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Impact of Plasmodium Falciparum Malaria Infection on Red Blood Cell Indices in Adults Living in Ido-Ekiti, South-West Nigeria

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Abstract Malaria remains a major public health problem in most countries of the tropics, It cause of morbidity and mortality accounting for an estimated of 300 to 500 million morbid episodes with 2 to 3 million death in a year worldwide. The aim of the study was to elucidate red blood cells indices patterns in adults infected with *Plasmodium falciparum* malaria and their impact on improved diagnosis and therapy. Malaria infected adult individuals within the age 15-64 years presented with signs and symptoms of malaria infection was used for the study, 4ml of blood sample was collected twice from each malaria infected individuals and dispensed into dipotassium ethylenediaminetetracetic acid vaccutainer bottles grouped as pre-treatment and post anti-malaria drug treatment sample. Red blood cell indices were analyzed using haematology analyzer, malaria parasite detection, malaria parasite count and malaria parasite species identification were carried out using stained thick and thin blood film. Data obtained was analyzed using SPSS version 16. Mean±SD of red blood cell indices was significantly ($p<0.05$) lower in age group A compared with other age groups in malaria infected (pre and post anti-malaria therapy) and non-malaria infected groups; red blood cell morphology shows normocytic and normochromic anaemia with a notable microcytosis and hypochromia. Parasite density was significantly reduced after taking anti-malaria drug. Anaemia is a major haematological disorder in patients infected with malaria at all age groups. Use of anti-malaria therapy would result in greater clinical and haematological benefits, after the recovery period of malaria infection.

Keywords: *red blood cell indices, anti-malaria therapy, anaemia in malaria*

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1. Introduction

Malaria is a major public health problem in tropical areas and it is estimated that malaria is responsible for 1 to 3 million deaths and 300-500 million infections annually [1]. The vast majority of morbidity and mortality from malaria is caused by infection with Plasmodium falciparum, although Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae also are responsible for human infections. Plasmodium falciparum has ability of infecting red blood cells (RBC) and to adhere the linings of small blood vessels, such sequestered parasites provide considerable obstruction to tissue perfusion [2,3]. Patients with malaria are often dehydrated and relatively hypovolemic, [4] potentially exacerbating micro-vascular obstruction by reducing perfusion pressure. Red blood cells destruction is also an inevitable part of malaria and anemia further compromises oxygen delivery. The sequence of invasion is probably similar for all *Plasmodium* species. The parasite must engage receptors [5] on red blood cells for binding and undergo apical reorientation, [6] junction formation, [7] and signaling. The parasite then induces a vacuole derived from the red blood cells plasma membrane and enters the vacuole by a moving junction. Three organelles on the invasive (apical) end of parasites (rhoptries, micronemes, and dense granules) define the phylum Apicomplexa. Receptors for invasion of red blood cells by merozoites and for invasion of liver by sporozoites are found in micronemes, [8] on the cell surface and in rhoptries. The distribution of these receptors within an organelle may protect the parasite from antibody-mediated neutralization, as the release after contact with the red blood cells may limit their exposure to antibody. *P*lasmodium *falciparum* and to a much lesser extent, *P*lasmodium *vivax* [9] are the main causes of disease and death from malaria. Mosquitoes inject parasites (sporozoites) into the subcutaneous tissue and less frequently directly into the bloodstream; from there, sporozoites travel to the liver. Recent evidence indicates that sporozoites pass through several hepatocytes before invasion is followed by parasite development [10]. The co-receptor on sporozoites for invasion involves, in part, the thrombospondin domains on the circumsporozoite protein and on thrombospondin-related adhesive protein (TRAP). These domains bind specifically to heparin sulfate proteoglycans on hepatocytes in the region in

apposition to sinusoidal endothelium and Kuppfer cells [11]. Within the hepatocyte, each sporozoite develops into tens of thousands of merozoites, each able to invade a red blood cells on release from the liver. Disease begins only once the asexual parasite multiplies within red blood cells. This is the only gateway to disease. *P*lasmodium *falciparum* and *P*lasmodium *vivax* within red blood cells develop over 48 hours, producing around 20 merozoites in a mature parasite, each able to invade other red blood cells. A small proportion of asexual parasites converts to gametocytes that are critical for the transmission of the infection to others through female anopheline mosquitoes but cause no disease. The signs and symptoms of malaria infection in humans are caused by the asexual blood stage of the parasite. The ratio of numbers of deaths to infections from malaria suggests that infection with blood stage parasites may result in a wide range of outcomes and pathologies. Indeed, the spectrum of severity ranges from asymptomatic infection to rapidly progressive, fatal illness. The clinical presentation of malaria infection is particularly influenced by host age, by immune status with respect to malaria and pregnancy, and by the species, genotype, and perhaps the geographical origin of the parasite. Symptoms may appear on average 12 days (but occasionally 6 months or more) after inoculation of sporozoites into the bloodstream. An infection may be asymptomatic in those with acquired or innate immunity to malaria; others with no or partial immunity may suffer from a severe acute illness. Prodromal symptoms of malaria include headache (which is often severe and dominates the presenting complaint), myalgia, and coughing that precede the typical sequence of shaking chills, fever, and sweating associated with a paroxysm of fever. The erythrocytic cycle in falciparum malaria is usually synchronized so, in the initial stages of infection, fever occurs on days 1 and 3 (and thus is a tertian fever). In advanced infections the pattern of fever becomes less regular, even continuous. Nausea, vomiting, diarrhea, and abdominal pain may accompany fever. In an uncomplicated infection, signs are few, with the notable absence of lymphadenopathy or rash, but include splenomegaly and mild jaundice. If the course of treatment is incomplete or if the parasites are resistant to the treatment given, then parasites may recrudesce and once more cause a patent infection. Follow-up of treated cases is therefore essential. Anemia may arise from multiple poorly understood processes including acute haemolysis of uninfected red blood cells and dyserythropoiesis, as well as through the interaction of malaria infection with other parasite infections and with nutritional deficiencies [12]. The blood stage of falciparum malaria may cause life-threatening anemia, and hemoglobin of less than 5 g/dL is considered to represent severe disease. Anemia may become worse after treatment begins, particularly if the parasitemia is high. The anemia is typically normocytic and normochromic, with a notable absence of reticulocytes, although microcytosis and hypochromia may be present due to the very high frequency of alpha and beta thalassemia traits and/or iron deficiency in many endemic areas [13]. The pathophysiology of anemia in malaria is a complex which gives ample reasons for both increased destruction and reduced production of red cells. Red blood cells are destroyed as parasites complete their growth cycle, although some parasites may be removed from erythrocytes as immature ring forms by phagocytic cells [14]. Infected erythrocytes may also be phagocytosed by macrophages following opsonization by immunoglobulins and/or complement components. Other effector cells and mechanisms are less well defined but may include antibody-dependent cytotoxicity and natural killer (NK) cells. Pathology of malaria anemia is not complete without consideration of 'blackwater fever.' Which is the sudden appearance of hemoglobin in the urine indicating severe intravascular hemolysis leading to hemoglobinemia and hemoglobinuria? There was an association between blackwater fever and the irregular use of quinine for chemoprophylaxis. This drug can act as a hapten and stimulate production of a drug-dependent complement-fixing antibody [15]. Microscopic examination of stained blood films is the gold standard for routine malaria diagnosis. Parasite density has to be reliably evaluated in order to deal with discriminatory thresholds of parasitemia; the level of parasitaemia is useful as one of the criteria in defining "severe *P*lasmodium *falciparum* malaria" and to monitor the effect of anti-malaria therapy [16,17]. Early treatment of clinical malaria attacks by anti-blood stage chemotherapy for *P*lasmodium *falciparum* kills the developing gametocytes. World Health Organization has recommended that artemisinin combination therapies (ACT) be first-line therapy for malaria worldwide [16,18]. Combinations are effective because the artemisinin component kills the majority of parasites at the start of the treatment while the more slowly eliminated partner drug clears the remaining parasites [16,19]. Artemisinin and its derivatives is extremely rapid and most patients show clinical improvement within 1-3 days after treatment. The aim of the study was to elucidate red blood cells indices patterns in adults infected with *Plasmodium falciparum* malaria and their impact on improved diagnosis and therapy.

2. Study Design

Two hundred and two confirmed malaria infected patients were recruited for the study within the age of 15 – 64 years at Federal Teaching Hospital, Ido-Ekiti, Nigeria. One hundred and two apparently healthy non-malaria infected individuals were used as control; patients were placed on Artemether and Lumefantrine combine therapy, taken one tablet two times daily for three days. The study was conducted with an informed consent of the patients; ethical approval was obtained from ethical committee of Federal Teaching Hospital, Ido-Ekiti. Four millimeters (4 ml) of blood sample was collected from patient clinically diagnosis for malaria infection; baseline blood sample was collected on the first day (grouped as pre anti-malaria therapy sample), another 4ml of blood sample was collected on the second or third day from the same patient after taking anti-malaria therapy (grouped as post anti-malaria therapy sample). Blood sample collected was dispensed into di-potassium ethylenediaminetetraacetic acid (K2EDTA) vacuitaner bottles used for red blood cell indices analysis using haematology analyzer (Sysmex model KX-21N) and to screen for malaria parasite using commercially prepared malaria rapid test kit; also thick and thin blood film was made for microscopic gold

standard diagnosis of malaria parasite infection; for malaria parasite detection, malaria parasite count and malaria parasite species identification [16].

3. Statistical Analysis

Results obtained were analyzed using student t-test to compare the means. Analysis was performed using computer database software from the statistical package for social sciences (version 16.0 SPSS). A P-value of < 0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

4. Results

Most of the participants were from rural residence and all of them were infected with *Plasmodium falciparum* species; red blood cell morphology shows normocytic and normochromic anaemia with a notable microcytosis and hypochromia.

Table 1. MEAN±SD OF RED BLOOD CELL INDICES IN PRE-TREATMENT MALARIA INFECTED SUBJECTS

Groups	RBC X10^9/L)	H B (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	MPC µ/l
A (n=68)	4.05± 0.30	10.74 ± 1.68	32.12 ± 5.04	75.40 ± 5.74	26.89 ± 2.71	31.18 ± 1.63	14.81± 2.34	2606.40 ± 526.03
B (n=59)	4.34± 0.49	11.52± 1.55	34.82 ± 4.58	78.32 ± 5.99	27.18 ± 2.74	31.84 ± 1.33	14.21 ± 1.83	2515.7 ± 436.72
C (n=34)	4.69 ± 0.45	12.59 ± 1.87	37.94 ± 5.48	80.40 ± 4.08	28.46 ± 2.27	32.08 ± 1.15	12.46± 1.62	2782.20 ± 285.19
D (n=17)	4.82± 0.53	13.88 ± 1.89	41.67 ± 5.72	84.61 ± 3.84	30.09 ± 2.31	33.03± 0.90	12.46 ± 1.60	2693.70 ± 18.43
E (n=24)	4.60 ± 0.55	12.73 ± 1.57	38.18 ± 4.52	80.91 ± 4.72	28.50 ± 2.05	32.38 ± 1.23	13.72± 1.52	2702.40 ± 123.99
F (p-value)	18.10 (0.00)	17.32 (0.00)	18.28 (0.00)	13.67 (0.00)	8.44 (0.00)	8.27 (0.00)	7.79 (0.00)	2.68 (0.03)
AvsB p-value	0.00	0.06	0.02	0.05	1.00	0.10	0.49	0.83
AvsC p-value	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.19
AvsD p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72
AvsE p-value	0.00	0.00	0.00	0.00	0.03	0.00	0.08	0.63
BvsC p-value	0.01	0.05	0.05	0.29	0.12	0.89	0.03	0.00
BvsD p-value	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.05
BvsE p-value	0.30	0.02	0.03	0.24	0.13	0.39	0.71	0.03
CvsD p-value	0.92	0.17	0.20	0.01	0.06	0.02	0.64	0.53
CvsE p-value	0.96	1.00	1.00	1.00	1.00	0.88	0.60	0.60
DvsE p-value	0.71	0.26	0.25	0.06	0.08	0.31	0.11	0.10

AGE GROUP A= (15-24)
AGE GROUP B = (25-34)
AGE GROUP C = (35- 44)
AGE GROUP D = (45-54)
AGE GROUP E = (55-64)
P-value: P<0.05 Significance, P>0.05 non Significant.

Table 1 show comparison of red blood cell indices in pre-treatment malaria infected subjects. The mean±SD of RBC, Hb, PCV, MCV MCH and MCHC were significantly (P<0.05) lower in age group A compared with other age groups; mean±SD of RDW in age group A was significantly (P<0.05) higher compared with other age groups, mean±SD of MPC was significantly (P<0.05) lower in age group B compared with other age groups. In post hock multiple comparison between age group A and B shows, mean±SD of RBC, PCV and MCV were significantly (P<0.05) lower in age group A compared with age group B, mean ±SD of Hb, MCH and MCHC were non significantly (P>0.05) lower in age group A compared with age group B; mean ±SD of RDW and MPC were non significantly (P>0.05) higher in age group A compared with age group B. Multiple comparison between age group A and C shows mean±SD of RBC, Hb, PCV, MCV, MCH and MCHC were significantly (P<0.05) lower in age group A compared with age group C, mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group C; mean ±SD of MPC in age group A was non significantly (P>0.05) lower compared with age group C. Multiple comparison between age group A and D shows mean±SD of RBC, Hb, PCV, MCV,MCH and MCHC were significantly (P<0.05) lower in age group A compared with age group D, mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group D; mean ±SD of MPC in age group A was non significantly (P>0.05) lower compared with age group D. Multiple comparison between age group A and E shows mean±SD of RBC, Hb, PCV, MCV,MCH, and MCHC were significantly (P<0.05) lower in age group A compared with age group E, mean ±SD of RDW was non significantly (P>0.05) higher in age group A compared with age group E; mean ±SD of MPC in age group A was non significantly (P>0.05) lower compared with age group E. Multiple comparison between age group B and C shows mean±SD of RBC, Hb, PCV and MPC were significantly (P<0.05) lower in age group B compared with age group C, mean ±SD of MCV, MCH and MCHC were non significantly (P>0.05) lower in age group B compared with age group C; mean ±SD of RDW

in age group B was significantly (P<0.05) higher compared with age group C. Multiple comparison between age group B and D shows mean±SD of RBC, Hb, PCV, MCV, MCH, MCHC and MPC were significantly (P<0.05) lower in age group B compared with age group D, mean ±SD of RDW was significantly (P<0.05) higher in age group B compared with age group D. Multiple comparison between age group B and E shows mean±SD of RBC, MCV,MCH, and MCHC were non significantly (P>0.05) lower in age group B compared with age group E, mean ±SD of Hb, PCV and MPC were significantly (P<0.05) lower in age group B compared with age group E; mean ±SD of RDW in age group B was not significantly (P>0.05) higher compared with age group E. Multiple comparison between age group C and D shows mean±SD of RBC, Hb, PCV and MCH were non significantly (P>0.05) lower in age group C compared with age group D, mean ±SD of MCV and MCHC were significantly (P<0.05) lower in age group C compared with age group D; mean ±SD of RDW and MPC in age group C was not significantly (P>0.05) higher compared with age group D. Multiple comparison between age group C and E shows mean±SD of RBC and MPC were non significantly (P>0.05) higher in age group C compared with age group E, mean ±SD of Hb, PCV, MCV, MCH, MCHC and RDW were non significantly (P>0.05) lower in age group C compared with age group E. Multiple comparison between age group D and E shows mean±SD of RBC, Hb, PCV, MCV,MCH, and MCHC were non significantly (P>0.05) higher in age group D compared with age group E, mean ±SD of RDW and MPC were non significantly (P>0.05) lower in age group D compared with age group E.

Table 2. MEAN±SD OF RED BLOOD CELL INDICES IN POST ANTI-MALARIA DRUG TREATMENT IN MALARIA INFECTED SUBJECTS

Groups	RBC ($X10^9$/L)	H B (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	MPC µ/l
A (n=68)	3.84±0.34	9.63±1.94	28.81± 5.73	73.23±5.92	25.50±2.72	30.78±1.65	14.12 ± 2.18	2410.30 ± 705.13
B (n=59)	4.09± 0.49	10.73±1.73	32.15 ± 5.13	75.95± 5.57	25.8 ± 2.75	30.92± 1.39	13.36 ± 1.88	2194.10 ± 480.79
C (n=34)	4.51± .42	11.9 ± 2.12	35.69± 6.12	78.26± 3.69	26.95± 2.73	31.26± 1.10	12.42 ± 1.63	2369.50 ± 495.15
D (n=17)	4.59 ±0.47	13.15 ± .93	39.31 ± 5.84	82.35± 4.03	29.23± 2.43	32.16± 0.96	11.79 ± 1.45	2172.60 ± 103.06
E (n=24)	4.39± 0.57	11.71 ±1.66	35.15± 5.01	78.80± 4.88	27.2 ± 2.02	31.65± 0.10	13.07 ± 1.65	2283.00 ± 247.47
F (p-value)	19.98 (0.00)	17.74 (0.00)	18.06 (0.00)	14.14 (0.0)	8.56 (0.00)	8.13 (0.00)	8.01 (0.00)	1.69 (0.16)
AvsB p-value	0.01	0.01	0.01	0.07	0.95	0.27	0.54	0.25
AvsC p-value	0.00	0.00	0.00	0.00	0.09	0.01	0.00	1.00
AvsD p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
AvsE p-value	0.00	0.00	0.00	0.00	0.01	0.00	0.12	0.00
BvsC p-value	0.00	0.05	0.05	0.12	0.34	0.68	0.03	0.46
BvsD p-value	0.01	0.00	0.01	0.00	0.00	0.00	0.00	1.00
BvsE p-value	0.22	0.13	0.12	0.15	0.09	0.10	0.77	0.80
CvsD p-value	0.97	0.27	0.27	0.01	0.04	0.04	0.64	0.19
CvsE p-value	0.89	0.99	0.27	1.00	0.99	0.68	0.57	0.91
DvsE p-value	0.71	0.12	0.15	0.10	0.07	0.53	0.09	0.31

AGE GROUP A= (15-24)
AGE GROUP B = (25-34)
AGE GROUP C = (35- 44)
AGE GROUP D = (45-54)
AGE GROUP E = (55-64)
P-value: P<0.05 Significance, P>0.05 non Significant.

Table 2 show comparison of red blood cell indices in post anti-malaria drug treatment in malaria infected subjects. The mean±SD of RBC, Hb, PCV, MCV MCH and MCHC were significantly (P<0.05) lower in age group A compared with other age groups; mean±SD of RDW in age group A was significantly (P<0.05) higher compared with other age groups, mean±SD of MPC was non significantly (P>0.05) lower in age group B compared with other age groups. Multiple comparison between age group A and B shows, mean±SD of RBC, Hb and PCV were significantly (P<0.05) lower in age group A compared with age group B, mean ±SD of MCV, MCH and MCHC were non significantly (P>0.05) lower in age group A compared with age group B; mean ±SD of RDW and MPC were non significantly (P>0.05) higher in age group A compared with age group B. Multiple comparison between age group A and C shows, mean±SD of RBC, Hb, PCV, MCV and MCHC were significantly (P<0.05) lower in age group A compared with age group C, mean ±SD of MCH was non significantly (P>0.05) lower in age group A compared with age group C; mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group C. Mean±SD of MPC was non significantly (P>0.05) higher in age group A compared with age group C. Multiple comparison between age group A and D shows, mean±SD of RBC, Hb, PCV, MCV, MCH and MCHC were significantly (P<0.05) lower in age group A compared with age group D, mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group D. Mean±SD of MPC was non

significantly (P>0.05) higher in age group A compared with age group D. Multiple comparison between age group A and E shows, mean±SD of RBC, Hb, PCV, MCV, MCH, MCHC and MPC were significantly (P<0.05) lower in age group A compared with age group E, mean ±SD of RDW was non significantly (P>0.05) higher in age group A compared with age group E. Multiple comparison between age group B and D shows, mean±SD of RBC, Hb and PCV were significantly (P<0.05) lower in age group B compared with age group C, mean ±SD of MCV, MCH, MCHC and MPC were non-significantly (P>0.05) lower in age group B compared with age group C. Mean±SD of RDW was significantly (P<0.05) higher in age group B compared with age group C. Multiple comparison between age group B and D shows, mean±SD of RBC, Hb, PCV, MCV, MCH and MCHC were significantly (P<0.05) lower in age group B compared with age group D, mean ±SD of RDW was significantly (P<0.05) higher in age group B compared with age group D. Mean±SD of MPC was non significantly (P>0.05) higher in age group B compared with age group D. Multiple comparison between age group B and E shows, mean±SD of RBC, Hb, PCV, MCV, MCH MCHC and MPC were non-significantly (P>0.05) lower in age group B compared with age group E, mean ±SD of RDW was non-significantly (P>0.05) higher in age group B compared with age group E. Multiple comparison between age group C and D shows, mean±SD of RBC, Hb and PCV were non-significantly (P>0.05) lower in age group C compared with age group D, mean ±SD of MCV, MCH and MCHC were significantly (P<0.05) lower in age group C compared with age group D. Mean±SD of RDW and MPC were non significantly (P>0.05) higher in age group C compared with age group D. Multiple comparison between age group C and E shows, mean±SD of RBC, Hb, PCV and MPC were non-significantly (P>0.05) higher in age group C compared with age group E, mean ±SD of MCV, MCH, MCHC and RDW were non-significantly (P>0.05) lower in age group C compared with age group E. Multiple comparison between age group D and E shows, mean±SD of RBC, Hb, PCV, MCV, MCH and MCHC were non-significantly (P>0.05) higher in age group D compared with age group E, mean ±SD of RDW and MPC were non-significantly (P>0.05) lower in age group D compared with age group E.

Table 3. MEAN±SD OF RED BLOOD CELL INDICES IN NON-MALARIA INFECTED SUBJECTS (CONTROL)

Groups	RBC ($X10^9$/L)	H B (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	MPC µ/l
A(n=36)	4.11± 0.36	12.48 ± 0.64	37.78±1.55	76.80±17.16	28.63±0.90	31.49±1.20	14.34±1.00	-
B(n=20)	4.41± 0.49	13.64± 1.16	40.78±3.39	84.42 ± 2.42	29.84±0.41	32.69±1.18	13.44±1.11	-
C(n=24)	5.06± 0.48	14.62 ± 0.85	43.86±2.57	83.98 ± 2.91	29.34±1.07	33.38±1.05	13.01±1.38	-
D(n=19)	5.25± 0.37	14.93 ± 0.72	45.04±1.99	85.68±22.31	30.61±0.85	33.48±0.90	12.17±1.15	-
E (n=03)	5.42 ± 0.43	15.10 ± 0.61	45.27±1.77	85.20 ± 1.82	29.20±1.59	33.93±0.76	12.33±2.41	-
F (p-value)	33.68 (0.00)	39.20 (0.00)	42.13 (0.00)	3.33 (0.01)	9.99 (0.00)	16.34 (0.00)	11.98 (0.00)	-
AvsB p-value	0.16	0.00	0.01	0.09	0.00	0.01	0.03	-
AvsC p-value	0.00	0.00	0.00	0.12	0.09	0.00	0.00	-
AvD p-value	0.00	0.00	0.00	0.03	0.00	0.00	0.00	-
AvsE p-value	0.09	0.04	0.05	0.07	0.96	0.06	0.67	-
BvsC p-value	0.00	0.03	0.02	0.98	0.24	0.27	0.79	-
BvsD p-value	0.00	0.00	0.00	0.46	0.94	0.14	0.01	-
BvsE p-value	0.13	0.10	0.09	0.95	0.94	0.28	0.92	-
CvsD p-value	0.58	0.71	0.45	0.22	0.17	0.99	0.21	-
CvsE p-value	0.69	0.75	0.74	0.84	1.00	0.78	0.98	-
DvsE p-value	0.95	0.99	1.00	0.99	0.89	0.88	1.00	-

AGE GROUP A= (15-24)
AGE GROUP B = (25-34)
AGE GROUP C = (35- 44)
AGE GROUP D = (45-54)
AGE GROUP E = (55-64)
P-value: P<0.05 Significance, P>0.05 non Significant.

Table 3 shows comparison of red blood cell indices in non-malaria infected subjects (control). Since control subjects were not infected with malaria parasite, their malaria parasite count was nil. The mean±SD of RBC, Hb, PCV, MCV MCH and MCHC were significantly (P<0.05) lower in age group A compared with other age groups; while mean±SD of RDW in age group A was significantly (P<0.05) higher compared with other age groups. Multiple comparison between age group A and B shows, mean±SD of Hb, PCV, MCH and MCHC were significantly (P<0.05) lower in age group A compared with age group B, mean ±SD of RBC and MCV were non significantly (P>0.05) lower in age group A compared with age group B; mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group B. Multiple comparison between age group A and C shows, mean±SD of RBC, Hb, PCV and MCHC were significantly (P<0.05) lower in age group A compared with age group C, mean ±SD of MCV

and MCH were non significantly (P>0.05) lower in age group A compared with age group C; mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group C. Multiple comparison between age group A and D shows, mean±SD of RBC, Hb, PCV,MCV, MCH and MCHC were significantly (P<0.05) lower in age group A compared with age group D, mean ±SD of RDW was significantly (P<0.05) higher in age group A compared with age group D. Multiple comparison between age group A and E shows, mean±SD of Hb and PCV were significantly (P<0.05) lower in age group A compared with age group E, mean ±SD of RBC, MCV, MCH and MCHC were non significantly (P>0.05) lower in age group A compared with age group E; mean ±SD of RDW was non-significantly (P>0.05) higher in age group A compared with age group E. Multiple comparison between age group B and C shows, mean±SD of RBC, Hb and PCV were significantly (P<0.05) lower in age group B compared with age group C, mean ±SD of MCV, MCH and RDW were non significantly (P>0.05) higher in age group B compared with age group C; mean ±SD of MCHC was non-significantly (P>0.05) lower in age group B compared with age group C. Multiple comparison between age group B and D shows, mean±SD of RBC, Hb and PCV were significantly (P<0.05) lower in age group B compared with age group D, mean ±SD of MCV, MCH and MCHC were non significantly (P>0.05) lower in age group B compared with age group D; mean ±SD of RDW was significantly (P<0.05) higher in age group B compared with age group D. Multiple comparison between age group B and E shows, mean±SD of RBC, Hb, PCV MCV and MCHC were non-significantly (P>0.05) lower in age group B compared with age group E, mean ±SD of MCH and RDW were non significantly (P>0.05) higher in age group B compared with age group E. Multiple comparison between age group C and D shows, mean±SD of RBC, Hb, PCV, MCV, MCH and MCHC were non-significantly (P>0.05) lower in age group C compared with age group D, mean ±SD of RDW was non-significantly (P>0.05) higher in age group C compared with age group D. Multiple comparison between age group C and E shows, mean±SD of RBC, Hb, PCV, MCV and MCHC were non-significantly (P>0.05) lower in age group C compared with age group E, mean ±SD of MCH and RDW were non significantly (P>0.05) higher in age group C compared with age group E. Multiple comparison between age group D and E shows, mean±SD of RBC, Hb, PCV, MCHC and RDW were non-significantly (P>0.05) lower in age group D compared with age group E, mean ±SD of MCV and MCH were non significantly (P>0.05) higher in age group D compared with age group E.

5. Discussion

In this present study, the level of anaemia at presentation (pre treatment) was lower as compared to anaemia observed during treatment (post-treatment). Normocytic and normochromic anaemia with a notable microcytosis and hypochromia were majorly observed in the study determined by red cell indices and red blood cells morphology. The increase in anaemia observed during treatment was due to effects of anti-malaria therapy used with high level of parasitaemia before treatment. Similar to this study, Bashawri reported normochromic normocytic microcytic hypochromic, in red cell morphology of malaria infected patients [20]. Anaemia in malaria infection was supported with the facts that, anaemia correlates with the severity of the malaria infection [21,22]. The pathogenesis of anaemia in malaria by the mechanisms of red cell destruction is thought to result from a combination of haemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocently unparasitized red blood cells, depressed as well as ineffective erythropoiesis with dyserythropoietic changes, RBCs destruction is an inevitable part of malaria and anaemia further compromises oxygen delivery, other factors contributing to anaemia in malaria infection include decreased deformability of infected red cells, membrane changes, immune mechanisms, splenic phagocytosis and/or pooling (reticuloendothelial hyperplasia) [23,24] resulted to increased rate of red blood cells clearance from the circulation. It should be borne in mind that red cell morphology in malaria patients may be influenced by their nutritional status i.e., patients could be iron deficient, folic acid or vitamin B_{12} deficient or they may have a concurrent thalassemia, or haemoglobinopathy (Hb SS) which aggravates the severity of the anemia. Similar to this study, Sowunmi *et al.*, 2009 reported that artemisinin drugs (anti-malaria drug) cause falls in haematocrit during treatment resulted in anaemia [25]. Sumbele *et al.*, 2010 stated that, a drop in prevalence of anaemia was observed during follow up; the prevalence of persistent anaemia (Hb concentration that remained below 11 g/dl for the duration of the follow up) was 9.7%. The untreated malaria recorded the highest prevalence of persistent anaemia (17.2%) when compared to the treated malaria, the prevalence of mild anaemia increased during follow up, a drop in prevalence of moderate and severe anaemia was observed. Although there was a general rise in Hb concentration in treated malaria compared to the untreated malaria. As parasites were cleared, an increase in Hb was observed in the treated malaria hence, prevalence of anaemia in untreated malaria was higher when compared to the treated malaria. The significantly high prevalence of anaemia in untreated malaria when compared to the treated malaria indicated that malaria treatment is necessary for haematological recovery [26]. Delayed parasite clearance was highlighted by Price *et al*., 2001 as a significant independent risk factor for anaemia; It was also identified as a risk factor for persistent anaemia [27,28]. In this present study, haematological parameters which includes red blood cells (RBC), Haemoglobin Concentration (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and red cell distribution width (RDW) were significantly difference in pre-treatment, post-treatment and control subject. The mean values of these parameters in post-treatment were lower compared with pre-treatment and control subject. Decrease of these parameters in post-treatment in this study was due to effects of anti- malaria therapy used and high level of parasitamia before treatment, this was supported by Sowunmi *et al.*, 2009, who stated that after the recovery period of malaria infection, MCV, MCHC and MCH were expected to be decrease as artemisinin drugs are reported to cause less anti-malaria drug-related falls in haematocrit

during treatment. Haematological parameters in this present study was used to determine anaemia induced by malaria parasite infection, according to Dondorp *et al.*, 2000 who stated that the severity and type of anaemia can be determined by the levels of haematological indices such as haemoglobin concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH). It is clear that in severe malaria there may be marked reductions in the deformability of uninfected RBCs [29]. The low haemoglobin concentrations may have triggered gametocytogenesis [30]. In this study, haematological parameters were significantly lower in age group A compared with other age groups in malaria infected (pre and post anti-malaria therapy) and non-malaria infected groups; this was supported by Akanbi *et al.*, 2010 who reported the highest parasite density was found among those within the age range 18-21 years while it was least among those within the age range 26-30 years which was similar to what observed in malaria parasite count of this present study. He then concluded that this could be due to the number of exposure to mosquito bites among people within the age range of 26-30 years as it has been reported that the number of exposure to mosquito bite increases with age [31]. This number of exposure to mosquito bites by individuals had been confirmed to increase the level of immunity against malaria infection and these increases with age [32]. Thus, in *Plasmodium falciparum* endemic areas, protective immunity against malaria infection is acquired slowly after a large number of infections and its maintenance requires a sustained exposure to infected mosquito [33]. The level of immunity against malaria has also been related to age of the individuals living in malaria endemic areas [34].

6. Conclusion

Parasite density was significantly reduced after taking anti-malaria drug. Anaemia is a major haematological disorder in patients infected with malaria at all age groups. Use of anti-malaria therapy would result in greater clinical and haematological benefits, after the recovery period of malaria infection.

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