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Table of Contents

Editorial Board **2**
General Information on Author Guidelines **3**
Editorial **6**

Original Articles
Oyibo WA, Samuel TA, Onwuachusi LG Oladosu OO, Iriemenam NC, Salisu M
C-Reactive Protein as a Likely Biomarker for Uncomplicated Malaria **7**

Iliyasu U, Sani S, Umar HD, Umar AK and Salisu S
Pharmacognostic Standardization of the Leaves of *Ficus Kamerunensis* Warb (Moraceae) **14**

Ibrahim SI, Fakhraddeen YM, Hauwa UA
Mosquito Repellent Activity of Leaf and Seed Extract of *Azadirachta Indica* (Neem) **19**

Harcourt AM, Nwauche CA, Okpani AOU, Okerengwo AA, Gbenga O, Harcourt SL
Assay of *Plasmodium falciparum* Specific Antibodies in Maternal and Cord Blood at the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria **24**

Opinion Article
Obunge O.K, Oboro I.L, Chijioke-Nwauche I.N
Improving Laboratory Capacity; A necessity towards elimination of malaria in Nigeria **34**

Profile of the Centre for Malaria Research and Phytomedicine **37**

Centre Leadership **38**

JOURNAL OF MALARIA AND PHYTOMEDICINE
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Disclosure of conflict of interest
Authors should disclose all financial/relevant interest that may have influenced the study.

Acknowledgement: Acknowledgement section may be included if the author wants to acknowledge the funding agency or any other person (s) as deemed necessary.
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EDITORIAL

Herbal medicine in the 21st century.

As the fight against malaria from all fronts continues in Nigeria, many areas of research are coming up in the bid to get the key to combating the disease and its many manifold effects. The importance of drug use in the treatment of malaria cannot be overemphasised. In many countries where there is stable transmission of malaria as obtained in many sub-Saharan African countries, the main tool for the war against malaria is the use of drugs for prophylaxis and treatment. As the threat of resistance to the artemisinins increases and its continued advancement towards Africa, there is an urgent need to begin to look at possible alternatives to the present drug combination therapies in use.

The current trend of the increased use of herbal and alternative medicine globally has re-ignited the need to revisit the local herbs indigenous to the country as possible sources of cure for malaria and indeed other diseases that are local to the environment. Herbal medicine is the oldest form of medicine in the world and has been of use in Nigeria just as in other countries. Many drugs that are currently used in medicine originated from herbs: Drugs like Digoxin was extracted from Digitalis foxglove, artemisinin from *artemisinin annua*, quinine from cinchona bark, etc. Most of these medicines have been handed down either through oral or written traditions in the environment where they were used before it gained global interest.

The richness of our forest with vast number of plants with promising medicinal effects to deal with the diseases around us calls for more attention and research for possible new discoveries and of course revisiting of the older known ones. Our duty as publishers is to bring to light that which is new or rediscovered akin to the oral tradition of old.

This third edition brings to the fore the mosquito repellent property of the old known local medicine *Azadiracthaindica* and highlights another plant of potential medicinal use *FicusKamrunensis.*

IfeyinwaChijioke-Nwauche. Ph.D
The Editor.

C-REACTIVE PROTEIN AS A LIKELY BIOMARKER FOR UNCOMPLICATED MALARIA.

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ABSTRACT

Introduction

Malaria is a public health disease that presents with symptoms that are similar to bacterial and viral infections that stimulates inflammation. C-reactive protein (CRP) is one of the markers of inflammation, which is identified to increase with high fever. The connection between malaria and C-reactive protein is still emerging. Therefore understanding the relationship between CRP and malaria parasitaemia is likely to guide its development as a possible marker for malaria. The objective of the study was to assess CRP and malaria parasitaemia in patients presenting with uncomplicated *Plasmodium falciparum* malaria in Lagos, Nigeria

Methodology: This was a cross-sectional study conducted in health facilities in Lagos State, Nigeria. The subsets of 100 patients enrolled in the study were randomly selected from participants who presented with fever or with history of fever in the last 48 hours. Patients presenting symptoms were captured in a designed case report form (CRF), blood smears done for malaria microscopy and plasma obtained from the 4mls of venous blood that was collected from each patient in Ethylenediaminetetraacetic acid (EDTA) bottles. Thick and thin blood smears were prepared for malaria microscopy on the same slide. Plasma separated from the blood in EDTA was used to run the CRP latex agglutination qualitative and semi quantification assay.

Result: This exploratory study recorded a positive correlation between CRP and parasitaemia which was statistically significant (Spearman's rho correlation coefficient= 0.845, P<0.001 and Pearson Correlation= 0.795, P<0.001), though with overall weak correlation (R^2=0.4492). Also, the highest level of CRP range (49->96mg/l) was seen in individuals with higher parasitaemia, which ranges from 244.4-21615.5mg/l.

Conclusion: High parasitaemia correlated positively with high CRP level though the overall correlation of all parasitaemia was weak. This is suggestive that CRP could be assessed for its potential as a biomarker for malaria using a larger sample

Key Words: C-Reactive Protein, Biomarker, Malaria

INTRODUCTION

Inflammation is a part of a complex biological response of the immune system to disease causing organisms, damaged cells or irritants which is grouped by five signs namely redness (rubor), swelling (tumor), heat (calor), pain (dolor) and loss of function (functiolaesa)[1]. Inflammation is a mechanism that helps the body get rid of foreign matter also known as non-self and dispose cells that are not useful, which could result to healing.

The immune system is a system that helps to protect the body from possible infectious agents as well as causing occasional harmful effects[2]. The immune system is composed of the innate or non-specific immune system and the adaptive or specific immune system[3,4.] During malaria infection, the immune system triggers an inflammatory response. As a result of these activities, certain proteins are formed to help fight off the infection, one of such protein is the C-reactive protein (CRP)[5-7].

CRP has been identified as a biomarker of inflammation[8], and a prognostic marker in malaria[7] that is known to induce adhesion molecule expression in human endothelial cells[9], ligand binding, activation of complement[6,8,10], opsonization and antigen presenting[11-12], protection against pre-erythrocytic stages of malaria[13], and could increase tolerance to malaria[14]. It has also been identified that CRP and the classical component acts together to promote non-inflammatory clearance of apoptotic cells[15]. These functionalities help in the prompt identification and clearance of the parasite by the immune system.

C reactive protein (CRP) level has been said to increase with the severity of malaria[16-19] and could be used as a diagnostic and management tool in malaria holoendermic areas like Nigeria to reduce disease burden[20-21]. CRP has also been identified to have adverse pathological effects like the clearance of RBC which results in severe anaemia[22-23], increasing susceptibility to *Plasmodium falciparum* malaria among Sudanese donors[24] and, playing a part in the expression of experimental cerebral malaria[25]. Malaria is a parasitic disease of importance in Nigeria and the better understanding of the "malaria-human immune" relationship will help the better management of the disease. In this study, we assessed the relationship between CRP and malaria parasitaemia in patients that presented with uncomplicated malaria with the aim of underscoring the utility of CRP as a potential biomarker for malaria diagnosis.

MATERIALS AND METHODS

Study Area and Participant Recruitment:

This study was carried out in Lagos state situated in the south-west zone of Nigeria where perennial malaria transmission occurs. Patients suspected to have uncomplicated malaria, including those with fever (>37.5^{0}C) or those with history of fever (<37.5^{0}C) in the last 48 hours were enrolled from four health facilities, namely: Regina Mundi Catholic Hospital, Mushin; Randle General Hospital, Surulere; Igando General Hospital, Igando; and Badagry General Hospital, Badagry, Lagos, Nigeria.

The symptoms of patients that consented to participate in the study were recorded in a case record form (CRF) after which venous blood was collected. Patients with signs of severe malaria and other severe diseases were excluded from the study.

Sample Collection:

Four millilitres of blood (4mls) was collected from each patient in EDTA containers. A total of 1,867 patients were enrolled from the four health facilities. The samples collected were transported in a cool sample carrier bag to the ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine University of Lagos, Idiaraba, Lagos where they were processed for malaria microscopy and assay for CRP determination.

Malaria Microscopy

Thick and thin malaria blood films (MBFs) were made from newly-collected blood in EDTA bottle of each patient on the same slide and stained following standard procedures. The patient's absolute white blood cell count was used to determine the patients' parasite density per micro-litre of blood (parasite/µL of blood). Essentially, two independent certified Malaria Microscopist read each slide with a third certified Microscopist that served as an arbiter where there was

discrepancy in detection, stage and species of the parasite, and parasite enumerated.

Separation of Plasma and Serological Assay for C-reactive protein

The blood samples collected in the EDTA bottles were spurned at 4000 revolutions per minute (RPM) for ten minutes to separate the plasma. Separated plasma was stored in cryovials at -20°C from which aliquots were taken for the CRP serological assay. A sub-set of 100 malaria parasite positive and negative samples from the suspected malaria patients from the four health facilities was randomly selected for the assay.

The Biotec Cambridge CRP latex test kit for 100 tests was used to determine the presence of CRP in the separated sera following the manufacturer's instructions. A serial dilution of the plasma samples were done and calculated for. Using normal saline,100µL of saline was placed in about 5 tubes each and another 100µLof sample was placed in the first tube, which is mixed properly. Then, 100µL of mixed sample with saline is taken using a new pipette tip and mixed in the next tube containing normal saline; thus a serial dilution was done on each positive sample (Table 1). A drop (40µL) of reagent is placed in the circle of the slide and the 50µL of sample prepared via serial dilution is added, the reagent and the serum is spread round the circle and tilted backwards and forward approximately once every 2 seconds for 2 minutes. This procedure was to determine the highest dilution that will show reaction. Once this is determined, the estimated level of CRP in the sample is calculated and recorded (Table 1).

Table 1: Serial dilution procedure and calculation

Dilutions	*½*	*¼*	*1/8*	*1/16*	*1/32*	*1/64*	*1/128*
Sample Serum	100µL	-	-	-	-	-	-
Saline	100µL	100µL	100µL	100µL	100µL	100µL	100µL
Volume of Sample used on test slide	50µL	50µL	50µL	50µL	50µL	50µL	50µL
6 x Titer	6 x 2	6 x 4	6 x 8	6 x 16	6 x 32	6 x 64	6x128
Mg/ml	12	24	48	96	192	384	768

Ethical Considerations

All patients enrolled in this study gave written informed consent after the purpose of the study was explained. Patients who refused to participate also received standard care in the presenting facilities. The study protocol was reviewed and approved by the Research Ethics Committee of the College of Medicine, University of Lagos, Lagos.

Data Analyses

The data obtained were entered in a computer and validated to ensure quality. The Spearman's rho and Person's correlation was used in the determination of association as appropriate. P-values was set at <0.001 for significance.

RESULTS

The subset of 100 participants made up of 61 females and 39 males was assayed from the 1,867 patients that were enrolled for significant level of C-reactive protein (CRP >6). Of the 100 participants, 75 (75%) had documented fever (temperature >37.5°C) while 25 (25%) had normal temperature but with history of fever. A total of 13 participants (13%) were microscopy positive from both groups. The 13 microscopy positive samples that was tested for CRP, 8 (61.5%) had CRP level of 0-95mg/L while 5 (38.5%) had CRP level of 96-384mg/L (Table 2).

Table 2. Baseline characteristics of the sub-set of the study population assayed for CRP.

Variables	CRP (0-95 mg/L) (n=73)	CRP (96-384 mg/L) (n=27)	P value
Age (Years)			
Mean ± SD	31.01 ± 16.01	22.30 ± 13.4	0.013
Sex			
Male	24 (32.9%)	15 (55.6%)	0.039
Female	49 (67.1)	12 (44.4%)	
Malaria			
Negative	65 (89%)	22 (81.5%)	0.318
Positive	8 (11%)	5 (18.5%)	
Febrile (Fever)			
No	22 (30.6)	3 (11.1)	0.047
Yes	50 (69.4)	24 (88.9)	
CRP			
Geometric mean	0	137.52	<0.001
Median (Range)	0 (0-48)	96 (96-384)	<0.001

Note: N = number of samples. SD = standard deviation. CRP = C-reactive protein (mg/L). P values were based on Pearson Chi-Squared test or Exact Chi-Square for categorical variables and ANOVA for the comparison of the mean of the continuous variables.

CRP was associated with an increase in temperature in individuals with fever (88.9%) when compared to those without fever (11.1%). The relationship between CRP level and parasite density using the Spearman's rho correlation (coefficient t= 0.845, P<0.001) and Pearson Correlation (0.795, P<0.001) was significant (Figure 1). Higher level of CRP (96-384mg/l) was seen in individuals with high parasitaemia, which ranged from 244.4 to 21615.5 P/µL of blood, while those with low parasite density had lower concentration of CRP (Table 3).

Table 3. CRP concentration compared with malaria parasitaemia

CRP Concentration (g/dl)	Malaria Parasitaemia (P/ml)
6-12	28
13-24	33
25-48	Nil
49-96	244.4-3017.2
>96	1560-21615.5

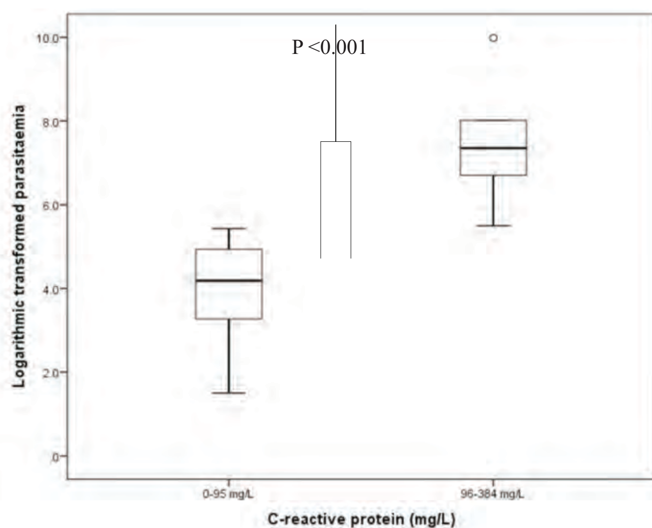

Figure 1. Logarithmic transformed parasitaemia level with CRP (mg/L). The boxes illustrate the total observations equivalent to the first quartile and the third quartile. The median is represented by the horizontal line. The outlier is shown as a circle point.

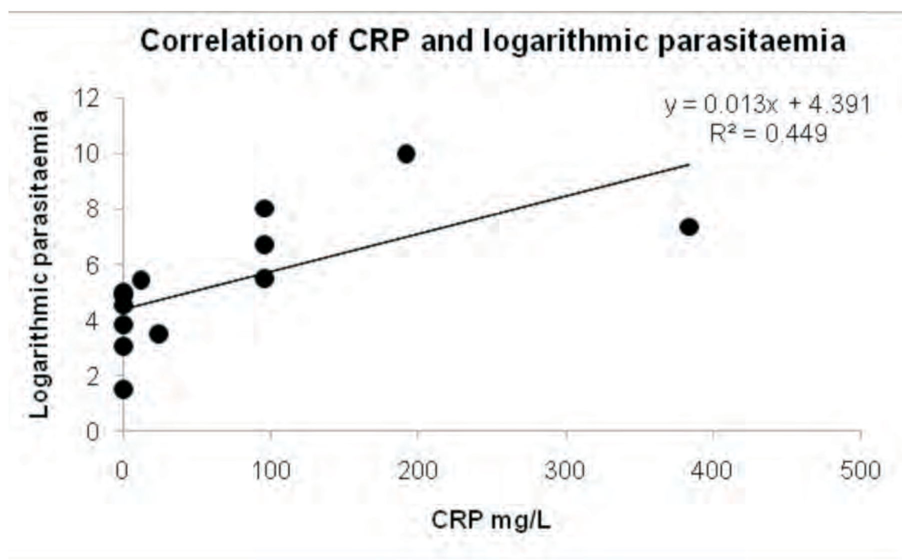

Figure 2: Overall correlation of CRP (mg/L) and Logarithmic parasitaemia

DISCUSSIONS AND CONCLUSIONS

CRP can be used as a prognostic marker since its concentration in human sera reduces with reduction in parasitaemia and increases with increased parasitaemia; this is supported by other studies[5,7] which affirmed that CRP can be used as an effective biomarker in assessing malaria severity and could also be used as a follow-up test for malaria patients to track recovery progress. Our study showed that CRP was associated with malaria since there was a significant increase in the level of CRP in participants with malaria as identified in other studies[5,14,23,26-27.]

This study further highlighted that CRP was associated with increased temperature as seen in the number of individuals without fever (<37.5^{0}C) at the point of sample collection that had high level of CRP when compared to those who presented with fever (>37.5^{0}C) at the point of sample collection (88.9%), thus identifying CRP as a marker of inflammation associated with fever since fever is a sign of systemic inflammation[8].

We noted that the highest level of CRP was seen in individuals with higher parasitaemia while those with low parasite density had lower concentration of CRP. This is consistent with some studies that reported increased C-reactive protein concentration with severity of malaria infection[16-19]. Consequently increased level of CRP and increased parasitaemia, which could be used for

diagnostic basis to differentiate malaria fever from non-malaria fever like Dengue fever in areas where other tests are not available[27].

CRP may also have an important role to play in the immune response to malaria since there is a possibility the marker is involved in the clearance of the parasite or the pathogenesis of the disease due to its high concentration during high parasitaemia and lower concentration in cases of low parasitaemia. CRP may play an important role in immune response to malaria through the process of inhibiting dendritic cells, neutrophils or complement regulatory proteins resulting in the clearance of red blood cells which results in severe anaemia[6,22,23]. Also C-reactive protein could bring about systemic autoimmunity through binding to apoptotic cells and protecting the cells from terminal complement Components, thereby sustaining an anti-inflammatory innate immune response[15].

Some other studies recorded a strong association between increase malaria susceptibility and presence of CRP-286 A-allele[24] and, the expression of experimental cerebral malaria (a sign of severe malaria) as being promoted by CRP[25]. Another study in Ghana suggested that CRP levels are positively related to immune responsiveness and malaria parasitaemia[28]. Furthermore, CRP can be used to track the acquisition of tolerance to malaria that is suggestive of a protective function in malaria patients[14]. In addition, CRP could be useful for malaria immunoepidemiology; however, it was not clear if CRP is beneficial or detrimental[18].

In this assessment study, we showed that the concentration of C-reactive protein in sera increased with increase in malaria parasite density in patients presenting with uncomplicated *Plasmodium falciparum* malaria in Lagos, Nigeria. This therefore, could indicate that CRP is associated with the functionality of parasite clearance in malaria patients since the protein level is seen to increase with an increase in parasite density or could also be involved in the disease pathogenesis of which further studies on the immunological role of CRP will help to clarify its precise immunological function. These observations suggest that CRP could be explored for its potential as a biomarker for malaria using a larger sample.

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PHARMACOGNOSTIC STANDARDIZATION OF THE LEAVES OF *FICUS KAMERUNENSIS* Warb (MORACEAE)

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ABSTRACT

Background: Standardisation of medicinal plants and herbal drugs by pharmacognostic procedures is still relevant despite the current modern techniques. The morphological and anatomical description of a medicinal plant is the first step towards standardization of the plant materials and should be carried out before the commencement of any experimental procedure for the detection of adulterations and impurities.

Methodology:Macroscopical, microscopical, physicochemical studies and preliminary phytochemical screening have been carried out on the leaves of *Ficus kamerunensis*. Qualitative leaf microscopy and physicochemical parameters of the leaves were evaluated. The leaf was screened for the presence of different classes of secondary metabolites.

Results: The leaf was found to be simple petiolate with entire margin, pinnate venation and acute apex. Stomata of anomocytic type were observed in both upper and lower epidermis of the leaf of the plant. Unicellular covering trichomes were present at the upper epidermis surrounded by polygonal epidermal cells with wavy anticlinal walls.Some phytochemicals such as flavonoids, saponins and tannins were present with the absence of alkaloids.

Conclusion: This study could serve as a useful tool in the identification, evaluation and standardization of this plant species.

Keywords: *Ficus kamerunensis,* Macroscopy, Microscopy, Physicochemical, Phytochemical

INTRODUCTION

Pharmacognosy is the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs substances of natural origin as well as the search for new drugs from natural sources [1]. Herbal drugs play a significant role in health care, especially in developing countries[2]. Despite the modern techniques, standardization and authentication of plant drugs by pharmacognostic procedures is more trustworthy. The morphological and anatomical description of a medicinal plant is the first step towards standardization of plant materials, which needed to be carried out before the commencement of any experimental procedure for the detection of adulterations and impurities[3]. According to WHO, standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Herbal materials are categorized according to sensory, macroscopic and microscopic characteristics [4]. An examination to determine these characteristics is the first step towards establishing the identity and the degree of purity of herbal materials. They are carried out before any further tests are undertaken[5]. The macroscopic identity of herbal materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface.

However, since these characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material, it is often necessary to substantiate the findings by microscopy and/or physicochemical analysis [6]**.**Microscopic inspection of herbal materials is indispensable for the identification of broken or powdered materials; the specimens are treated with chemical reagents.

An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence[7]**.** Comparison with a reference material reveals, characteristics not described in the requirements, which might otherwise have been attributed to foreign matter, rather than normal constituents[6]**.**

Ficus is a genus of about 800 species and 2000 varieties of woody trees, shrubs and vines in the family Moraceae occurring in most tropical and subtropical forests worldwide[8]. In Africa, the plants are widely distributed in South Africa, Mozambique, Zimbabwe, Botswana, Nigeria, and Sudan [9]and over 45 different species of *Ficus* recorded in Nigeria [10]. *Ficus kamerunensis*is an epiphytic shrub, strangling, sometimes and lianescent growing up to 20 m high producing copious white latex [11]. Traditionally, the plant is used for the treatment of sexually transmitted infections, gastrointestinal, respiratory, inflammatory, cardiovascular disorders, ulcerative diseases, and cancers [12]. However, there has not been a reported pharmacognostic study on the leaves of the plant.

MATERIALS AND METHODS

Plant Collection and Identification

The aerial parts*F. kamerunensis* was collected in September 2013 from Samaru, Zaria, Nigeria. The plant was identified and authenticated by a taxonomist at the Herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria. Its voucher specimen number is 900308.

Macroscopical Examinations

The Macro-morphological features of the leaf were observed with naked eyes and under magnifying lens. They were described using terminologies in [13].

Microscopical Examinations

Freshleaves of the plant were studied transversely and longitudinally using surface preparation and sections. They were cleared and mounted on the microscope slide with dilute glycerol as described by [14]. Quantitative evaluations of the leaf microscopy has been carried out as described in [13]and [15].

Physico-chemical Parameters evaluation

The physicochemical parameters of the dried powdered leaves which includes; moisture content, ash values, extractive values were studied as outlined by the World Health Organization guideline [16].

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the powdered leaves were carried out as described by [15]and [17].

RESULTS

The macro-morphological features of the leaves of the plant appeared to be dark green coloured, with pinnate venation ovate shape and entire margin. It has a dimension of 9.2-6.7-4.2 cm X 5.4-3.9-2.4 cm. with an acute apex, cordate base, glaborous surface and petiole (2.9-2.4-1.9 cm). Micro-morphologically, features of the fresh leaves shows anomocytic stomata on both upper and lower epidermis but stomata were more in the lower epidermis. The epidermal cells were polygonal in shape with straight and wavy anticlinal walls. Numerous unicellular, uniserrate covering trichomes as well as parenchymatous mesophyll were observed.

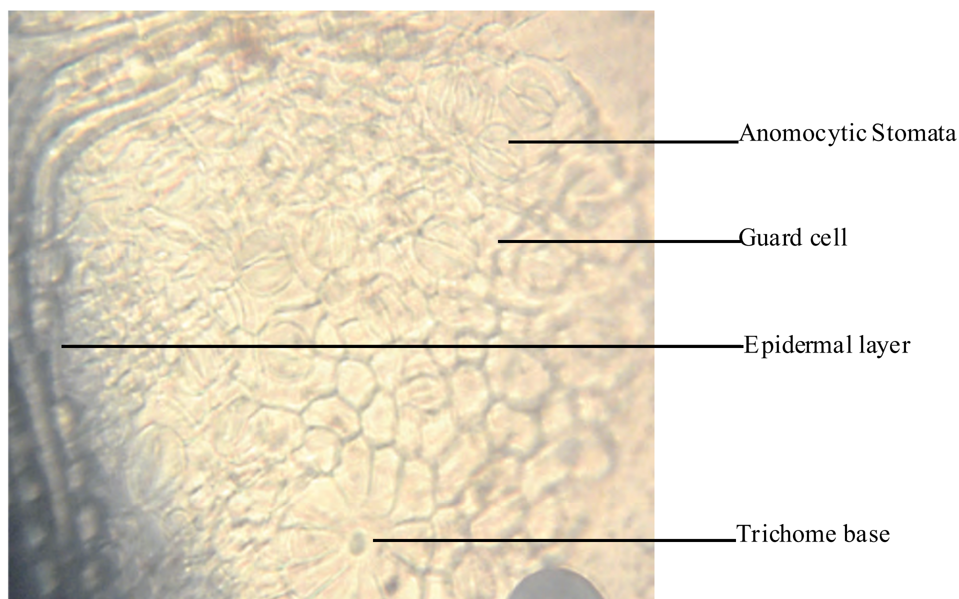

Figure 1: Lower Epidermal layer of *F.kamerunensis*

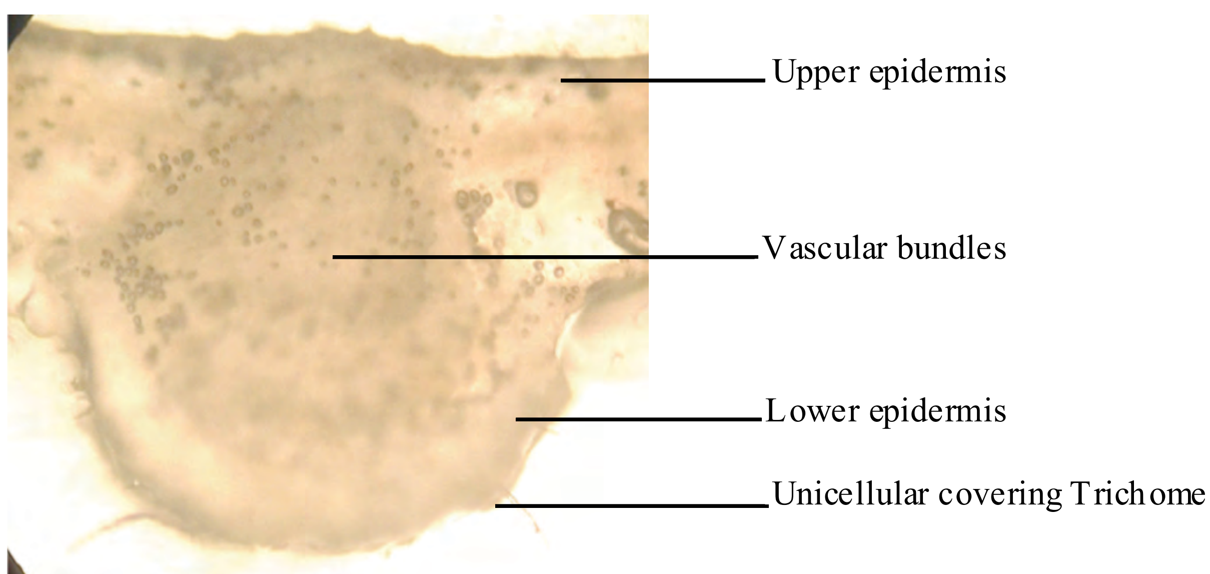

Figure 2: Transverse Section (T.S) through the mid rib of *F.kamerunensis*

The result of the qualitative leaf microscopy and physicochemical parameter were shown in table 1 and 2 respectively.
The preliminary phytochemical screening of the leaves of indicated the presence of anthraquinones, flavonoids, triterpenes, saponins, cardiac glycosides and tannins with absent of alkaloids.

Table 1: Quantitative leaf Microscopy of *F. kamerunensis*

Parameter	Mean ± SEM
Stomatal number	47.2 ± 0.58
Stomatal index	22.72 ± 0.54
Vein termination number	16.40 ± 0.51
Veinlet termination number	40.20 ± 0.37
Palisade ratio	8.00 ± 0.32

*Average of five determinations.

Table 2: Physicochemical parameters of the leaf of *F. kamerunensis*

Parameters	Values ($\%^{w}/_{w}$)
Moisture Content	9.67
Total Ash	9.5
Acid Insoluble Ash	2.2
Water Soluble Ash	1.2
Ethanol Extractive Value	2.3
Water extractive Value	2.7

DISCUSSIONS AND CONCLUSIONS

Pharmacognostic evaluation of a plant or plant parts is considered an important step that provides valuable information in terms of its morphological, microscopical and physical characteristics. This is important in the study of crude drugs being the first step in establishing the correct identity of the plant. Macroscopically, the leaves features of the plant were in conformity with the characteristic features of some plant members of the genus Ficus such as *Ficus bengalensis* Linn as described by [18].

Microscopical features of the transverse section of the fresh leaves are consistent with those of *Ficus hispida* [19]. The occurrence of anomocytic stomata on both the abaxially and adaxially is a diagnostic importance, likewise the result obtained from the quantitative leaf microscopy which is being reported for the first time for the plant *F. kamerunensis*. The physicochemical parameters of the leaves of the plant were determined and can be used in development of a monograph for the plant.

The preliminary phytochemical screening of the leaves reveals the presence of anthraquinones, saponins, cardiac glycosides, triterpenes, flavonoids, tannins and absence of alkaloids, which is consistent with some members of the genus Ficus as reported by earlier studies [20-22].

Based on these pharmacognostic studies carried out, the results obtained could usefully aid in the identification, evaluation and standardization of *Ficus kamerunensis*.

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MOSQUITO REPELLENT ACTIVITY OF LEAF AND SEED EXTRACT OF *AZADIRACHTA INDICA* (NEEM)

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ABSTRACT

Introduction: Prevention of mosquito bites is one of the main strategies to control or minimize incidence of malaria in Africa. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. The present study was conducted to evaluate mosquito repellent activity of neem leaf and seed extract formulated in form of a cream against mosquito under laboratory conditions using human volunteers.

Methodology: The leaf and seeds were extracted using ethanol and n-Hexane for three (3) days, then the resulting oil extracted from the leaf and seeds were used to formulate a cream at two different concentrations, 15 and 25% w/w respectively.

Results: The result showed that all the extract at all concentration repelled mosquitoes with the n-Hexane seed oil-based cream showing the highest repellency of (92.8%and 85.7%),followed by ethanol neem seed-based cream (88.2% and 78.82%). Then n-Hexane leaves based cream (73% and 57.3%) and ethanol neem leaves showing the least of (68% and 55%) both at concentration (25%w/w and 15%w/w) respectively. The n-Hexane seed fraction was found to have higher mosquitoes-repellency activity.

Conclusion: n-Hexane seed oil extract was found to be more potent against repelling mosquito, and could thus be used as a better oil for formulating mosquito repellant creams.

Keywords: Malaria, Mosquito, *Azadirachta indica, Oil, Repellent, cream*

INTRODUCTION

Many mosquito-borne diseases, such as malaria, dengue fever (DF),denguehemorrahagic fever (DHF) and filariasis, are serious public health problems in tropical regions, especially in Africa and Asia. These diseases are transmitted to human beings through mosquito bite only and there is no effective vaccine available for the control of these diseases. Prevention of mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. It is important not only for local people in disease risk areas especially in tropical countries, but also for travellers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from their home countries.

DEET(N, N-diethyl-3-methylbenzamide) is the most widely used mosquito repellents which are commercially available[1]. Spray mosquito repellents are very common and may be sprayed on the clothing or skin. Cream or lotion repellents are applied directly to the skin and rubbed in thereby creating a repellent barrier. Mosquito repellent clothing is specially designed with tight fiber weave and infused with a long-lasting natural mosquito repellent to prevent mosquito bites[2].

Additionally, mosquito coils are also widely known as efficient mosquito repellents. Even

though mosquito repellents based on chemicals have remarkable safety profiles, they are toxic against the human skin and nervous system and may cause rashes, swelling and eye irritations. This has therefore necessitated the need for research and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control which can be used with minimum care by individuals and communities[3]. Studies have shown that extracts from plant sources possess insecticidal[4,5] and repellent[6,7] properties. The neem, *Azadirachta indica*, is a draught-resistant tree that provides many useful compounds that are used as pesticides[8].

Research by Moser[9] indicated that globally the most important use of neem was as an insecticide. Neem contains several aromatic compounds that can be used to repel insects from biting humans and animals. Neem oil mixed with coconut oil gave up to 98.03% protection against the mosquito, Anopheles culicifacies, in all-night biting tests conducted in Gujarat, India[10]. Neem oil also provided more than 75% protection against *A. fluviatilis, Aedestaeniorhynchoides* and *Mansonia uniformis*. Burning neem oil in a room is also said to repel mosquitos and other biting insects[11].

MATERIALS AND METHOD

Mosquito Larval Collection: - Larval collections were made from stagnant water within Federal University, Dutse in the month of August, 2019, during rainy season. Collections consist of systematic dipping into the habitats using ladle.

Rearing of Mosquitoes:-Mosquito larvae and pupae were collected and reared at room temperature around 25-27^{0}C which is similar to the environment from which they are isolated. Approximately 200 larvae were reared in white plastic container. Net is used to cover the container so as to allow sufficient oxygen and light penetration. The larvae and pupae were kept in the water medium and fed with small quantities of bakers or brewer's yeast once in every two days so as to avoid fermentation and development of fungus. (Larvae pupate within 3-4days and pupae emerges as adult in a day or two under favorable condition. Immediately the adult emerges they leave the water surface and attach themselves to the walls of the container and net).The container was placed in the rearing cage and the net was removed carefully so as to transfer all the emerged adult mosquitoes into the cage[12].

Leaf and seed collection:-Fully developed leaves and seeds of *Azadirachta indica* were collected in the month of June, 2019 in the beginning of rainy season within the Federal University Dutse campus and were air-dried under shade in Biotechnology laboratory for 3Weeks.

Extraction of oil from seed:-n-Hexane and Ethanol were used as solvent for the extraction processes according to the method described by Aremu[13] with some modifications. Neem seed kernel were removed and grounded into powder. The extracts were prepared by taking 250g each of the grounded kernel and put into two separate containers, 400ml of n-Hexane was added into one container and 400ml ethanol into the other. The seeds kernel were allowed to soak in the solvent for 2 days at room temperature with periodic shaking. The solution was then filtered through a piece of fabric to retain the debris and the filtrate into a volumetric flask. The filtrate was then poured into a silver plate and allowed to evaporate for 2weeks at room temperature to obtain solvent free oil[13].

Leaves extract (ethanol and n-Hexane):-The dried leaves were grounded and sieved to get fine powder, n-Hexane and ethanol extract were prepared by taking 150g of the powered leaves and added into two separate containers, 400ml of the solvents were poured into one container each. Then they were allowed to soak for 2weeks with periodic shaking. The solution was then filtered through a piece of fabric into a volumetric flask. The filtrate was then poured into a sliver plate and allowed to evaporate for 2weeks at room temperature according to method described by Charmaine[14].

Making seed extract based cream: A cream was made out of the oil and leaf extract to serve as the oil component at the same containing a mosquito repelling constituent of *Azadirachta indica*. Four different creams were made from the ethanol and n-hexane extract o*f Azadirachta indica* seed at 15% and 25% w/w concentration. The following steps

were followed for the cream making:
3g of beeswax and 5g stearic acid were melted over low heat, followed by addition of 15g of petroleum jelly which was allowed to melt. 7.5ml and 12.5ml oil extract of n-hexane (at conc. 15% and 25%) were added to two separate containers. This was followed by mixing of 2g baking soda and 8g borax and 20ml of boiled water together which was then finally sieved and the mixture poured into the oil mixture and mixed until evenly and completely cooled. The same process was made for the leaf extract fraction.

Mosquito repellent test:-The repellency test of the four extracts was assessed using human-bait techniques. Forty volunteers (from age 17-27 years) participated in the test with each volunteer exposed to only one of the extracts at a time. The evaluation was carried out at room temperature. The test extract 15% and 25% w/w(n-Hexane/ethanol) were applied on one hand while the other forearm was treated with only ethanol free from the extract to serve as a control. Each volunteer put the test forearm in the mosquito cage containing 50 female mosquitoes. Before the start of each exposure, the bare hand that was used as a control area was exposed for up to 30 seconds. If at least 2 mosquitoes landed on the hand, the repellency test will then be continued. The number of mosquitoes probing the treated area was noted for five minutes as described by Tawatsin[6].

The percentage repellency (% repellency) in the field evaluation was analyzed according to the formula described by Yap *etal*, (1998).

$$\frac{C-T}{T}x100$$

Where C is the number of mosquitoes that landed on the control and T is the mean number of mosquitoes that landed on the treated volunteers.

Data analysis

Data was analyzed using statistical software package, SPSS version 20.0. Data was presented as mean ± SD. For comparisons of means, student t-test was used to determine the significance between groups and. P–value of <0.05 was considered statistically significant.

RESULTS

Yield and colour of leaf and seed extract of *Azadiracta indica*

The result of the present study revealed that n-Hexane extract of neem seed has the highest yield of (20%),followed by ethanol extract of neem seed (15.5%) as shown in the table 1 below:

Table 1: Percentage yield and colour of leaf and seed extract of *Azadiracta indica*

S/N	Extract	% yield	Colour
1	Neem Leaves Ethanol Extract	2.8	Army Green
2	Neem Leaves n-Hexane Extract	2.7	Dark Green
3	Neem Seed Ethanol Extract	15.5	Lemon Green
4	Neem seed n-Hexane Extract	20	Amber/yellow

Repellency activity of n-Hexane and ethanol leaf and seed extract of *Azadiracta indica*

The result of the Mosquito repellents activity test showed that n- Hexane seed extract was found to have higher mosquito-repellency at 25% concentration (Table 2) than at 15% concentration. This is followed by ethanol seed extract of neem at 25% concentration. Ethanol leaves extract of neem showed the lowest percentage of mosquito-repellency as shown in Table 2.

Table 2: Repellent activity test of n-Hexane and Ethanol leaf and seed extract of *Azadirachta indica*

S/N	Fraction	Conc (%)	Mean no of Mosquitoes	% Repellency
1	Ethanol leaves	15	6.4±1.1	55
		25		
		0	4.8±0.8	68
	CONTROL		15	
2	Ethanol seed	15	3.6±0.5	78.82
		25		
		0	2±1	88.23
	CONTROL		17	
3	n-Hexaneleaves	15	5.8±0.8	57.3
		25		
		0	3.4±1.1	73
	CONTROL		13	
4	n-Hexane seed	15	2±1	85.7
		25		
		0	1±0.7*	92.8
	CONTROL		14	

Result was expressed as Mean ± SD *$p<0.05$

Paired sample t-test showed that n-Hexane seed extract at 15% concentration is significantly ($p<0.05$)different from ethanol seed extract of neem at 15% concentration. Analysis of Variance using Turkey's posc -hoc analysis showed thatn-Hexane seed extract at 25% concentration was found to be significantly($p<0.05$) higher than all the extracts. n-Hexane seed extract at 15% concentration was also found to be significantly($p<0.05$) higher than ethanol leaves extract at 25% concentration and ethanol seed extract at 25% concentration.

DISCUSSIONS AND CONCLUSIONS

One of the active compounds contained in neem oil is azadirachtin, which presumably has the ability to act as a natural insecticide[13,15]. It was reported that when mosquito eats azadirachtin, it actively attacks its reproductive cycle, its feeding pattern, its bodily development, as well as acting as direct toxin[16].It was observed that from the result obtained (Table 2), that different extracts show certain level of repellency, with n-Hexane seed extract showing the highest degree of repellency of 85.7%(at 15% concentration) and 92.8 (at 25% concentration). This is not surprising as it has been previously reported that neem (*Azadiracta indica*) seeds oil and leaves extract in appropriate amount when smeared on the surface of the hand showed excellent repellent action against mosquitoes[17], their finding was in agreement with our study where they reported that the degree of repellency was in increasing order as the amount of extracts is increased. A similar study observed that at a concentration of 5% neem oil in cream formulation, showed a marked improvement in the repellent activity against *Aedes aegypti* and the activity was significant when compared to concentrations of 1%, 2%, 3%, and 4%, hence 5%. as a topical application on humans[18]. Similar results were also reported by Sharma[19] using 5% neem oil against *Culex quinguefasciatus* and *Anopheles culicifacies* mosquitoes.

Probably, the superiority of Neem seed oil extract could be attributed to a much higher concentration of Azadirachtin. This could be explained thus that as soon as alighting mosquitoes sense the discomfort or lethal effect of the chemical constituent of neem seed oil and leaves cream, they left. That could be the reason why the forearms that were treated with neem seed oil extract were better at repellent mosquitoes than the ones treated with leaves extract because of the presence of much higher Azadirachtin concentration. It is evident from the result of this study that among the four extracts tested for repellent activities, the seed extract based-cream of Neem (*Azadirachta indica)* was found to be the most effective in repelling mosquitoes.

DISCLOSURE

The authors declare no conflict of interest

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ASSAY OF *PLASMODIUM FALCIPARUM* SPECIFIC ANTIBODIES IN MATERNAL AND CORD BLOOD AT THE UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL, PORT HARCOURT , NIGERIA.

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ABSTRACT

Introduction: Detection of antibodies specific to an antigen, especially IgM is a retrospective confirmation of exposure to a particular infection. Malaria infection is confirmed by detection of *Plasmodium falciparum* specific antibodies**.**

The aim of the study was to assess the occurrence of transplacental transmission of malaria parasites and provide evidence that the foetus is able to elicit humoral immune responses to the antigen *in-utero.*

Methodology: Malaria parasites rates in maternal, placental and cord blood samples from 45 deliveries were investigated microscopically.Levels of IgM and IgG specific to *P. falciparum* were measured by Enzyme-linked immunosorbent assay (ELISA) and the seropositivity rates were determined. Socio-demographic and obstetrics data of the parturient women was collected.

Results*:* *Plasmodium falciparum* was the only malaria parasite specie detected. Rates of parasitaemia in maternal, placental and cord blood were 28.9%, 20.0% and 13.3% respectively.ELISA seropositivity rates *for P.falciparum* specific IgG and IgM antibodies in maternal sera were 73.3% and 64.4% respectively while those of cord sera were 64.4% and 11.1% respectively.Malaria parasites rates detected in maternal blood by microscopy differ significantly from that of ELISA, but not so in cord blood (P=0.7475).

Conclusion: Transplacental transmission of *Plasmodium falciparum* occurred in the foetuses and is confirmed by detection of IgM specific antibody in their cord blood.

Keywords: Assay, specific, maternal, cord, antibodies, seropositivity.

INTRODUCTION

Plasmodium falciparum is the most virulent and most wide spread of the five species of human malaria parasite. The other species are *Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae and Plasmodium knowlesi which* is less common in human beings[1].

Plasmodium falciparum is the predominant specie in the sub-Saharan African. The female mosquito of *Anopheles gambiae* has been found to be its very effective vector [2] and it is transmitted to human beings during a blood meal by the mosquito[3,4].

The various forms of *Plasmodium* develop at different phases and encode hundreds of proteins which are antigenic and evoke different immunologic responses. MSP-1 is the most bountiful surface protein of the invasive merozoite stage of the *P.falciparum* life cycle[5,6]. Detection of *Plasmodium falciparum* specific antibodies, especially IgM is a retrospective confirmation of an attack of malaria infection[7-9]. IgM is the first immunoglobulin produced in response to invading antigen. It does not cross the placenta because of its pentameric nature and large molecular weight. IgG appears following the process of antibody

class switch[10]. Maternal IgG is known to cross placental barrier passively to attain nearly equal titre in cord blood. This has been attributed to the small molecular weight of IgG and the presence of neonatal fc receptor (FcnR) on the placenta which facilitates the passage of IgG through the syncytiotrophoblastic membrane[11].

The parasite is relatively protected from attack by the immune system because most of its human life cycle is within the liver and blood cells, making it relatively invisible to immune surveillance. The adhesive proteins produced by *Plasmodium falciparum* on infected red cells enable the red blood cells to adhere to the walls of the small blood vessels, thereby isolating (sequestering) the parasite from passing through the general circulation and the spleen[12].Occurrence of sequestration of parasites in the placenta is a virulent factor exclusively observed in *Plasmodium falciparum* infection (13). This sequestration of the parasites in the placenta may block the microvasculature, thereby causing symptoms of placental malaria[14]. Syncytiotrophoblastic membrane damage may result from inflammation caused by the parasites. This may increase transplacental passage of infected red cells to the foetus during pregnancy, and may result in congenital malaria[15,16].

Malaria in pregnancy with its accompanying risk for the pregnant woman and her foetus is a significant public health challenge. Congenital malaria may result in severe anaemia, metabolic acidosis and death in infants[17-19]. Studies have shown that early gestational encounter with the blood stage malaria antigen may have very serious long term effects as a result of immune tolerance which can lead to increased susceptibility to infection in infancy and childhood[20,21]. Immune responses may be activated prior to birth by late gestational exposure to malaria antigen and this seems to improve responses to future attacks[22]. Congenital exposure to malaria antigen elicits immune responses leading to production of antibodies against the antigen. Assay of *Plasmodium falciparum*-specific antibodies in paired maternal and cord sera may reveal the actual burden of congenital malaria in the population better than microscopic examination of blood films in which low density parasitaemia most times go undetected[23,24].

The aim of the study is to assess the occurrence of transplacental transmission of malaria parasites and provide evidence that the foetus is able to elicit humoral immune responses when exposed to malaria parasite antigen. The results will help obstetricians and paediatricians intensify malaria preventive measures in antenatal and postnatal care before the neonate is exposed to mosquito bites and malaria infection.

MATERIALS AND METHODS

Study design, study area, Ethical considerations

A cross-sectional hospital based study was conducted among parturient women after delivery at the labour ward of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria between February and May 2016. Approval for the study was obtained from the Ethical Committee of the above mentioned hospital. The purpose of the study was explained to each woman and informed consent was given by each of them.

Source of data, sampling technique and Sample size

Socio-demographic data questionnaires were used to record relevant information including maternal age, blood group, parity, gestational age, and birth weight. Systematic random sampling technique was used to obtain the women. 45 parturient women were recruited for the study.

Sample collection and Preparation:Five (5mls) each of maternal and cord blood were taken and dispensed into EDTA and plain bottles respectively. EDTA blood samples were used for blood films. Sera were stored at -20°C until used for the assay. Pooled blood from a slit in the placenta was used to make thick smears. Thick blood smears were stained with Giemsa stain while thin blood films were stained with Giemsa and Leishman stain. Two smears were made for each sample for quality assurance[25].

Investigation of Malaria Parasitaemia at delivery

Microscopic Examination: Stained thick blood films of maternal blood, cord blood and the placental blood were viewed under oil immersion (at X100) for malaria parasites. A minimum of hundred thick film fields were examined for a sample to be considered negative. A blood smear was considered to have malaria infection if any of the asexual blood parasites were seen in more than three thick film fields. Thin blood smears were used for species identification. The contrast between parasite cytoplasm and red cell cytoplasm is more pronounced with Leishman stain than Giemsa stain [26].

Assay of *P.falciparum* specific IgG and IgM antibodies: Seropositivity rates of *P. falciparum* specific IgM and IgG antibodies in paired maternal and cord sera were determined using indirect ELISA technique. Human anti-merozoite surface protein-1 IgM and IgG ELISA kits manufactured by Alpha Diagnostic International, USA were used to analyse the samples. All samples were assayed for IgM and IgG at a dilution of 1:100 in Low non-specific binding (NSB) diluents. Net optical density (OD) < 0.5 was selected as sensitivity for negative non- immune samples. The threshold for positivity was an absorbance >3 standard deviation above the mean of the negative non-immune samples. This threshold improves the reliability of the results [27].

Statistical Analysis: All tests were carried out with the Epi Info v7 (CDC, Atlanta, USA) software at a 95% confidence interval and a p-value less than 0.05 was considered significant. Methods of analysis for comparisons of distributed variables are described in Results.

RESULTS

A total of 45 parturient women were included in the study. The age range was 20-46 years with mean age of 30.3$\pm$ 0.8 years. The gestational age range was 36-41 weeks. Only 4 babies were born at 36 weeks. The babies were all of normal birth weight (2.8-4.7kg).14 (31.1%) of the women were nullipara (primigravida), 10 (22.2%) were secundigravida while 21 (46.7%) were classified as multigravida or multipara.

Table 1: Frequency of *P. falciparum* infection by different methods of detection

Method of detection	No (%) in maternal blood	No. (%) in cord blood
Microscopic examination	13 (28.9%)	6 (13.3%)
Anti-MSP-1 IgM ELISA	29 (64.4%)	5 (11.1%)

Table 1 shows the frequency of P. falciparum infection by two methods of detection. By microscopic examination, 13 women (28.9%) and 6 cord samples (13.3%)were positive. Anti- Merozoite surface protein-1 IgM ELISA detected seropositivity in 29 (64.4%) maternal blood and in 5 (11.1%) cord blood.

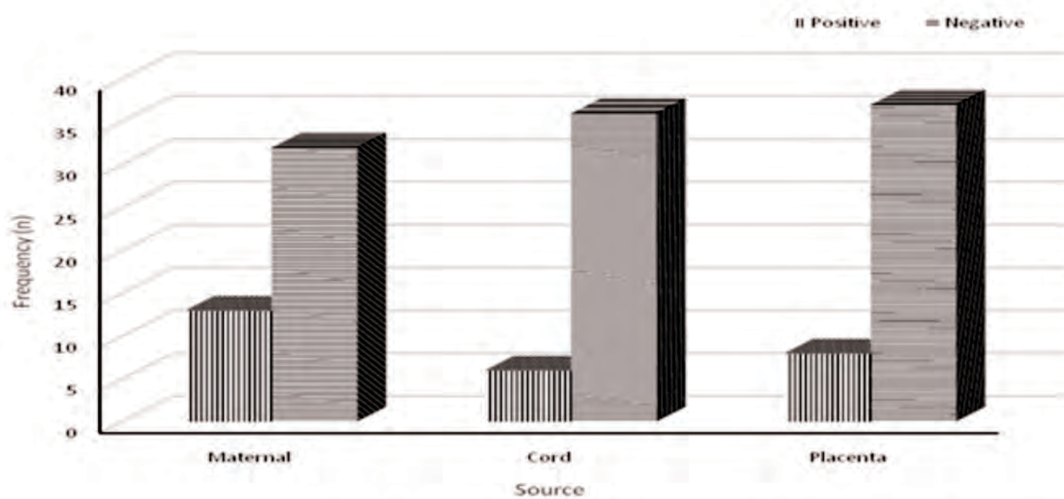

Fig 1. Detection of *Plasmodium falciparum* by microscopy.

Fig 1 shows frequency of *Plasmodium falciparum* in maternal, cord and placental blood smears by microscopy. The parasites were seen in peripheral thick blood films of 13 of the 45 parturient women (28.9%).Cord blood parasiteamia was observed in 6 samples (13.3%). Only 9 (20%) of the 45 placental smears had malaria parasite

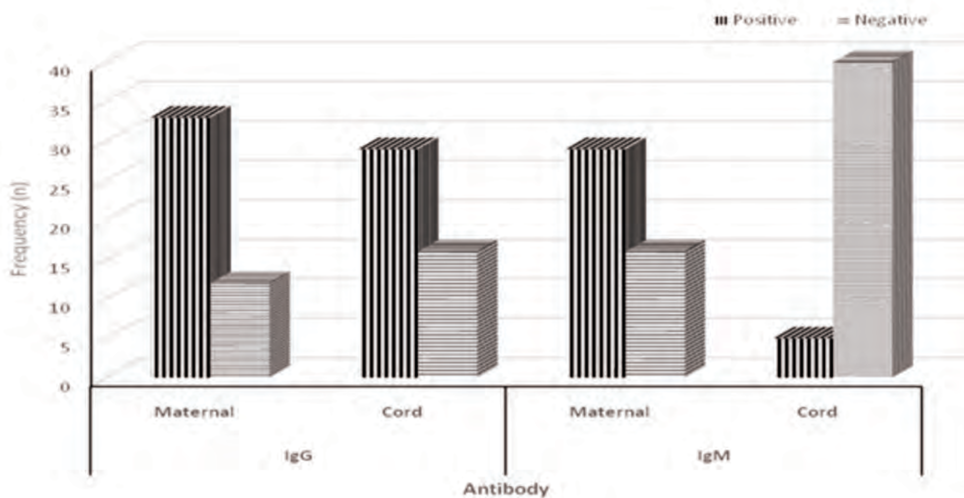

Fig 2. Anti MSP-1 seropositivity by ELISA

Fig 2 shows the frequency (seropositivity rates) of Anti MSP-1 IgG and IgM in maternal and cord sera analysed by ELISA .The seropositivity rates of IgG in maternal and cord sera were 33(73.3%) and 29(64.4%) respectively. The seropositivity rates of IgM in maternal and cord sera were 29(64.4%) and 5(11.1%) respectively. All samples that were IgM positive were also ELISA positive for IgG antibody.

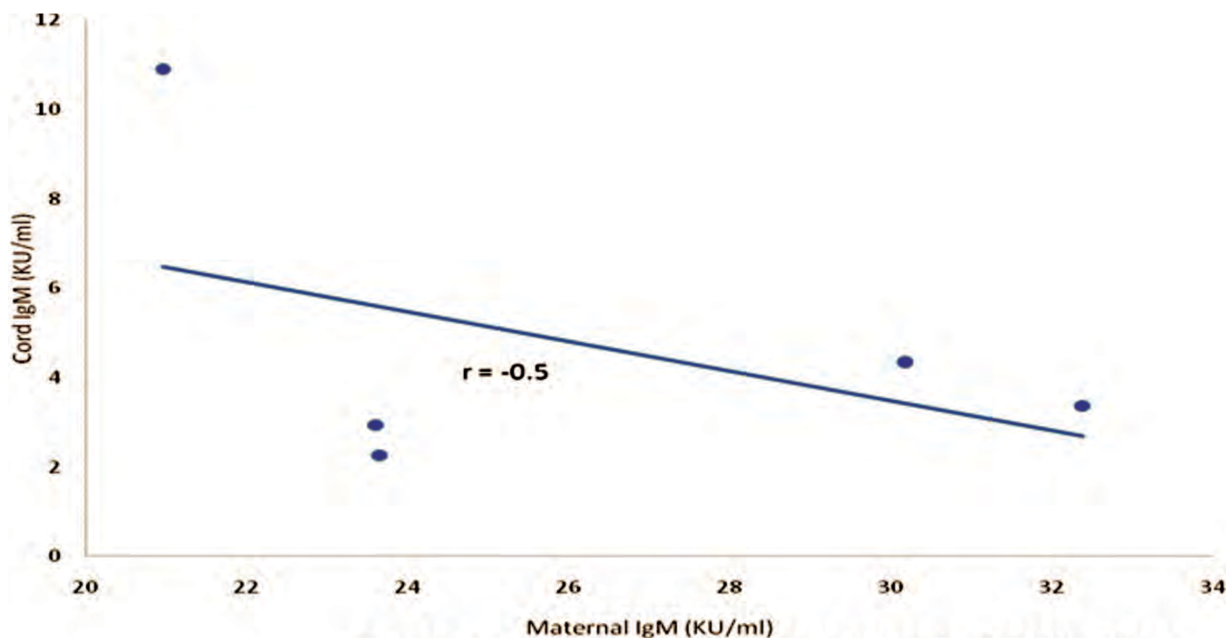

Fig 3. Correlation of Cord and Maternal Anti-MSP 1 IgM

Fig 3 is a scatter plot of correlation of cord and maternal Anti-MSP-1 IgM concentrations showing inverse correlation (r = -0.5).

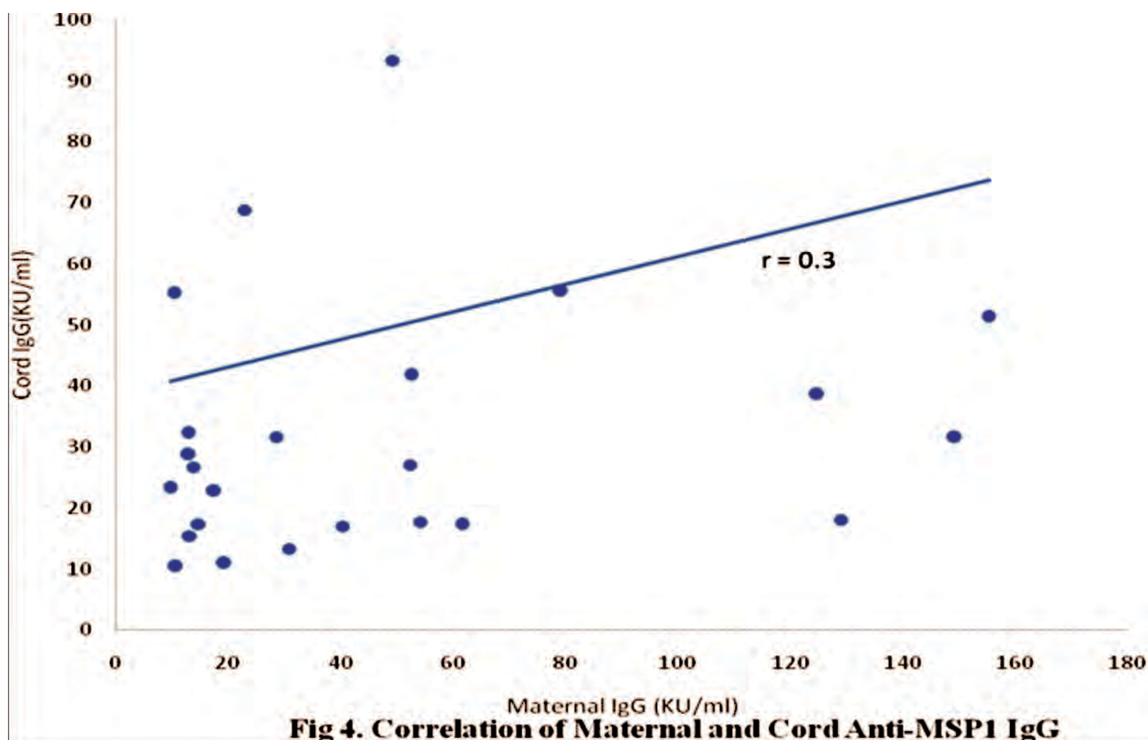

Fig 4. Correlation of Maternal and Cord Anti-MSP1 IgG

Fig 4 is a scatter plot showing significant positive correlation of cord Anti-MSP-1 IgG concentration with increase in maternal IgG concentration (r =0.3, P< 0.05).

Table 2: Comparison of Mean Antibody concentration in Primigravid women and women with history of previous pregnancies.

Antibody	Primigravid	Previous Pregnancy	T-test (*p*-value)
IgM (KU/ml)	173.5±84.5	175.5±82.7	0.6426**
IgG (KU/ml)	130.6±74.1	410.1±44.2	0.0151*

**Difference between the groups is statistically significant (p < 0.05)*
***Difference between the groups is not statistically significant (p >0.05)*
Table 2 shows comparison of mean antibody concentration in primigravid women and women with history of previous pregnancy. The difference in IgG concentration is statistically significant but not in IgM.

Table 3: Comparison of Mean maternal antibody concentration in placental parasiteamia

Placental parasiteamia	Antibody concentration (KU/ml)	
	IgG	IgM
Positive	42.6±14.9	18.1±3.1
Negative	32.7±7.1	16.5±1.5
T-test (p-value)	0.5448**	0.0422*

All values are expressed in Mean ± SEM (standard error of mean)
**Difference between the groups is statistically significant (p<0.05)*
***Difference between the groups is not statistically significant (p>0.05)*
Table 3 shows comparison of antibody concentrations in placental parasiteamia.The difference between antibody concentrations in placental malaria positive and negative subjects is statistically significant (T-test =0.0422, P<0.05).

DISCUSSIONS AND CONCLUSIONS

Studies have shown that malaria parasite antigen passes through the placenta barrier and provoke acquired immune response in the foetus [28, 29]. Different immunoglobulins have been assayed in the sera of subjects in malaria endemic area [30, 31, 28]. In this study, the levels of IgM and IgG antibodies in maternal and cord blood were evaluated to investigate transplacental transmission of malaria parasites and immunological evidence that foetuses can elicit humoral immune responses when stimulated by malaria parasite antigen.

Plasmodium falciparum was the only species detected in this study. The study showed that the rates of malaria parasitaemia obtained in maternal and cord samples respectively by microscopy were higher than that obtained by George *et al. (32)* in Port Harcourt, but within the ranges of 19.7% to 72% for maternal blood and 2.6% to 54.2% for cord blood obtained by various studies done in Nigeria [33, 2,34].

Enzyme linked immunosorbent assay of *Plasmodium falciparum*-specific IgG and IgM revealed that seropositivity rate was higher in maternal than in cord samples and that all samples that were IgM positive were also ELISA positive for IgG antibody. Desowitz*et al,*[35] in a study in Papua, New Guinea reported same for maternal samples but unlike this study which detected 11.1% IgM seropositivity in cord blood samples, IgM was not present in cord blood samples of Papua New Guinea newborn. The variation may be attributed to differences in geographical location. *Plasmodium falciparum* is more endemic in Africa [36]. The presence of IgM in cord blood has been reported. In Gabon, anti- *Plasmodium*

*falciparum*IgM was present in 11.9% of cord serum samples examined (31). Xi *et al.(9)* reported in Yaounde, Cameroon, *P. faliciparum* specific IgM in 14% of cord blood samples. The results obtained by the two methods used to investigate malaria parasite rate in this study confirms the finding by George *et al*,[32] that congenital malaria in not uncommon in Port Harcourt, Nigeria.

The seropositivity rates of *Plasmodium falciparum*-specific IgG and its concentrations were similar in paired maternal and cord sera (P>0.05). The positive relationship between maternal and cord IgG concentrations was not surprising because 1gG crosses the placenta (37). On the contrary, the parasite specific IgM seropositivity rate and concentration in cord blood were lower than maternal level, giving an inverse correlation (r=-0.05). This suggests that the foetuses produced IgM *in utero* in response to antigenic challenge [38,39]. The mechanism of entry of these antigens into foetal circulation was not investigated in this study, but the predilection of *P.falciparum* for placenta and the accompanying histopathological changes suggested by previous studies may have allowed the passage of malaria antigen from maternal to foetal circulation [28, 15, 14]. The intimate association between placental and feotal tissue may also afford the malaria antigen entry into foetal circulation when a pregnant women gets infected with malaria parasite.

The significant difference in antibody concentrations between seropositive and seronegative subjects supports the fact that Anti-MSP-1 antibody production occurs when the immune system is challenged with MSP-1 antigen [40]. Antibodies specific to these blood stage variant surface antigens would indicate that an attack of malaria infection had occurred. Anti-MSP-1 seropositivity rate in the parturient women indicated a high level of exposure which also reflects in the high level of malaria antibodies found in cord blood. Pasay *et al*, [41] obtained similar results.

The observed high concentration of IgG antibodies in multiparae may be associated with the age of the mothers because antibody concentrations have been observed to increase with age and frequency of malaria attacks [42]. This may also be the reason for the significant difference in IgG concentrations between primigravid and multigravid women (women with previous pregnancies). Low 1gG concentration seems to make the primigravida more susceptible to malaria infection [43]. IgM concentrations did not differ between primiparous and multiparous women because production of IgM is soon followed by production of IgG and other classes of immunoglobulin in a phenomenon known as antibody class switch [27]. Moreover, women with previous pregnancies were more in the study than primigravid women.

IgM concentrations were approximately half the concentrations of 1gG. This result differs slightly from that reported by Desowitz*et al*,[35] in showing that there was significant difference in IgM concentrations between placental parasitaemia-positive and negative subjects. Again, the variation in the two studies may be attributed to differences in acquired immune factors between populations (44).

The result indicted high rate of IgM seropositivity in the mothers which may have resulted from the incessant attacks of the parasite in endemic areas. It has been demonstrated that IgM antibodies may commonly be present in patients exposed to frequent malaria attacks [30,31].

The ELISA measures anti-MSP-1 activity, a combination of antibody concentration and avidity for the MSP-1 antigen. The use of a high threshold of positivity, an absorbance of three standard deviations above the mean of non-immune samples improves the reliability of the results [27]. ELISA detected IgG and IgM seropositivity in multiparae but by microscopic examination, no parasite was detected. This suggests that ELISA may bemore sensitive than light microscopy in malaria parasite studies. It is also interesting to note that the rate of *P. falciparum* parasitaemia in cord blood detected by the two methods clearly indicated that prenatal exposure to malaria antigen and *in utero* priming of immune system of the foetus is not an infrequent event.

Transplacental transmission of malaria parasite antigens occurred in the foetuses and they were

able to produce IgM antibodies– aconfirmation of active malaria infection. With the finding of up to 11.1% of congenital malaria in the study, it may be necessary to investigate febrile illness in neonates further with ELISA to rule out low density parasitaemia. Further studies should characterize the antigenic specificity of foetal *P. falciparum IgM* antibody. More sero-epidemiological studies of IgM antibodies especially as it relates to malaria and pregnancy should be carried out and factors associated with *in utero* immune priming should be identified. Assay of other immunoglobulin classes to determine the full extent of malaria parasitaemia on humoral immune mechanism in the foetus may be necessary.

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Improving Laboratory Capacity; A necessity towards elimination of malaria in Nigeria

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ABSTRACT
The quest to achieve malaria elimination requires that all the steps leading to its achievement should be strengthened. One of the key areas of focus is assessment of diagnostic capabilities. Strengthening diagnostic testing will reduce mortality through administration of the exact recommended treatment. The article addresses the challenge of diagnosis within the context of the current concerted drive to achieve malaria elimination in Nigeria. One of the key facts emphasized is the need for the establishment of Reference laboratories in various parts of the country with the required facilities, expertise, supply chain, funding, political and administrative support.

Key Words: Elimination, diagnosis, molecular techniques, reference laboratories.

Malaria is caused by Plasmodium parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. Though it is a life-threatening disease, it is preventable and curable. The WHO African Region where Nigeria lies, continues to bear a disproportionately high share of the global malaria burden, accounting for 93% of cases and 94% of deaths from malaria in 2018 [1].

In line with the World Health organization's vision of achieving malaria elimination in various regions of the world and its eradication ultimately, the Nigerian government launched the National malaria elimination program (NMEP), domiciled in the Federal and State Ministries of health, through which various efforts have been made towards achieving malaria elimination in Nigeria. In 2015, the director of the NMEP shared the National malaria strategic plan in which the goal was to reduce malaria burden to pre-elimination levels and bring malaria-related mortality to zero by 2020 [2].

Elimination defined as Interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite species in a defined geographical area as a result of deliberate activities implies zero transmission of malaria in a geographical region because only after transmission of all human Plasmodium parasites is interrupted is a certificate of elimination issued [3]. In the past decade, 10 countries (including Algeria) were certified by WHO to have eliminated malaria[1]. This proves that elimination is possible, however; it is a task that requires a well-coordinated approach, which should be designed after critically appraising the various factors that determine transmission of malaria in Nigeria.

The steps to elimination starts from malaria control through pre-elimination and elimination phases before finally working to prevent reintroduction. Key considerations and thus areas of focus should include unique transmission dynamics of local settings (effects of seasonal variations, weather pattern and differing topography), diagnostic capabilities, therapeutic options and control efforts including surveillance systems. Post- eradication management is also critical to prevent a re-introduction of malaria. Hence, long-term strategic planning has to be made.

Thankfully, a lot of progress has been made as incidence and mortality rates in Nigeria and globally, continues to drop; most of these gains being attributed to the increased use of insecticide treated bed nets and indoor residual spraying, and the global push to test and treat [1,3].

With regards to diagnosis, the World Health

Organization (WHO) Global malaria program's framework for malaria elimination which provides guidance for countries as they work towards elimination, buttresses the need to optimize currently and commonly available laboratory techniques such as malaria microscopy and the use of rapid diagnostic tests (RDTs) for diagnosis and monitoring of infections reiterating that these are veritable tools as evidenced in regions that have successfully achieved elimination [3].

Molecular method is a tool that plays a role both in the area of clinical diagnosis and malaria epidemiology. This approach questions the use of microscopy as the gold standard for malaria diagnosis being of higher sensitivity and specificity while molecular epidemiology leverages on genetic information to study risk factors and transmission dynamics [4].

The need for molecular methods such as polymerase chain reaction (PCR) arises due to but not limited to the identification of asymptomatically infected persons with low parasite density who serve as reservoirs of the plasmodium parasite [3,4]. The PCR is also useful for the identification of molecular markers of malaria parasites that are resistant to treatment. These markers have been proven to be tools for surveillance of resistance, provide additional data that compliment clinical observations of the *in vivo* efficacy of a drug and has been instrumental to policy making with regards to control of malaria epidemic[6].

Also, following treatment of symptomatic infected persons, treatment failures versus re-infection can be elucidated more effectively using molecular methods to determine the genetic relatedness of the parasite before and after treatment[7].

Understanding the genetic diversity and structure of malaria parasite populations is the key for predicting the emergence and spread of phenotypes of interest, such as new antigenic or drug resistance variants[8, 9]. Sequencing is aimed to better understand the diversity of a gene under consideration as a vaccine candidate or because it harbors mutations linked to drug resistance[10,11].

As part of the integrated vector approach, molecular identification of species and sibling species of Anopheline mosquitoes is an important tool to appreciate divergence of vector siblings and early detection and monitoring of insecticide resistance in local vectors[11].

In molecular characterization of progress made during pre and post-elimination, measures such as the complexity of infection, genetic relatedness of parasites causing infection, sources and sinks of outbreaks as well as anti-malarial drug resistance genes can be monitored effectively using genetic toolkits such as genetic barcodes and sequencing among others[7, 11]. This would certainly prove beneficial in sustenance of elimination

The major limitation associated with the use of molecular techniques would be their application to large numbers of specimens principally because of the associated high cost implication and technical expertise required [4], which at present is limited in Nigeria. It is therefore most effective to maximally apply them from the pre-elimination phase where we have fewer numbers of infections, however, we must invest in preparation now. To overcome PCR limitations, a more field-friendly and cost-effective diagnostic tool, the loop-mediated isothermal amplification (LAMP) which has no major capital equipment requirement, simplified DNA extraction methods, was developed[12]. The technique facilitates running amplification reactions in difficult test environments, enabling use outside of a high-tech laboratory and requires minimal training in addition to requiring less time compared to PCR[13].

One of the key roles of a reference laboratory as detailed in the WHO elimination framework is coordinating the referral of samples from district laboratories and providing confirmatory testing and special testing services (e.g. molecular and serological tests, expert microscopy) [3].

Therefore, in the move towards elimination, there is need to build capacity across the span of the country for ease of access, reduced turnaround time and better control. Reference laboratories with the required facilities, expertise, supply chain, funding and political and administrative support must be

established and equipped adequately. There should be a network for collaboration and easy exchange/ sharing of information necessary for technical development in requisite areas so all centers in the country progress at a similar pace.

Most importantly, these should be decentralized because failure of elimination in some parts of the country could strongly undermine elimination achieved in other regions of the country.

The combination of conventional and molecular techniques is helpful to ascertain how malaria incidence is affected by parasites, vectors, and human host populations and therefore contribute significantly in the elimination phase of malaria in Nigeria[7,11].

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Profile of the Centre for Malaria Research and Phytomedicine

INTRODUCTION

The Centre for Malaria Research and Phytomedicine (CMRAP) was set up by the 7th Vice Chancellor of the University of Port Harcourt, Prof J.A.Ajienka, to achieve malaria control and elimination through research. Originally conceptualized as a Malaria Research Laboratory, sponsored by the World Health Organisation (WHO/TDR) and the Multilateral Initiative on Malaria in Africa (MIM) in 2001, the purpose of which was to carry out phyto-medicinal studies aimed at the identification and clinical evaluation of potential anti-malarial components from the Nigerian phytomedicine compendium. Following the re-designation of the Malaria Research Laboratory and approved by the Governing Council of the University of Port Harcourt in December, 2010, the Vice Chancellor, Prof. J.A. Ajienka, appointed Prof. O.O.Ebong as the Pioneer Director and Prof. C. A. Nwauche as Assistant Director for CMRAP in March, 2011. Prof. C.A. Nwauche succeeded Prof Ebong as Director in April 2015 and was himself succeeded Prof I.M. Siminialayi on August 1, 2016. Dr. Hamilton Opurum was appointed Assistant Director in 2015 and was replaced by Dr. Balafama Alex-Hart in 2018. The current Director Prof. Orikomaba Obunge was appointed to take over the headship of the Centre in April, 2019 with Dr. Ibinabo Oboro as the Assistant Director.

CMRAP is responsible for leading and coordinating research activities in the University of Port Harcourt aimed at finding effective and sustainable means of not only ending malaria in Nigeria in particular but related diseases as well.

The offices of CMRAP are located on the 2nd floor of the right wing of the School of Basic Studies Building at the University Park, University of Port Harcourt, Port Harcourt.

MISSION
To achieve quality research in malaria and related diseases towards better health for all.

VISION
To be a leading Centre of excellence in malaria research and related diseases as well as in medicinal plant research.

GOAL
To achieve malaria control and better health for all through basic and operational research.

To explore the indigenous plant sources of new medicinal compounds for the treatment of malaria and other related diseases.

Professiorial Chairs in the Centre

1. Sylvanus JS Cookey Professor of Malaria Studies, with Professor Iyeopu M. Siminialayi as the current Chair Occupant who succeeded Professor Omotayo, the first Chair Ooccupant. The research focus of the Chair includes Malaria Vector Control in the campaign for malaria elimination in Nigeria.

2. NDDC Progessorial Chair on Malaria Elimination and Phytomedicine Research, with Professor Chijioke Adonye Nwauche as the Pioneer Chair Occupant. The Chair focuses on research amongst the vulnerable groups as pregnant women, under-five children and Sickle Cell Anaemia patients; and Power Tube TENS research.

Centre Leadership

Director:	Prof. O. Obunge
Asst. Director:	Dr. I. L. Oboro
Pioneer Director:	Prof. O.O. Ebong
Past Directors:	Prof. C.A. Nwauche and Prof. I. Siminialayi

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