133 – 155	0.929 ^{ns}
Bilirubin (mg/dl)	0.45 ± 0.17	0.48 ± 0.15	0.02 – 10	0.548 ^{ns}
Total protein (g/dl)	36.02 ± 140.39	11.44 ± 15.03	6.0 – 8.0	0.398 ^{ns}
Total iron (ug/dl)	438.87 ± 617.98	284.50 ± 79.68	6 – 150	0.231 ^{ns}
PCV (%)	30.39 ± 4.92	30.50 ± 4.41	25	0.939 ^{ns}
Hb (gm/dl)	10.33 ± 1.65	10.471 ± 1.37	10 – 13	0.752 ^{ns}
WBC(x10³/l)	4782.61 ± 1021.43	4316.67 ± 899.76	4,500 – 10,000	0.102 ^{ns}
10 – 14.9 years				
Sampled size (n)	4	5	-	0.989 ^{ns}
ALP (iu/l)	58.33 ± 18.69	46.40 ± 1.67	20 – 140	0.192 ^{ns}
SGOT (u/l)	7.82 ± 3.02	5.48 ± 1.21	8 – 40	0.152 ^{ns}
SGPT (u/l)	5.43 ± 1.77	4.58 ± 0.89	5 – 35 u/l	0.377 ^{ns}
Sodium (mmol/l)	182.00 ± 109.526	135.40 ± 3.578	133 – 155	0.365 ^{ns}
Bilirubin (mg/dl)	0.78 ± 0.49	0.60 ± 0.53	0.02 – 10	0.439 ^{ns}
Total protein (g/dl)	6.10 ± 1.54	6.90 ± 0.43	6.0 – 8.0	0.293 ^{ns}
Total iron (ug/dl)	273.75 ± 134.25	358.60 ± 48.58	6 – 150	0.226 ^{ns}
PCV (%)	30.50 ± 1.73	31.6 ± 1.14	25	0.287 ^{ns}

Hb (gm/dl)	10.30 ± 0.29	10.90 ± 0.26	10 – 13	0.013*
WBC(x10³/l)	5450.0 ± 1517.67	4690.0 ± 712.74	4,500 – 10,000	0.350 ^{ns}
Hospitals				
0 – 4.9years				
Sampled size (n)	93	6		
ALP (iu/l)	65.77 ± 56.85	81.83 ± 47.52	20 – 140	0.501 ^{ns}
SGOT (µ /l)	26.88 ± 30.21	30.42 ± 22.60	8 – 40	0.779 ^{ns}
SGPT (µ /l)	18.50 ± 55.22	24.68 ± 18.10	5 – 35	0.786 ^{ns}
Na⁺ (mmol/l)	255.24 ± 90.53	188.33 ± 93.90	133 – 155	0.83 ^{ns}
Total protein (g/dl)	8.17 ± 10.58	15.90 ± 21.19	6.0 – 8.0	0.110 ^{ns}
Bilirubin (mg/dl)	2.21 ± 13.10	1.37 ± 0.82	0.02 – 10.0	0.876 ^{ns}
Total iron (µg/dl)	211.22 ± 163.69	325.80 ± 120.48	6 – 150	0.096 ^{ns}
PCV (%)	29.90 ± 10.98	27.00 ± 3.46	25	0.522 ^{ns}
Hb (gm/dl)	9.61 ± 2.08	9.15 ± 1.32	10 – 13	0.598 ^{ns}
WBC (x10³/l)	8909.14 ± 2544.13	8383.33 ± 5330.45	4,500-10,000	0.652 ^{ns}
5 – 9.9 years				
Sampled size (n)	41	3	-	0.01*
ALP (iu/l)	16.33 ± 9999.93	63.67 ± 14.15	20 – 140	0.789 ^{ns}
SGOT (µ /l)	31.53 ± 18.49	4.73 ± 1.37	8 – 40 µ /l	0.330 ^{ns}
Na⁺ (mmol/l)	252.5 ± 74.62	291.00 ± 7.94	133 - 155	0.382 ^{ns}
Total protein (g/dl)	10.65 ± 13.61	6.30 ± 2.86	6.0 – 8.0	0.588 ^{ns}
Bilirubin (mg/dl)	0.75 ± 0.28	0.68 ± 0.19	0.02 – 10	0.710 ^{ns}
Total iron (µg/dl)	204.25 ± 114.69	94.67 ± 2.136	6 – 150	0.109 ^{ns}
PCV (%)	29.00 ± 4.03	34.00 ± 5.29	25	0.048*
Hb (gm/dl)	9.90 ± 1.32	11.67 ± 1.67	10 – 13	0.032*
WBC (x10³/l)	8029.27 ± 2482.36	5300.00 ± 1819.341	4,500 - 10,000	0.070 ^{ns}



0 – 4.9 year

5 – 9.9 years



10 – 14.9 years

Figure 5: Biochemical and haematological indices of malaria infected and uninfected children of different age groups from the communities in Anambra State, Nigeria.

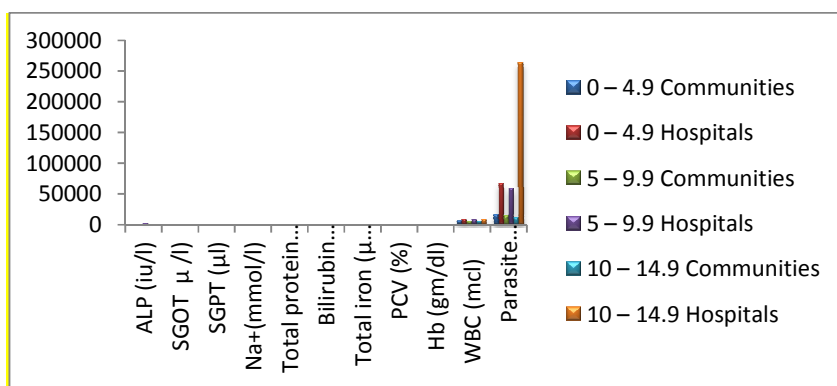
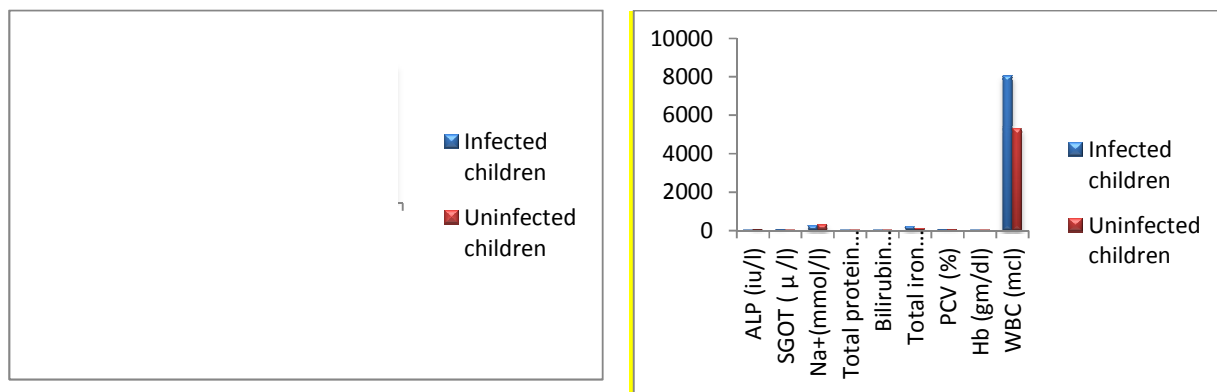


Figure 6: Biochemical and haematological indices of malaria infected and uninfected children of different age groups from the hospitals (a and b) and comparison of communities and hospitals (c) in Anambra State, Nigeria.

Table 6: Comparison of age - related biochemical and haematological indices, and parasite density of malaria infected children in the communities and hospitals in Anambra State, Nigeria

Variables	Age Groups (Years)					
	0 – 4.9		5 – 9.9		10 – 14.9	
	Communities	Hospitals	Communities	Hospitals	Communities	Hospitals
ALP (iu/l)	57.30 ± 4.78 ^a	66.67 ± 5.93 ^a	50.26 ± 2.07 ^a	1632 ± 1561.76 ^a	58.50 ± 9.43 ^a	64.89 ± 2,97 ^a
SGOT μ/l)	9.88 ± 3.76 ^{bc}	27.15 ± 3.16 ^{ab}	5.00 ± 0.69 ^c	31.39± 3.6 ^a	7.83 ± 1.51 ^{bc}	35.36 ± 5.85 ^a
SGPT (μl)	4.65 ± 1.11 ^a	18.93 ± 5.85 ^a	7.09 ± 2.47 ^a	14.86 ± 2.90 ^a	5.43 ± 0.88 ^a	21.84 ± 8.25 ^a
Na+(mmol/l)	160.70 ±15.38 ^b	258.35 ± 9.65 ^a	146.98 ± 8.65 ^b	248.10 ± 10.56 ^a	182.00 ± 54.77 ^b	213.26 ± 12.94 ^{ab}
Total protein (g/dl)	6.35 ± 0,20 ^a	8.22 ± 1.11 ^a	36.02 ± 29.27 ^a	10.46 ± 2.13 ^a	6.10 ± 0.77 ^a	8.91 ± 1.35 ^a
Bilirubin (mg/dl)	0.57 ± 0.82 ^a	2.23 ± 1.38 ^a	0.45 ± 0.34 ^a	0.75 ± 0,44 ^a	0.78 ± 0.26 ^a	0.96 ± 0.11 ^a
Total iron (μ g/dl)	319.90 ± 24.97 ^{ab}	212.94 ± 17.06 ^b	438.87 ± 128.86 ^a	202.64 ± 18.15 ^b	273.75 ± 67.13 ^{ab}	289.44 ± 23.92 ^{ab}
PCV (%)	29.80 ± 1.56 ^a	29.88 ± 1.15 ^a	30.39 ± 1.04 ^a	29.12 ±0.63 ^a	30.50 ± 0.87 ^a	28.74 ± 0.92 ^a
Hb (gm/dl)	9.89 ± 0.61 ^a	9.61 ± 0.22 ^a	10.33 ± 0.39 ^a	9.90 ± 0.21 ^a	10.30 ± 0.15 ^a	13.45 ± 4.05 ^a
WBC (x10³/l)	5850.00 ± 311.72 ^b	8873.43 ± 282.22 ^a	4782.61 ± 212.98 ^b	8007.32 ± 382.36 ^a	5450.00 ± 758.84 ^b	8213.04 ± 427.30 ^a
Parasite density (μ /l)	15870.00 ± 4525.11 ^b	66513.00 ± 4858.88 ^b	14021.74 ± 3127 ^b	59258.54 ± 8110.37 ^b	11400.00±4248.14 ^b	263252.17 ± 84566.84 ^a

N/B: Mean values in a row with different alphabets as superscript are significantly different.

Discussion

The assessment of haematological profile can be used to determine the effect of malaria parasite on the blood of male and female children. The significant decrease in male PCV in the communities showed that the males were slightly anaemic. The decreased PCV levels were expected based on the previous reports (Adesina *et al.*, 2009; Ogbodo *et al.* 2010; Kayode *et al.*, 2011; Akaninwor, *et al.*, 2013). Genetic factors and even host – parasite interactions play key role in disease susceptibility progression and outcome. Results from independent segregation analysis of blood parasitaemia showed that there are complex genetic factors controlling infection levels and it shows dramatic influence of age with putative major gene controlling the trait (Fortin *et al.*, 2002). Also there was a significant difference in the WBC count of both sexes even though both were within the normal range (4,500 – 10,000). WBC had a positive linear relation with parasite density this means that as the parasite density is increasing there is a corresponding increase in total white cell count. This result is consistent with Laura *et al.* (2004). This may be attributed to many factors such as increase in breakdown of red blood cells, acuteness of infection, parasitemia, disease severity, state of the host immunity to malaria, concurrent infections and malnutrition (Nkuo-Akenji, 2008 and Haruna *et al.*, 2013). Anaemia in malaria is a major cause of infant death in malaria endemic Africa.

The insignificant difference in the serum levels of total protein, bilirubin and total iron between male and female infected and uninfected children respectively in these communities is in disagreement with Ikekpezazu *et al.* 2010. Both sexes had hypernatraemia without significant difference in the serum level. However, infected females from the communities had significant higher total protein more than infected males and females from the hospitals. Sodium (Na^+) is a major cation of extra cellular fluid that plays a central role in the maintenance of normal distribution of water and osmotic pressure in various fluid compartments. Significant reduction can occur as a result of high temperature which may lead to disorder in water balance. Serum sodium concentration higher than normal can be caused by gastroenteritis, osmotic diuresis and pronounced sweating (Sood, 2006). The insignificant changes observed in cation concentration between uninfected and asymptomatic children could explain why they are asymptomatic.

Based on parasite count, there was no significant difference in the mean serum level of total protein, total iron, ALP, SGOT, bilirubin and SGPT of the infected children with 1-10 gametocytes and the control group. The significant difference in the mean WBC count of infected children of 1-10 gametocytes is in line with Ladhani *et al.* (2002) and Pavithran

(2005). Leucocytosis is associated with severity and mortality of childhood *falciparum* malaria (Pavithran, 2005).

The values obtained for plasma bilirubin in the age group 0 – 4.9 years was significantly higher in malarious children than in non - parasitaemic children. The result is in line with Orimadegun *et al.* (2008). A primary reason is that this age group are most vulnerable to *P. falciparum* morbidity and mortality because of their immune system. Also a high proportion of children with fever are not taken to the hospital for care or the caregivers use the informal private sector, where they are less likely to obtain ACTs for treatment (WHO, 2015). High level of bilirubin can be caused by liver diseases, excessive haemolysis/destruction of RBCs (haemolytic jaundice), obstruction of the biliary tract (obstructive jaundice) and drug induced reactions (Sood, 2006).

In the effects of malaria infection on haematology based on age groups 0 - 4.9 and 5 - 9.9 years in the communities, there were no significant difference in the PCV, Hb and WBC, but the infected children in the age group 10 – 14.9 years had a significant lower Hb than the control. This is comparable with the reports of Ekvall *et al.* (2001) and Adedotun, *et al.* (2013) who observed a higher susceptibility of children in the age group of 0 - 15 years to anaemic condition than the adult population Oyo state, Nigeria. Also Nwaorgu, and Orajaka, (2011) reported 11.24g/dl and 12.18g/dl mean Hb values in the infected and uninfected children in the age range of 6 – 10 years in communities in Awka North Local Government Area, Anambra state South East Nigeria. Malaria parasite impacted negatively on Hb concentration of infected children, also the level of Hb was positively associated with age. The drop in Hb may be due to selective removal of parasites from infected RBCs by macrophages (pitting), and deleterious effects of haeme released during the oxidation of cell-free hemoglobin under inflammatory conditions (Ferreira *et al.*, 2008 and Ekvall *et al.* 2001). Also in population subject to very high inoculation rate year round, severe anaemia is the most common complication of *P. falciparum* infection affecting mainly infants and very young children (WHO, 2014).

In the hospital infection there was significant elevation in the serum level of the liver enzyme SGOT in the *Plasmodium* infected male more than their female counterpart. This significant rise signifies hepatic dysfunction. This result was expected because previous reports showed that renal and hepatic dysfunctions in *falciparum* malaria are common in both children and adults living in malaria endemic regions (Ogbadoyi *et al.*, 2009; Uzugbue *et al.*, 2011; Garba and Gregory 2006; Ashok *et al.*, 1979). This showed that malaria can bring about the inhibition of certain liver functions even in the absence of normal clinical signs of

hepatic insufficiency; however, normal liver function may be restored after termination of the malaria attack. Long continued low-grade malaria infection can cause permanent liver damage (Ashok *et al.*, 1979).

Similarly the significant difference between the PCV of the infected female and male in the hospitals is in line with Opara *et al.* (2006) and Jayant and Mani (2010). Generally, mild anaemia is very common in childhood malaria but may vary with Hb <5g/dl or Hct <15% in the presence of parasite count >10,000 (symptomatic and asymptomatic). Anaemia in malaria, however, is associated with combination of haemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocent non parasitized red blood cells, deprived and ineffective erythropoiesis due to tumour necrosis factor and spleen phagocytises or pooling. This haematological assessment showed that sex can be a determinant of anaemia (PCV <15%g/dl). This is in line with Gayawan *et al.* (2014) who reported that other important risk factors to anaemia apart from sex include household wealth index and presence of fever in the last two weeks preceding the survey. Malaria parasitaemia significantly lowers the PCV of infants especially between the first six to ten months of life (Afolabi *et al.*, 1997). Further evaluation of the effects of sex on the haemoglobin concentration of malaria infected children in Anambra State showed no significant difference. In the hospital assessment of the effects of malaria infection on the biochemical profile of children based on age, there was no significant difference between the infected and controlled. This result is in disagreement with Zaki *et al.* (2012) who reported a significant decrease in ALP, SGOT, total protein and total iron of malaria infected children of different age groups compared to the uninfected. The inconsistency may be attributed to the fact that the children were already receiving treatment, because these parameters were normally found to be at their normal levels six weeks after treatment (Ashok *et al.*, 1979).

Age group study showed that age group 5 – 9.9 years had a significant low PCV and Hb and this is in line with Opara *et al.*, (2006). This could be due to the fact that this age group expose themselves more than the other groups to malaria vector bites because PCV had a negative linear relationship with parasite density. This means that as the parasite density is increasing there is a corresponding decrease in PCV. Anaemia in childhood malaria is more prevalent in lower age groups of 0 - 10 months with fatal consequences (Afolabi *et al.*, 1997). Their WBC count showed no significant difference in this study and is in agreement with Opara *et al.* (2006). Divergent views, have been expressed on WBC in malaria, some have reported leucopenia (Laura *et al.*, 2004), while others have reported Leukocytosis (Pavithran, 2005).

No alteration was observed in both the liver enzymes, and some other biochemical and haematological parameters of infected children with gametocyte 1-10 and 11-100 counts. This is in disagreement with Adeosun *et al.* (2007) and Kitua *et al.* (1997). Alteration in haematology (PCV % and Hb gm/dl) was related to parasite count and is very common in the presence of chronic and recurrent malaria (Jayant and Mani, 2010). Difference in the WBC blood level was also observed in the assessment based on parasite count and is in line with Ladhani *et al.* (2003). This may be associated with concurrent infections and/or poor prognosis. White blood cell count during malaria are generally characterized as being low (Laura *et al.*, 2004) a phenomenon that is widely thought to reflect localization of leucocytes away from the peripheral circulation and to the spleen and other marginal pools rather than actual depletion or stasis. Also it has been observed that in endemic and hyperendemic area, the parasite rate increases with age from 0 – 10% during first three months of life to 80% - 90% by one year of age and the rate persists at a high level during early childhood. Also inadequate immunity results in rapid increase in the parasite count and development of complications (Kakkilaya, 2015).

Conclusion

Malaria parasite infection (symptomatic and asymptomatic) may be associated with anaemia, and haemoglobinuria which may be as a result of increase in breakdown of parasitized and non parasitized red blood cells. Serum level of WBC in malaria infection is gametocyte count dependent. Sex and age may be a determining factor for low PCV since the male infected children had a significant lower PCV in both community and hospital surveys. Hypernatraemia was generally observed in malaria infection amongst children in Anambra State. It is recommended that sex as determinant of anaemia in children should be further investigated.

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