Available online at www.worldscientificnews.com

World Scientific News

WSN 74 (2017) 80-93 EISSN 2392-2192

Impact of Rectal Artesunate on Haematological Parameters of Children of Ogun State, South-Western Nigeria

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ABSTRACT

Impact of Rectal Artesunate on haematological parameters was assessed in 905 children below the age of 5 years. Grid systematic method was employed in selecting sixteen study centres from 8 Local Government Areas of Ogun State. Ethical approvals were obtained in addition to interactive sessions with parents of the children and PHCs medical practitioners. Drug was administered at a dose of 5 to 10mg/kg of the body weight per rectum at 0 hour, 24 hours and 48 hours. Data obtained were analyzed using SPSS version 20 to assess association with *p*-value of < 0.05. Impact of Rectal Artesunate was monitored by analysing blood samples taken at 0 hour, 24 hours and 48 hours for Packed Cells Volume (PCV) and Haemoglobin concentration (Hb). There was a significant ($p < 0.05$) increase in baseline PCV (L/L) and Hb (g/L) during 48 hours across the LGAs. Parasitaemia (MPC/µl) in children has a significant ($p < 0.05$) association with anaemia (PCV and Hb) and was positively correlated ($r = +0.348$ and $r =+ 0.201$). A significant ($p < 0.05$) difference observed between MCV (fl), MCH (pg) and MCHC (g/L) at 0 hour and MCV (fl), MCH (pg) and MCHC (g/L) at 48 hours. More awareness on the use of Rectal Artesunate in reducing anaemia due to malaria in children should be created since it is highly effective antimalarial suppository.

***Keywords*:** Effectiveness, Anaemia, Rectal Artesunates, Malaria, Children, Ogun State, South-western Nigeria

1. INTRODUCTION

Anaemia due to malaria Hb <110 g/dL or PCV <0.33L/L is a major public health problem affecting 1.62 billion people globally (McLean, *et al.*, 2009). Africa and Asia are the most affected regions with more than 85% of the absolute anaemia burden (McLean, *et al.*, 2009). Children and women of reproductive Age are most at risk, with global anaemia prevalence estimates of 47% in children younger than 5 years, 42% in pregnant woman and 30% in non-pregnant woman Aged 15-49 years (McLean, *et al.*, 2005; Balarajan, *et al.*, 2011). Causes of anaemia can be broadly classified into decreased erythrocyte production or increased loss of erythrocytes through increased destruction (haemolysis) or blood loss or both (Balarajan, *et al.*, 2011). Severe malaria anaemia is defined as Haemoglobin concentration <5g/dl associated with *P. falciparum* parasitaemia (WHO, 2012). Severe anaemia may exist alone or in combination with other complications particularly cerebral malaria and respiratory distress in which it portends worse prognosis (WHO, 2004). Although, anaemia is a known complication of malaria disease, it has a profound effect on the quality of life of people by inducing such symptoms as loss of stamina, rapid heart rate and shortness of breath. It has also been reported that over half of malaria related deaths are attributable to severe anaemia (MIS, 2013). It was also reported that ignorance, poverty and gender bias also significantly contribute to high prevalence of anaemia (Jaleel and Khan, 2008). These factors are rife in rural communities of Ogun State and indeed most other rural settings in Nigeria. Although few study exist on malaria and anaemia among children in Nigeria. Anaemia is increasingly being used as an indicator of the impact of malaria control in intervention trials (ter Kuile, *et al.*, 2003 and Chandramohan, *et al.*, 2005) and for monitoring and evaluation by the Roll Back Malaria Partnership (Korenromp, *et al.*, 2004). Anaemia is assessed either by measurement of the PCV or the Hb concentration. Clinical and epidemiological studies of malaria use either measure. The WHO definition of anaemia in children between 6 and 60 months of Age is a Hb level lower than 110 g/dL which is equivalent to a Hct/PCV lower than 0.33L/L (Erhabor, *et al.*, 2010; Knoblauch, *et al.*, 2014). Besides, their calculation depends on the method used for their determination. Typically anaemia is determined by measuring Hb concentration (WHO, 2011a). However, PCV has been widely used as an alternative to Haemoglobin in malaria studies in Africa countries (Chandramohan, *et al.*, 2005) and in Nigeria (Imoru, *et al.*, 2013; Ifeanyichukwu and Esan, 2014 and Nwagu, *et al.*, 2014).

2. MATERIALS AND METHODS

Study Areas

The study was conducted in Ogun State, Nigeria; a Tropical Rain Forest Zone, lies approximately between Longitude 2°31' W and 4°31' E, and Latitude 6°31' S and 8° N, its bounded in the south partly by the Atlantic Ocean, and sharing common boundaries with Oyo, Osun, Ondo, Lagos States and Republic of Benin (Plate 5).The state is made up of three Senatorial Zones i.e. Ogun Central, Ogun East and Ogun West) and four Geo-Political Zones (GPZs) i.e. Yewa-Awori, Egba, Ijebu and Remo) with five main ethnic groups namely, Egba, Ijebu, Egbado, Awori and Egun. The main occupations are Farming, Textile production (tie and dye), Fishing, Trading, Civil Servant, Public Servant and Pottering. It has an area of

16,980.55 Square Kilometres (km^2) of the 196,000 km^2 land area of the South-West Zone of the 192,803.07 km^2 of the Southern Nigeria in overall land area of 937,052.16 km^2 of Nigeria. It has a population of 3,751,140, (1,864,907 Males and 1,886,233 Females) (NPC, 2010).

Selection of Study Sites

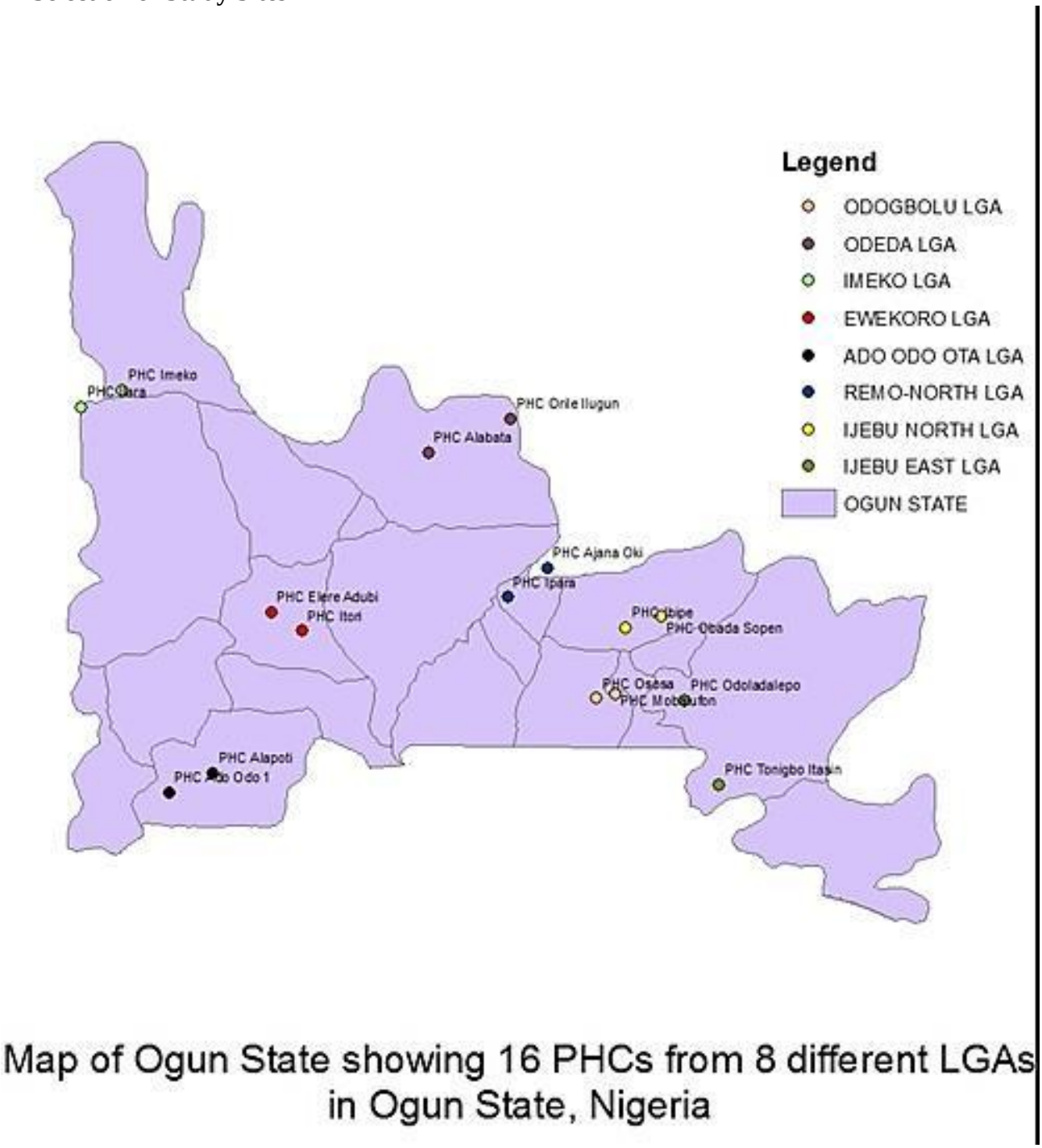

Figure 1. Map of Ogun State Showing the Selected LGAs and their PHCs Used for the Study.

Grid Systematic Method was employed in selecting sixteen (16) study centres which comprised two Primary Health Centres (PHCs) from each eight Local Government Areas (LGA) namely, Ado-Odo-Ota (ADT), Imeko-Afon (IMA), Ewekoro (EWK), Odeda (ODD), Ijebu-East (IJE), Ijebu-North (IJN), Odogbolu (ODG) and Remo-North (RMN). GIS instrument was used to obtained co-ordinates of the PHCs and map was drawn using ArcGIS 9.3 software (Figure 1).

Consent and Ethical Approvals

Ethical approval was obtained from Ethics Committee of Department of Biological Sciences, and Federal Medical Centre Idi-Aba Abeokuta, Ogun State, Nigeria. Permission for study was obtained from Ogun State Ministry of Health and Local Government Service Commission, Abeokuta Ogun State, Nigeria.

A Certificate of approval was obtained from National Agency for Food and Drug Administration and Control (NAFDAC) for import permission for the Plasmotrim-50/200mg (produced by Acino Pharma Ltd Dornacherstrasse 114/ch-4147 Aesch Switzerland) used for the study and multi-centre clinical trial permission to carry out the research.

Determination of Sample Size

From the 2014 immunization data obtained from Ogun State Ministry of Health, 905 children were sampled (Table 1). This was calculated using formula by Kothari (2005).

$$N = \frac{Z^2 pq}{d^2}$$

Table 1. 2014 Immunization Data of under Five Years Children, Calculated Sample Size, Parents of ≤5 Years Treated, Interviewed Health Workers and Interviewed Patent Medicine Vendors

Local Governments	Selected PHCs	Pop. of Communities	Pop. of <5 Years Children in PHCs	Sample Size	≤5 Years Treated
Ado-Odo Ota	Alapoti.	19023	3804	130	74
	Ado-odo 1.	23888	4778		56
Imeko-Afon	Imeko.	18577	3715	110	62
	Ilara.	13033	2607		48
Ewekoro	Itori.	9735	1947	100	50
	Elere-Adubi.	7815	1563		50
Odeda	Ilugun.	16173	3235	110	64
	Alabata.	12889	2578		46

Ijebu-East	Odoladalepo	15696	3139	110	56
	Tonigbo.	8080	1616		54
Ijebu North	Obada.	39273	7855	120	64
	Ibipe.	32500	6500		56
Odogbolu	Ososa.	14868	2974	115	52
	Mobalufon.	25163	5032		63
Remo-North	Ipara	8809	1762	110	59
	Ajana	3856	771		51
Total	**16**	**269378**	**52876**	**905**	**905**

Inclusion Criteria

The inclusion signs/features for selecting children for this study are:

- Children from 1 month to 5 years
- Temperature ≥ 37.5 °C,
- Feverish condition,
- Nausea,
- Repeated vomiting
- Unable to eat or drink (or suck, in the case of infants).
- General weakness
- No history of watery stools, diarrhoea and anal disease.

Blood Collection and Examination

Pre-treatment blood samples were collected (0 hour) from each child that met the inclusion criteria and at 24 and 48 hours during treatment to determine Packed Corpuscular Volume and Haemoglobin concentration as described by Flegg *et al.* (2013). Venous blood was collected from antecubital vein of the children at enrolment to provide blood for the haematological investigations. 2ml of venous blood of the children was collected using sterile needle and syringes, as described by Carter and Lema (1993); Cheesbrough (2009) and WHO (2010). Blood collected was transferred immediately into a labeled Ethylene-Diamine-Tetra-Acetate Acid (EDTA) bottle and mixed gently by inverting the stopper tube several time to prevent blood from clotting and kept at temperature of 4-8 °C and transferred to laboratory within 2-3 days of collection (Cheesbrough, 2009).

The severity and type of anaemia were determined through the following haematological indices; Haemoglobin concentration (Hb) and Packed Corpuscular Volume (PCV) (Kimbi *et al.*, 2013).

Packed Corpuscular Volume **(**PCV) is the percent volume of packed red cells following centrifugation. PCV of each child was determined by centrifugation of blood in heparinised capillary tube (with one end sealed) using Haematocrit Centrifuge and spun at 10,000 rpm (revolution per minute) for 5 minutes. The capillary containing the centrifuge blood was placed on a haematocrit reader, following procedure of reading as described by Cheesbrough (2009).

Haemoglobin concentration was determined using the cyanomethamoglobin method as described by Cheesbrough (2005). Ferricyanide present in Drabkins solution oxidizes the iron (II) present in Haemoglobin, oxy-haemoglobin and carboxyhaemoglobin into iron (III) giving rise to Methaemoglobin which in the presence of cyanide ion produces cyanomethaemoglobin a stable red compound that was photometrically determined at 540nm.

3. RESULTS

Packed Corpuscular Volume (PCV L/L) and Haemoglobin (Hb g/L) among the Studied Group at 0 Hour and during 48 hours across the LGAs

Result from Table 2 showed that children had low anaemia (PCV L/L) at 0 hour with more children observed with moderate anaemia (PCV L/L) (0.21-0.30L/L) (44.5%) and mild anaemia (PCV L/L) (0.31-0.33L/L) (42.0%), lower percentage observed with severe anaemia (PCV L/L) (<0.21L/L) (5.7%) and 7.8% had normal anaemia (PCV L/L) (>33L/L).

Participated children under study at Ewekoro LGA (30%) had higher normal anaemia (PCV L/L) (>33L/L) with lower normal anaemia (PCV L/L) (>33L/L) observed at Remo-North LGA (1.8%). Higher moderate anaemia (PCV L/L) (0.21-0.30L/L) was observed in Ado-Odo-Otta LGA (62.2%). Also, higher severe anaemia (PCV L/L) (<0.21L/L) was observed in Ewekoro LGA (11.0%).

Result revealed reduction in the level of Anaemia (PCV L/L) at 48 hours with 49.1% children had normal anaemia (PCV L/L) (>33L/L), and no children with severe anaemia (PCV L/L) (<0.21L/L) (0%). Lower normal anaemia (PCV L/L) (>33L/L) was observed in Remo-North LGA (18.2%).

There was a significant (p = 0.000) reduction in baseline anaemia (PCV L/L) during 48 hours across all the LGAs. A higher percentage of low Haemoglobin concentration (Hb g/L) observed across the LGAs, with more children presented moderate anaemia (Hb g/L) Hb (70-99g/L) (60.0%), 12.9% had mild anaemia (Hb g/L) (110-109g/L), 26.3% had severe anaemia (Hb g/L) (<70g/L), and 0.8% had normal anaemia (Hb g/L) (>110g/L). Analysis of blood also revealed increase in the level of Haemoglobin (Hb g/L) at 48 hours with 29.2% children had normal anaemia (Hb g/L) (>110g/L), and 5.9% children still had severe anaemia (Hb g/L) (<70g/L). There was a significant (p = 0.001) reduction in baseline anaemia (Hb g/L) during 48 hours across the LGAs.

Parasitaemia (MPC/µl) in Relation to Anaemia (PCV and Hb)

Children with no parasitaemia (MPC/µl) presented normal anaemia (PCV L/L) (17.1% and 48.7%) both at 0 hour and during 48 hours of treatment (Figure 2) while children with severe parasitaemia (MPC/µl) presented severe anaemia (PCV L/L) (55.8%) at 0 hour. Decrease in parasitaemia (MPC/µl) during 48 hours resulted in reduction in severity of anaemia with no children presented severe anaemia (PCV L/L) (0%).

Parasitaemia (MPC/µl) in children has a significant (p = 0.003) association with anaemia (PCV L/L) and was positively correlated (r = +0.348). Children with no parasitaemia (MPC/µl) presented normal anaemia (Hb g/L) (71.4% and 84.2%) both at 0 hour and during 48 hours of treatmrnt (Figure 3) while children with severe parasitaemia (MPC/µl) presented more of moderate anaemia (Hb g/L) (72.2%) and severe anaemia (Hb g/L) (36.1%) at 0 hour. Decrease in parasitaemia during48 hours (MPC/µl) resulted in reduction in anaemia with more children presented normal anaemia (Hb g/L) (84.2%) and mild anaemia (Hb g/L) (71.7%). Parasitaemia (MPC/µl) in children has a significant (p = 0.008) association with anaemia (Hb g/L) and was positively correlated (r = +0.201).

MCV(fL), MCH (pg) and MCHC (g/L) among the Studied Group at 0 Hour and during 48 hours across the LGAs

A higher Microcytosis and hypochromasia Anaemia were observed among children under study before treatment (0 hour) across all the LGAs (MCV (<73fl) (68.6%), MCH (<27pg) (97.2%) and MCHC (<305g/L) (95.1%)) and fewer Normocytic Anaemia, MCV (73-96fl) (31.4%), MCH (27-32pg) (2.2%) and Normochromic Anaemia, MCHC (305-345g/L) (4.9%).

A significant percentage of Normocytic Anaemia, MCV (73-96fl) (74.8%), MCH (27-32pg) (27.3%) and Normochromic Anaemia, MCHC (305-345g/L) (30.2%) were observed among children under study across all the LGAs after treatment (48 hours) (Table 3).

Pearson's Chi-square (χ^2) analysis also showed that there was a significant (MCV- p = 0.0265 MCH- p = 0.0443 MCHC- p = 0.0387) difference between MCV (fl), MCH (pg) and MCHC (g/L) before treatment (0 hour) and after treatment (48 hours) across all the LGAs.

4. DISCUSSION AND CONCLUSIONS

The observed result on anaemia in relation to parasitaemia revealed that children of rural communities of Ogun State were anaemic may be due to excessive destruction of the Red Blood Cells by the malaria parasites harboured by the children, related results has been reported by Anumudu *et al.* (2008) in pre school children of rural communities of Odogbolu in Ogun State, and Sam-Wobo and Asiwaju (2014) reported that presence or increase in parasiteamia could decrease PCV since blood samples without malaria parasites had normal PCV which was statistically shown a significance difference (p < 0.05) between malaria parasites and PCV among children and adult in rural communities of Odeda in Ogun State. Osonuga, *et al.*, (2006) also reported high anaemic condition in <5 years children treated with Artesunate derivatives in Ikenne-Remo of Ogun State. Many reports on malaria-anaemia in other parts of Nigeria had shown that anaemia was one of the common complication in malaria especially in younger children and pregnant women in high transmission areas which cause PCV to has a significant decrease with increasing parasites (Erhabor *et al.*, 2014; Ajayi *et al.*, 2015). In Africa, malaria-anaemia (PCV) had been reported (Takem, *et al.*, 2010; Assefa *et al.*, 2013; Gansane *et al.*, 2013; Kimbi *et al.*, 2013).

However, the increase in the level of PCV (L/L) observed during treatment (48 hours) indicated that rectal artesunate had positive influence (efficacy) on PCV (anaemia) in children (Awad *et al.*, 2003).

Also the higher percentage observed in children with low haemoglobin concentration before treatment (0 hour) indicated that haemoglobin is a very common presentation of malaria in children. It was believed to be due to direct depression of erythropoiesis by malaria disease and actual parasitization of red cells by malaria parasites leading to shortened survival or death of erythrocytes suggested to be a consequence of excessive destruction of the red blood cells by the malaria parasites (Dondorp *et al.,* 2000) and this had also been reported by Anumudu *et al.* (2008) in pre-school children of rural communities of Odogbolu in Ogun State. Corresponding results had also been reported in children in other parts of Nigeria (Abduazeez and Muhibi, 2014; Erhabor *et al.,* 2014; Ifeanyichukwu and Esan, 2014; Nwagu *et al.,* 2014).

Increase in the level of haemoglobin concentration during treatment (48 hours) showed that the use of rectal artesunate reduced parasitaemia which might have destroy Red Blood Cell, thereby increase haemoglobin concentration as was reported by Hendriksen *et al.*(2012).

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(Received 22 April 2017; accepted 10 May 2017)

Table 2. Anaemia (PCV and Hb) among the Studied Group at 0 Hour and during 48 hours across the LGAs

Parameters	Local Government Areas							TOTAL
	EWK	ODD	IMA	ADT	ODG	RMN	IJN	IJE	
	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)	F (%)
PCV (L/L) (Anaemia) 0 Hour									
Normal (>0.33)	23(23.0)	3(2.7)	14(12.7)	5(3.8)	14(12.2)	2(1.8)	4(3.3)	5(4.5)	70(7.8)
Mild (0.31-0.33)	22(22.0)	40(36.3)	47(42.7)	46(35.4)	53(46.1)	52(47.2)	68(56.7)	52(47.2)	380(42.0)
Moderate (0.21-0.30)	44(44.0)	56(50.9)	41(37.3)	72(55.4)	45(39.1)	50(45.5)	45(37.5)	50(45.5)	403(44.5)
Severe (<0.21)	11(11.0)	10(9.1)	8(7.3)	7(5.4)	3(2.6)	6(5.5)	3(2.5)	4(3.6)	52(5.7)
PCV (L/L) (Anaemia) 48 Hours			p = 0.000						
Normal (>0.33)	61(61.0)	37(33.6)	67(60.9)	72(55.4)	73(63.5)	20(18.2)	61(50.8)	53(48.2)	444(49.1)
Mild (0.31-0.33)	20(20.0)	57(51.8)	29(26.4)	38(29.2)	35(30.4)	75(68.2)	47(39.2)	48(43.6)	351(38.8)
Moderate (0.21-0.30)	17(17.0)	16(14.6)	14(12.7)	20(15.4)	7(6.1)	15(13.6)	12(10.0)	9(8.2)	110(12.1)
Severe (<0.21)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Hb (g/L) (Anaemia) 0 Hour									
Normal (>110)	0(0)	0(0)	2(1.8)	0(0)	0(0)	2(1.8)	2(1.7)	1(0.9)	7(0.8)
Mild (100-109)	22(22.0)	15(13.6)	10(9.1)	32(23.8)	18(15.7)	11(10.0)	14(11.6)	55(50.0)	117(12.9)
Moderate (70-99)	43(43.0)	76(69.1)	78(70.9)	67(73.8)	78(67.8)	61(55.5)	89(74.2)	51(46.4)	543(60.0)
Severe (<70)	35(35.0)	19(17.3)	20(18.2)	31(23.8)	19(16.5)	36(32.7)	15(12.5)	3(2.7)	238(26.3)
Hb (g/L) (Anaemia) 48 Hours			p = 0.001						
Normal (>110)	28(28.0)	35(31.8)	21(19.1)	38(29.2)	41(35.7)	35(31.8)	33(27.5)	34(30.9)	265(29.2)
Mild (100-109)	32(32.0)	62(57.4)	49(44.5)	49(37.7)	57(49.6)	38(34.5)	42(35.0)	50(45.5)	379(41.9)
Moderate (70-99)	29(29.0)	9(8.2)	34(30.9)	32(24.6)	11(9.5)	31(28.2)	40(33.3)	22(20.0)	208(23.0)
Severe (<70)	11(11.0)	4(3.6)	6(5.5)	11(8.5)	6(5.2)	6(5.5)	5(4.2)	4(3.6)	53(5.9)

F- Frequency, EWK-Ewekoro, ODD-Odeda, IMA-Imeko-Afon, ADT-Ado-Odo-Ota, ODG-Odogbolu, RMN-Remo-North, IJN-Ijebu-North and IJE-Ijebu-East.

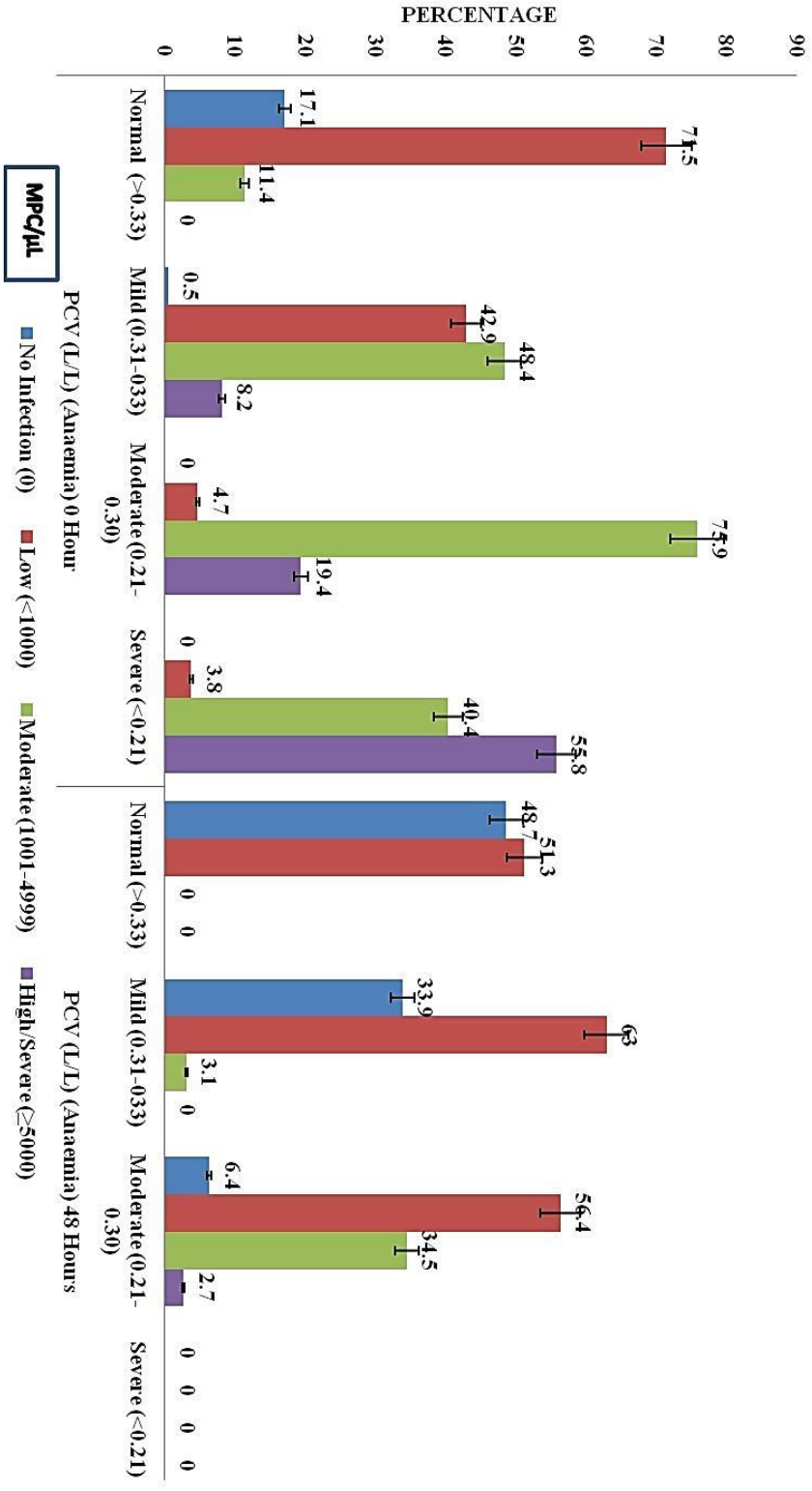

Figure 2. Parasitaemia (MPC/µl) in Relation to Anaemia (PCV L/L)

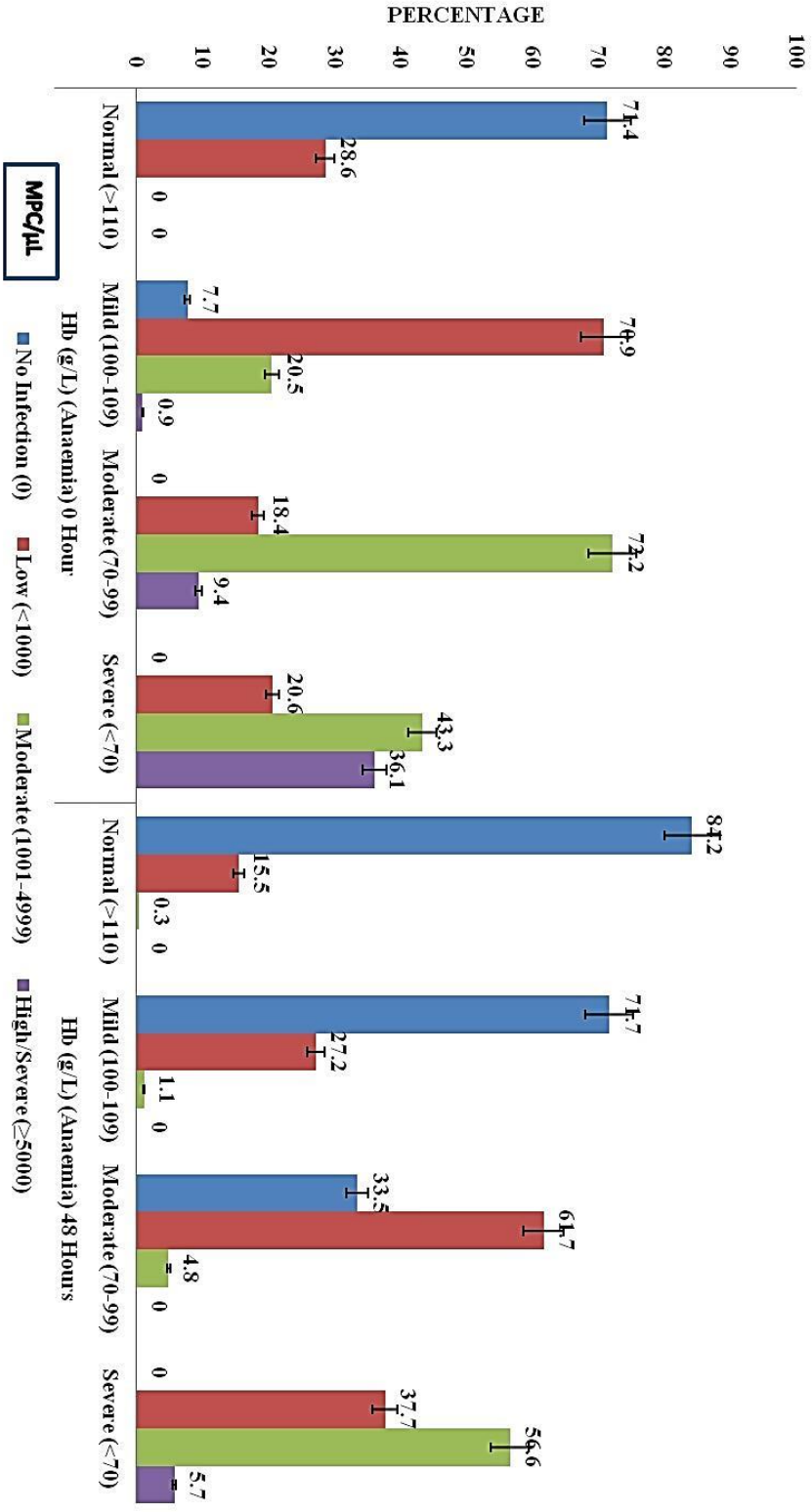

Figure 3. Parasitaemia (MPC/µl) in Relation to Anaemia (Hbg/L)

Table 3. Impact of Rectal Artesunate on Anaemia (PCV, HB, MCV, MCH and MCHC) at 0 hour and 48 Hours of Treatment across the LGAs

Parameters	Local Government Areas							TOTAL 905 (%)
	EWK F (%)	ODD F (%)	IMA F (%)	ADT F (%)	ODG F (%)	RMN F (%)	IJN F (%)	IJE F (%)	
MCV (fL) 0 Hour									
Normocytic (73-96)	46(46.0)	28(25.5)	30(27.3)	26(20.0)	46(40.0)	25(22.7)	42(35.0)	41(37.3)	284(31.4)
Microcytics (<73)	54(54.0)	82(74.5)	79(71.8)	104(80.0)	69(60.0)	85(77.3)	78(65.0)	69(62.7)	621(68.6)
Macrocytics (>96)	0(0)	0(0)	1(0.9)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
MCH (pg) 0 Hour									
Normocytic (27-32)	1(1.0)	1(0.9)	2(1.8)	1(0.8)	1(0.9)	2(1.8)	2(1.7)	10(9.1)	20(2.2)
Microcytics (<27)	99(99.0)	109(99.1)	108(98.2)	129(99.2)	114(99.1)	108(98.2)	118(98.3)	100(90.9)	885(97.2)
Macrocytics (>32)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
MCHC (g/L) 0 Hour									
Normochromic (305-345)	13(3.0)	2(1.8)	8(7.3)	3(2.3)	6(5.2)	2(1.8)	5(4.2)	5(4.5)	44(4.9)
Hypochromasia (<305)	87(87.0)	108(98.2)	102(72.7)	127(97.7)	109(94.8)	108(98.2)	115(95.8)	105(95.5)	861(95.1)
MCV (fL) 48 Hours	***p = 0.0265***								
Normocytic (73-96)	70(70.0)	82(74.5)	88(80.0)	96(73.8)	94(81.7)	68(61.8)	95(79.2)	84(76.4)	677(74.8)
Microcytics (<73)	28(28.0)	28(25.5)	21(19.1)	34(26.2)	21(18.3)	42(38.2)	25(20.8)	26(23.6)	225(24.9)
Macrocytics (>96)	2(2.0)	0(0)	1(0.9)	0(0)	0(0)	0(0)	0(0)	0(0)	3(0.3)
MCH (pg) 48 Hours	***p = 0.0443***								
Normocytic (27-32)	27(27.0)	34(30.9)	19(17.3)	27(20.8)	38(33.0)	25(22.7)	43(35.8)	34(30.9)	247(27.3)
Microcytics (<27)	71(71.0)	76(69.1)	91(82.7)	103(79.2)	77(70.0)	85(77.3)	77(64.2)	76(69.1)	656(72.5)
Macrocytics (>32)	2(2.0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(0.2)
MCHC (g/L) 48 Hours	***p = 0.037***								
Normochromic (305-345)	23(23.0)	40(36.4)	28(25.5)	41(31.5)	41(35.7)	28(25.5)	45(37.5	27(24.5)	273(30.2)
Hypochromasia (<305)	77(77.0)	70(63.6)	82(74.5)	89(68.5)	74(64.3)	82(74.5)	75(62.5)	83(75.5)	632(69.8)