

Influence of Parasitaemia on Anaemia in *Plasmodium Falciparum* Treated Children at Lake-Alau, Borno State

M. M. Kokori,¹ Z. S. G. TurakI², A. M. Garba³

¹Department of Biological Sciences, Faculty of Science, University of Maiduguri, Borno State, Nigeria

²Lake Chad Research Institute, Maiduguri, Borno State, Nigeria

³Department of Paediatrics, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria

Abstract: *The relationship between malaria parasite density and anaemia was investigated on 313 children (6-59 months). Drug resistance in Plasmodium falciparum malaria is a major obstacle to malaria control. Plasmodium falciparum infection is a major contributory factor to the etiology of anaemia in malaria endemic areas of world, and this resulted in parasite resistance to drug therapy. Drug combinations for the treatment of P. falciparum malaria can delay the emergence and spread of resistance. Most of the monotherapies of anti-malarial drugs have gradually become less efficacious over time, while the most efficacious ones gradually lost their efficacies. The present study assessed packed cell volume (PCV level) as indicators for recovery from malaria anaemia (PCV ≤ 33%) in children treated with AT+SP and AQ+SP between days 0,3,7,14 and 28 days follow-up. The standard protocol for therapeutic efficacy studies by the World Health Organization was strictly followed, parasite densities and PCV on days 0, 3, 7, 14 and 28 in children (6-59 months) treated for Plasmodium falciparum malaria with AT+SP and AQ+SP. There was a sharp parasite clearance phase between days 0 to 3 after treatment then a more stable clearance from 7 to 28 days. Similarly, for each µl of blood / parasites cleared there was a PCV recovery of 0.0214% versus 0.018% for AT + SP versus AQ + SP, respectively over 28 days of follow-up.*

Keywords: influence, Malaria, Parasitaemia, PCV, *Plasmodium falciparum*, Efficacy

1. Introduction

Malaria is a systematic protozoan parasitic disease of the genus *Plasmodium* that causes infections of the red blood cells [20]. It is a major cause of morbidity and mortality in children under the age of five years in Sub-Saharan Africa and responsible for the death of over one million children annually [22]. The risk associated with malaria infection constitutes 10% overall disease burden which accounts for 40% public expenditure, 30-50% of inpatient admissions and up to 50% outpatient visits in areas with high malaria transmissions of Africa [18]. Drug combinations for the treatment of *P. falciparum* malaria might delay the emergence and spread of resistance [1]. Anaemia is relative to *Plasmodium falciparum* malaria especially severe in younger (<5 years) children [7] than pregnant women [10] as compared to normal adults [13] and really associated with increased risk of death due to malaria of *Plasmodium falciparum* infections [4]. Anaemia as a result of *Plasmodium falciparum* infection in children could impair cognitive and motor development [21], growth and immune function in children malaria [12]. Changes in haematological profiles such as PCV (PCV<33%) are strongly associated with malaria infections because of red blood cells destruction [30] and a phenomenal process [21] which is linked to the frequency and degree of anaemia in malaria infected children and varies with intensities of parasite densities in malaria infected children during follow-up periods [3].

2. Literature Survey

In a study conducted in Nigeria on the relationship between Packed Cell Volume (PCV) and malaria parasitaemia showed a marginally significantly ($P>0.05$), results further showed that parasitaemic positive (33.8 ± 5.0) children had lower PCV compared to negative parasitaemia (35.7 ± 5.0) [29]. Anaemia is caused by increased hemolysis or a decreased rate of erythrocyte production [25]. Anaemia is associated with changes in hemoglobin among malaria cases and reports from Gambia and Nigeria indicated significant decreases in haemoglobin and packed cell volume [3]. Malaria-related anaemia is more severe in younger children and pregnant women rather than adults [10]. Anaemia may vary with level of malaria endemicity, background hemoglobinopathy, nutritional status, demographic factors, and malaria immunity [7]. Severe anaemia is associated with an increased risk of death [20]. A recent study in two large hospitals in Malawi indicated that cases of malaria associated with severe children anaemia were 5.2 to 8.5% of all paediatric admissions and accounted for 32 to 54% of malaria related deaths and malaria associated severe anaemia peaked at the age group 6 to 11 months [31]. Anaemia and possibly iron deficiency can impair cognitive and motor development [14], growth [21] immune function [17] and physical capacity [12].

3. Materials and Methods

3.1. Study site

This study was carried out at primary Health Center of Kayamla village settlement, Lake-Alau in Konduga Local Government Area of Borno State Nigeria. It is located at Lat: 120N and 130N; Long: 110E and 130E (Fig. 1) Prior to the commencement of the project, ethical clearance was sought from the Borno state ministry of health.

3.2. Recruitment Procedure

A complete physical examination was performed and a full medical history obtained by the clinical personnel. Detailed information's concerning the history of present illness, past and present drug history such as hypersensitivity to anti-malarial drugs were recorded into case record form (CRF) according to [26] as modified [27].

3.3. Inclusion and Exclusion Criteria

The inclusion criteria for the admission into study was strictly based on [27] for evaluating anti-malarial drugs in children (6 – 59 months). Informed consent provided by patient or parent/guardian, mono-infection with a slide confirmed *Plasmodium falciparum* infected children with asexual blood stage parasitaemia, absolute absence of history of hypersensitivity reactions to either of study drugs, enrolment parasite density of > 2,000 and < 200,000 asexual parasites/µl of blood). Measured axillaries temperature ≥ 37.5 °C with the ability to attend the stipulated follow-up visits and a packed cell volume > 15%.

3.4.1. Group one

(AT+SP), each child orally received 4 mg/kg body weight Artesunate daily for three days and a combined 25 mg/kg body weight Sulphadoxine and 1.25 mg/kg body weight Pyrimethamine as single oral do se on the first day of treatment.

3.4.2 Group two

(AQ+SP), each child orally received 10 mg/kg body weights of Amodiaquine daily for three days and also a combined 25 mg/kg body weight Sulphadoxine and 1.25 mg/kg body weight Pyrimethamine as a single oral dose on the first day of treatment.

3.5 Experimental Procedures

3.5.1 Physical parameters

The age of each child was determined by interviews with the parents or birth certificates while the body weight (kg) was expressed in (kg) using weighing balance (Model: Hansen H60 5500/11494798, UK) [26].

3.5.2 Blood sampling

Blood was sampled by pricking the third phalanx with a sharp sterile needle after cleaning with spirit-moistened cotton, smear slides on days 0, 1, 2, 3, 4, 7, 14 and 28 for the assessment of parasite densities [11].

3.5.3 Determination of Parasite density (per µl)

The thick film slide was stained for 30 to 45 minutes with 3% Giemsa for the assessment of parasite density. The samples were examined using objectives of a research microscope (x100) asexual parasites were counted alongside with 200 leukocytes. In an even that parasite count was < 10 parasites/200 leukocytes; count was continued per 500 leucocytes. The parasite density was expressed as the number of asexual parasites per ml of blood by assuming a mean normal leukocyte count of 8000/µl of blood [11and modified by [28]. Parasitaemia (per µl) = number of parasites x 8000 / number of leucocytes (200/500)

3.5.4 Determination of packed cell volume (PCV %)

The EDTA anti coagulated blood sample in a glass capillary was centrifuged in a microhaematocrit centrifuge at 12000 xg (rpm) for 5 minutes to obtain constant packing of the red cells. The PCV value was then read-off using a hand held microhaematocrit reader and the values were expressed as percentages of the PCV (%) on days 0, 3,7,14 and 28 [23].

3.5.5 Data management and analysis: Data management and analysis

Data collected were subjected to descriptive statistics using the analytical software Staistix Version 8.0 (Microsoft, 2003). Measures of central tendencies (standard deviations and percentages) were determined. Charts were drawn using Microsoft Excel (2007) and the regression equations on the relationships between *P. falciparum* parasitaemia (per µl) and PCV (%) over 28 days of follow-up.

4. Results

4.1. Baseline Parameters Before the admonstraion of drugs

Table 1: Baseline characteristics of patients at enrolment

S. No	Parameter	Baseline data
1	No. enrolled (N)	313
2	Gender (No. /%)	
	Male	149 (47.6)
	Female	164 (52.4)
3	Age (months)	
	Mean ± SD	43.3 ± 14.4
	Range	Aug-59
4	Body weight (kg)	
	Mean ± SD	18.4 ± 8.5
	Range	3.0 - 50.0
5	Temperature (°C)	
	Mean ± SD	38.15 ± 0.47
	Range	37.0 - 39.6
6	Haematological	
	a. Parasite count (µl)	
	Mean ± SD	20820 ± 5277.7
	Range	2304 - 36800
	b. Haematocrit (PCV %)	
	Mean ± SD	27.0 ± 0.5
Range	14.0 - 48.0	

Table - 1 shows the baseline characteristics of the patients at enrolment. A total of 313 children were enrolled for the study, 149 (47.6%) were males and 164 (52.4%) were females. Mean of standard deviation (SD) and measures of dispersion to estimate variability in the data set to observed ranges. Consequently, the age of the children was highly dispersed between 6 - 59 months from the mean of 43.3 + 14.4 months. Results showed a total mean parasite count of 2304 - 36800/μl on admission with a mean range of 20820 ± 5277.7/μl. The mean PCV of the children on admission was 27.0 ± 5.0%, with a range of 14.0 - 48.0%.

4.1. Influence of Parasitaemia on Anaemia:

Results of figures 1 and 2 shows a relationship between the PCV build - up and parasite depletion over 28 days of follow-up, The initial (day 0) with mean PCV levels for AT+SP as (25.69%) while slightly higher for AQ+SP (25.87%) patients, both treated groups were almost at par at enrollment. Influence of parasitaemia on PCV was higher (98.41%) for AT+SP (fig-1) compared to AQ+SP (93.51%) (Figure 2). The results further draws a respective daily build-up in PCV by 0.0214% and 0.018% per microliter (μl) of blood, which depicts almost twice as fast rate of recovery from anemia in AT+SP than AQ+SP patients.

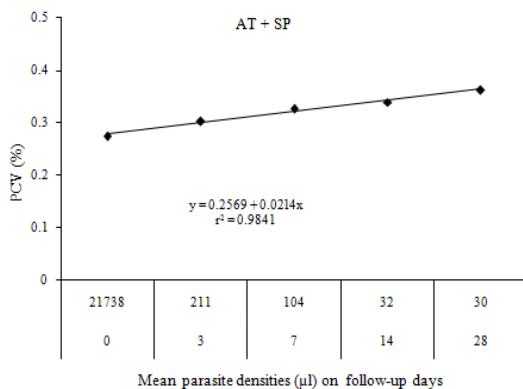


Figure 1: The influence of mean parasite densities (P. falcifarum) on treated (AT+SP) children during follow-up period (0-28).

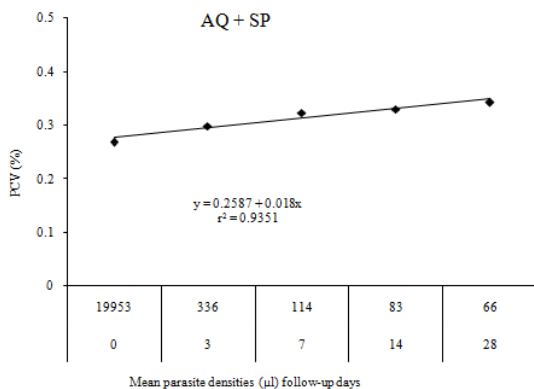


Figure 2: The influence of mean parasite densities (P. falcifarum) on treated (AQ+SP) children during follow-up days (0-28)

5. Discussion

The results of this study have clearly showed that anaemia has correlated directly with *Plasmodium falciparum* parasitaemia. The global malaria control strategy advocates a prompt and adequate treatment as essential measure to reduce morbidity and mortality arising from anaemia related complications [2]. *Plasmodium falciparum* infection is a major contributory factor to the etiology of anaemia in malaria endemic areas of the world (Kwadwo *et al.*, 2000). The result trend shows that despite high mean parasite density at inception (day-0) in the two treated groups (fig. 1 and 2) and characterized by low PCV (<33%) there was a rapid parasite clearance in less than 72 hours in both treated groups, this coincides with observations by [9] in Rwanda, as further confirmed by the potentials of combination therapies in the clearance of parasites within the shortest possible time as reported by [5] in Uganda and [15] in Nigeria. This remarks on artemisinin derivatives on its efficacy and the rapid and substantial decrease in the parasite load when in used for treating malaria patients is in agreement with Rwagacondo *et al.* (2003).

5.1. Pattern of recoveries from anaemia

The mean recoveries from anaemia in the two treated groups showed a reciprocal recovery from anaemia between the two drug groups as evident from regression coefficient (r²) values (fig 1 and 2), this is in congruent with [9] which lends credence to the fact that with ascending PCV level there will be a descending parasite density during the 28 days of follow-up period. Malarial infections normally relates with a decline in haemoglobin concentration of less than 8g/dl/PCV (<33%) [20]. This further concurs with reported studies by [16] and using AQ+SP treatment of children *Plasmodium falciparum* malaria in relation to anaemia. The cumulative mean influence of parasite densities on PCV during the follow-up days was higher (98.41%) for AT+SP (fig- 1) compared to AQ+SP (93.51% (fig- 2). In a similar trend, the speed of parasite clearance was faster for AT+SP than in AQ+SP, as revealed by the coefficient of regression (r²) with faster recovery from anemia in the older drug combination than in the later. The results further shows that parasitaemia had higher effects on the occurrence of higher frequency of anaemia in AQ+SP than AT+SP which concurs with the findings by [20] and [3] on the relationship of parasitaemia to anemia frequencies in *Plasmodium falciparum* infected treated children.

5. 2. The speed of recoveries from anaemia

The results on the speed of recovery from anaemia (fig-1 and 2) showed a respective build-up in PCV by 0.0214% and 0.018% in the two respective drug combinations which depicts almost twice as faster rate of PCV recovery in AT+SP compared to AQ+SP patients (Dorsey *et al.*(2007). In a similar trend, the relative parasite clearance rate was generally faster within the early phase of days 0 – 3 compared to the later phase (days 7- 28) in AT+SP as compared to AQ+SP treated groups and equally corresponding PCV levels for the respective days(fig 1 and 2). The explanations for this strong reciprocal relationship

between and malaria parasite densities and the anemia (PCV < 33%) trends on follow-up days was malaria-induced [6], [9] and parasitaemia dependent.

6. Acknowledgment

The authors acknowledged the tremendous contributions of the children who donated blood samples for the study and equally, their parents whose consent allowed their children to participate in the trial study from enrolment to the completion stage. We also wish to acknowledge the immense contributions of the staff and management of Kayamla Health Care Center and Konduga Local Government. We are grateful to laboratory technologists particularly, A. M. Garba, J. Sani, Y. Duniya and Bunu Karumi Kayamla all of the paediatrics unit, University of Maiduguri Teaching Hospital, for the strong commitment to the research work.

References

- [1] P. U., Agomo,.; Meremekwu, M.; Watila, I. M.; Omeh, I. J.; Odey, F. A.; Oguche, S.; Ezeiru, V. J. and Aina, O. "Efficacy safety and tolerability of Artesunate and Mefloquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in four geographical zones of Nigeria". *Malaria Journal*. **7**: 172-177, 2008.
- [2] J.A., Carter, A. J. Ross, Neville, B. G., Obiero, E., Katana, K., Mung'ala-Odera, V., Lees, J. A. and Newton, C. R., "Developmental impairments following severe falciparum malaria in children". *Tropical Medicine and International Health*. **10**: 3-10, 2005.
- [3] I., A., Chiaka, I. A.; Christian, N. Victor, I. N., Roseangela, and N. Mark, "Epidemiological factors that promote the development of malaria anaemia in children in Ibadan". *African Health Science*. **7(2)**: 80-85, 2007.
- [4] E. De-Maeyer, and M. Adiels-Tegman, "The prevalence of anaemia in the world". *World Health Statistics*. **38**: 302-316, 1985.
- [5] G. Dorsey, S. Staedke, T.D. Clark, D. Njama-Meya, B. Nzarubara, C. Maiteki-Sebuguzi, C. Dokomajilar, M. R. Kanya, and P. J. Rosenthal, "Combination therapy for uncomplicated *Plasmodium falciparum* malaria in Ugandan children: A randomized trial". *Journal of American Medical Association*. **297**: 2210-2219, 2007.
- [6] L. M. Erhart, K. Yingyuen, N. Chuanak, N. Buathong, A. Laoboonchai, R.S. Miller, S. R. Meshwick, R.A. Gasser, and C. Wongsrichanalai, "Hematologic and clinical indices of malaria in a semi-immune population of Western Thailand". *American Journal of Tropical Medicine and Hygiene* **70**: 8-14, 2004.
- [7] K. J. Evans, D.S. Hansen, N. van Rooijen, L. A. Buckingham, and L. Schofield, "Severe malarial anaemia of low parasite burden in rodent models results from accelerated clearance of uninfected erythrocytes". *Blood*. **107**: 1192-1199, 2006.
- [8] C. I. Fanello, C. Karema, W. van Doren, C. E. Rwagacondo, and U. D'Alessandro, "Tolerability of Amodiaquine and Sulphadoxine-Pyrimethamine, alone or in combination for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwandan adults". *Tropical and Medicine and International Health*. **11**:589-596, 2006.
- [9] C.I. Fanello, C. Karema, W. Van Doren, C. Van Overmeir, D. Ngamije, and U. D'Alessandro, "A randomised trial to assess the safety and efficacy of Artemether-Lumefantrine (Coartem) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwanda". *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **101**: 344-350, 2007.
- [10] J. P. Forney, C. Wongsrichanalai, A.J. Magill, L. G. Craig, J. Sirichaisinthop, C. T. Bautista, R.S. Miller, C.F. Ockenhouse, K.E. Kester, N.E. Aronson, E. M. Andersen, H. A. Quino-Ascurra, C. Vidal, K.A. Moran, C.K. Murray, C.C. DeWitt, D. G. Heppner, K.C. Kain, W.R. Ballou, and R.A, jr Gasser, "Devices for rapid diagnosis of malaria: evaluation of prototype assays that detect *Plasmodium falciparum* histidine-rich protein 2 and a *Plasmodium vivax*-specific antigen". *Journal of Clinical Microbiology*. **41**: 2358-2366, 2003.
- [11] H. Gilles, *Diagnostic methods in malaria*. In: H. M Gilles and D. A. Warrell (Eds) Essential malariology, 3rd ed. P. Edwards Arnold London, United Kingdom. pp342, 1993
- [12] J.D. Haas, and T. T. Brownlie, "Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship". *Journal of Nutrition*. **131**: 676-688, 2001.
- [13] J.A.V Kurtzhals, B.Q. Adabayeri, B. D. Goka, J.O. Akanmori, F.K. Oliver-Commey, C. Nkrumah, and L. H. Behr, Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria". *Lancet* **351**: 1768-1772, 1998.
- [14] J.W. Lawless, M. C. Latham, L.S. Stephenson, S. N. Kinoti, and A. M. Pertet, Iron supplementation improves appetite and growth in anemic Kenyan primary school children". *Journal of Nutrition*. **124**: 645-654, 1994.
- [15] M. Meremekwu, A. Alaribe, R. Ejemot, A. Oyo-Ita, E. Ekenjoku, N. Chukwuemeka, O. Donald, and E. Ezedinachi, "Artemether-Lumefantrine versus Artesunate plus Amodiaquine for treating uncomplicated childhood malaria in Nigeria: Randomized control trial". *Malaria Journal*. **5**: 43, 2005.
- [16] T.K. Mutabingwa, D. Anthony, A. Heller, R. Hallett, J. Ahmed, C. Drakeley, B.M. Greenwood, and C.J. Whitty, "Amodiaquine alone, Amodiaquine + Sulfadoxine-Pyrimethamine, Amodiaquine + Artesunate, and Artemether-Lumefantrine for outpatient treatment of malaria in Tanzanian children: A four-arm randomised effectiveness trial". *Lancet*. **365(3)**: 1474-1480, 2005.
- [17] S.J. Oppenheimer, "Iron and its relationship to immunity and infectious disease". *Journal of Nutrition*. **131**: 616S-633S, 2001.
- [18] Roll Back Malaria (RBM), "Scaling-up Insecticide-Treated Netting Programmes in Africa: Strategic Frame work for Coordinated National Action. Geneva": WHO/CDS/RBM/2002, 2002.
- [19] C.E. Rwagacondo, F. Niyitegeka, J. Sarushi, C. Karema, V. Mugisha, J.C. Dujardin, C. Van Overmeir, J. van den Ende, and U. D'Alessandro, "Efficacy of

Amodiaquine alone and combined with Sulfadoxine-Pyrimethamine and of Sulphadoxine Pyrimethamine combined with Artesunate". *American Journal of Tropical Medicine and Hygiene*. **68**: 743-747, 2003.

- [20] D. Schellenberg, J.R. Schellenberg, A. Mushi, D. Savigny, L. Mgalula, and C. Mbuya, C.G. Victora, "The silent burden of anaemia in Tanzanian children: a community-based study". *Bull World Health Organ*. **81**: 581-590, 2001..
- [21] Sheriff, A. Emond, J.C. Bell, and J. Golding, "Should infants be screened for anaemia? A prospective study investigating the relation between haemoglobin at 8, 12, and 18 months and development at 18 months". *Arch Dis Child*. **84**: 480-485, 2001.
- [22] R.W. Snow, E. Eckert, and A. Teklehaimanot, "Estimating the needs for artesunate-based combination therapy for malaria case-management in Africa". *Trends Parasitol*. **19**: 363-369, 2003.
- [23] M. M. Strumia, Sample, A.B., and Hart, eds (1954). An improved microhaematocrit method. *Am. J. Clin. Path*, **24**, 1016.
- [24] K. J., Evans, D.S., Hansen, N. van Rooijen, L.A. Buckingham, and L. Schofield, " Severe malarial anaemia of low parasite burden in rodent models results from accelerated clearance of uninfected erythrocytes". *Blood*. **107**: 1192-1199, 2005.
- [25] R. N. Price, J. A. Simpson, and F. Nosten, F "Factors contributing to anaemia after uncomplicated falciparum malaria". *American Journal of Tropical Medicine and Hygiene*. **65**: 614-622, 2001.
- [26] World Health Organization. "Assessment of therapeutic efficacy for uncomplicated falciparum malaria in areas with intense transmission. Geneva: World Health Organization. Unpublished document", *WHO/MAL/96.1077.PP-32*, 1996
- [27] World Health Organization, "Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated Falciparum Malaria". Geneva, Switzerland: WHO; 2003. *Technical document*, *WHO/RBM/HTM/2003.50*, 2003
- [28] World Health Organization, "WHO Guidelines for the Treatment of Malaria. Geneva, Switzerland": *Technical document*, *WHO/HTM/MAL/2006.1108*, 2008.
- [29] B.S.A. Oseni, V.A. Togun, A.O. Olowu and D.I. Okoli "Impact of Severe *Plasmodium falciparum* Malaria on Red Cell Indices in Children below 10 Years of Age in Lagos, Nigeria" *International Digital Organization for Scientific Information*, Volume 1 Number (1) : 04-07, Jan-Jun, 2006, ISSN: 1818-4952
- [30] J. W. Lawless, M. C. Latham, L. S. Stephenson, S.N. Kinoti, N. and A. M. Pertet, "Iron supplementation improves appetite and growth in anaemic Kenyan primary school children," *Journal of Nutrition*. **124**: 645-654, 1994.
- [31] L. Slutsker, T. E. Taylor, J.J Wirima, R. W. Steketee, "In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection" *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 548-51, 1994.

Author Profile

Dr. Mustapha Kokori, received B. Sc. degree. Zoology and General Biology, University of Maiduguri in 1990, M.Sc. in Applied Medical Parasitology of university of Ibadan, Nigeria in 1994. He did Ph.D in Applied Medical Parasitology - with reference to "Efficacies of Combination Therapies of Antimalarial Drug Safety in Children of Tropical Africa in 2004: A case study on Rural settlements of Lake alau, Borno State, Nigeria. Currently he is Senior lecturer in the department of Biological Sciences, faculty of science, University of Maiduguri, Nigeria.

Dr. Zakari Silas Goji Turaki did Higher National Diploma in Medical Science Laboratory Technology (HND). He did B.Sc. Agriculture in 1987, M.Sc. Agriculture in 1992 and Ph. D Crop protection Technology, Faculty of Agriculture, University of Maiduguri, Borno state, Nigeria. Presently he is director of research at the Lake Tchad Research Institute, Maiduguri, Borno State

Abdulmuminu Garba did Higher National Diploma in Medical Science Laboratory Technology (HND). He received Bachelor Degree in Medical Laboratory science (BMLS), Medical Science Laboratory Department, College of Medical Sciences, University of Maiduguri Teaching Hospital, Borno State, Nigeria. Currently he is a senior laboratory personal at the pediatrics 'department, on children malaria and control programme, University of Maiduguri Teaching Hospital, Borno State, Nigeria.