



## **Blood Cell Profiling of Malaria Patients Attending Gaya General Hospital, Kano State, Northern Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author MUA designed the study. Authors MUA and AAP performed the statistical analysis. Authors UAU and AAP wrote the protocol, author SMY wrote the first draft of the manuscript. Authors MUA and UAU managed the analyses of the study. Authors AAP, YSA and SI managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Changes in blood cell profile were common findings in malaria. In the rural community of Kano State, Nigeria, information on haematological changes in human malaria was scanty in spite of their role in the pathophysiology of malaria. This cross-sectional study was undertaken to determine blood cell profiles in malaria patients attending a rural hospital in malaria-endemic region.

**Methods:** Blood samples (3 ml each) were collected in EDTA-containers from 150 randomly selected outpatients attending Gaya General Hospital, screened for malaria using RDT kit (CareStart Malaria HRP 2, Access Bio Inc., USA) based on Histidine-rich protein 2 (PfHRP-2), and blood cell profiles determined using automated Sysmex haematologic analyser. Data on socio-demographics and medical history related to the study objectives, such as taking antimalarial regimen and/or haematinic, and direct involvement in blood transfusion, were obtained by questionnaire administration supplemented with oral interview.

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**Findings:** The study revealed a malaria prevalence of 67.33%, with highest in 11-20years (80.95%) and lowest (55.00%) in 1-10years age-groups; slightly higher in females (68.25%) than in males (66.67%) without significant difference ( $P<0.05$ ). For blood parameters, malaria positive patients have a significantly lower mean PCV of 32.2% as compared to 38.18% obtained for malaria negative patients ( $P<0.05$ ). The mean Hb was  $10.76\pm 2.27\text{g/dL}$  and  $12.65\pm 2.38\text{g/dL}$  ( $P<0.05$ ), while WBC revealed  $6.91\times 10^9/\text{L}$  and  $6.56\times 10^9/\text{L}$  in malaria positive and negative patients, respectively. Platelet counts recorded  $179.24\times 10^9/\text{L}$  and  $230.47\times 10^9/\text{L}$  ( $P<0.05$ ). Socio-demographic factors such as level of education, occupation and marital status did not significantly influence malaria prevalence.

**Interpretation:** Low PCV and Hb in malaria patients indicate mild anaemia due to malaria-related haemolysis. The occurrence of thrombocytopenia may be due to other underlying pathology as further studies with larger sample size are needed to ascertain the cause of low platelet counts in malaria patients in the study area.

**Keywords:** Malaria prevalence; blood parameters; anaemia; rural community; thrombocytopenia.

## 1. INTRODUCTION

Human malaria is a mosquito-borne infectious disease caused by parasitic protozoans belonging to the genus *Plasmodium* [1]. Malaria is currently endemic in the tropical zones with extensions into the sub-tropical regions of Asia, Africa, South and Central America. Nigeria suffers the world's greatest malaria burden, with approximately 51 million cases and 207,000 deaths reported annually (approximately 30% of the total malaria burden in Africa), while 97% of the total population (approximately 173 million) is at risk of infection [2]. Malaria causes symptoms that typically include fever, fatigue, vomiting, and headache. In severe cases it can cause yellow skin, seizures, coma, or death [3]. The disease is most commonly transmitted by an infected female *Anopheles* mosquito through a bite that introduces the parasites from the mosquito's saliva into a person's blood [1]. Four primary species of malaria parasites infect humans: *Plasmodium falciparum*, *P. ovale*, *P. malariae* and *P. vivax*. In addition, studies in Southeast Asia have shown that *P. knowlesi*, a malaria parasite that typically infects monkeys as the natural reservoir, can also infect humans, and in some cases, result in fatal disease [4]. Malaria due to *P. vivax*, *P. ovale* and *P. malariae* is less severe than that experienced by *P. falciparum* infections which is the most virulent of the human malaria parasites responsible for the bulk of the malaria-related morbidity and mortality. *P. falciparum* accounts for 91% of malaria cases worldwide of which the majority (with about 86%) occurs in the African region [5]. Consistent with the high rate of morbidity 90% of the *P. falciparum* attributable-malaria deaths also occur in sub-Saharan Africa [5]. Haematological changes are some of the most common

complications in malaria as the changes involve the major cell lines such as red blood cells, white blood cells and blood platelets [6]. Haematological abnormalities are considered a hallmark of malaria and many of these haematological values may lead to increased clinical suspicion for malaria, thus initiating a prompt institution of specific therapy even in the absence of a positive smear report for malaria. Prediction of the haematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications. These parameters are measurable indices of blood that serve as a marker for disease diagnosis [7]. In the rural area of Kano State, where the study was undertaken information on haematological changes occurring in malaria patients is scanty in spite of their roles in the pathophysiology of malaria, hence the need to determine the profile of blood cells in malaria patients attending a semi-urban health facility in Gaya Local Government Area, Kano State, North-western Nigeria, with a view to evaluating the extent of haematological changes associated with malaria in the study area.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out at Gaya General Hospital in Gaya Local Government Area of Kano State, Nigeria, which is located between latitude  $11.87^\circ\text{N}$  and longitude  $9.01^\circ\text{E}$ . It is surrounded by three local government areas, namely; Ajingi to the north, Albasu to the south and Wudil to the west. It has an area of  $613\text{ km}^2$  and a population of 201,016 at the 2006 population census [8].

## 2.2 Study Population

The study was designed to recruit >200 patients to constitute the study population. However, only 150 patients satisfied the aim of the study and made full compliance. Of this study population, 87 were males and 63 were females, and of different age-groups. The patients attended outpatient department of Gaya General Hospital and were prescribed blood malaria test by the health personnel on presenting with high fever and other symptoms which would suggest malaria for confirmation. The patients came from Gaya Town and its neighbourhood. Majority of the patients are of low socioeconomic background with simple trading and peasant farming as the major occupation for subsistence.

## 2.3 Inclusion and Exclusion Criteria

All patients who were presumptively diagnosed as having malaria and were prescribed blood malaria confirmatory test during the period of the study and gave their informed consent were included in the study. Patients who were not prescribed malaria diagnosis, were prescribed malaria diagnosis but were taking haematinic (blood tonic/iron supplement/folate) and antimalarial regimen or involved in blood transfusion in recent time (previous 10-14 days), either donated or received blood, were excluded from the study; female patients who were pregnant during the period of the study were also excluded.

## 2.4 Questionnaire Administration

A structured questionnaire designed for the purpose of the research was administered to the study subjects who had given a written consent. The questionnaire contained information relevant to the study objectives which included socio-demographics, history of blood transfusion, being on blood tonics and/or antimalarial treatment. Information contained in the questionnaire was translated into a local language, Hausa, in order to poster understanding and facilitate appropriate response. Where necessary the subjects were orally interviewed to supplement the administration of the questionnaire.

## 2.5 Collection of Blood Samples

Two hundred and seven (207) subjects were initially enrolled for the study. However, only 150

subjects fully complied with the study objectives. Using 5 ml disposable syringe, 2-3 ml blood sample was collected from each subject, placed into a well-labelled ethylene diamine tetra-acetic acid (EDTA) bottle, and analysed for malaria confirmatory test and blood cell profiling. Using the procedure by Cheesbrough [9] the venepuncture site was disinfected with 70% ethanol to cleanse the area of about 50 mm in diameter, prior to blood collection. A tourniquet was used to tie the arm of the individual so as to locate the suitable vein in the arm. The collected blood samples were then stored until use.

## 2.6 Determination of Malaria Parasites

Rapid Diagnostic Test (RDT) kit (CareStart Malaria HRP 2 (Pf), Access Bio, Inc., USA) was placed to test for the presence of *Plasmodium* antigen (PfHRP-2) in the blood samples. A drop of fresh blood sample was placed into a Sample (S) well and 2 drops of malaria assay buffer added into a Buffer (A) well on the kit, according to the manufacturer's instruction. Appearance of double lines (control and test lines) indicated the presence of *Plasmodium* antigen; a positive result, while appearance of a single line (control line) only, indicated the absence of *Plasmodium* antigen; a negative result.

## 2.7 Determination of Haematological Parameters

For the packed cell volume (PCV), capillary tubes were used to take the blood samples. Three-quarter of the capillary tube was filled with blood and sealed from the side of collection. The samples were then centrifuged at 2000rpm in a haematocrit machine for 5 minutes to separate the blood plasma and the blood cells. Haematocrit reader was used to read the percentage of the red blood cells. Other blood parameters such as white blood cell count (WBC), platelets count and haemoglobin concentrations were determined using an automated haematologic analyser (Sysmex KX-21N).

## 2.8 Data Analysis

The data collected were analysed using simple frequencies, means and percentages. Moreover, comparative analysis of the parameters was carried out using chi-square and student's t-test at 95% level of confidence. All data analyses were run by Microsoft Excel, 2007.

### 3. RESULTS

#### 3.1 Prevalence of Malaria in the Study Population

The result in Table 1 presents information on presumptive diagnosis of malaria generated from questionnaire which was translated in Hausa, a local language of the study subjects and supplemented with oral interview. Fever, headache and chills were the main complaints reported by the study subjects, with convulsion being the least, reported in younger age groups. In older age groups, headache, fever and body pain were the most frequent complaints. Prevalence of malaria recorded against socio-demographics was presented in Table 2. The study reveals a 67.33% malaria prevalence, although, no statistically significant difference was observed between the infection and age of the study subjects, as well as sex. However, infection prevalence was slightly higher in 11-20 age group (80.95%); the age group 1-10years had the least malaria prevalence of 55.00%. moreover, the prevalence was higher in female subjects (68.25%) than males with 66.67%. Moreover, there was no association between malaria prevalence and the suspected risk factors; occupation, education and marital status, at  $P < 0.05$ , although subjects without any form of education had the highest infection prevalence.

#### 3.2 Effect of Malaria on Haematological Parameters

The blood cell profile of the study subjects was presented in Tables 3 and 4, showing the mean packed cell volume (PCV), haemoglobin concentration (Hb), white blood cells (WBC) and blood platelets. The mean PCV values for malaria-positive individuals was  $33.67 \pm 6.77$  and  $30.37 \pm 6.54$  for males and females respectively; the values were lower than in malaria-negative individuals, males ( $39.72 \pm 7.24$ ) and female ( $35.95 \pm 6.36$ ). Table 4 shows the effect of malaria on packed cell volume. The prevalence of anaemia in malaria-infected patients is 81(84.38%). The result reveals a statistically significant difference at 5% level of significance ( $P < 0.05$ ). The mean Hb concentration for malaria-positive individuals also varied with sex, with males having 11.26 g/dL and females 10.1 g/dL. There was a significant difference in the haemoglobin concentration between malaria positive and negative cases at  $P < 0.05$  (Table 3). The prevalence of anaemia in malaria positive patients is 77(81.08%) The slight decrease in the mean PCV and Hb in malaria-positive individuals

indicated the development of mild anaemia. Moreover, the mean WBC Counts for malaria-positive individuals were  $6.87 \times 10^9/L$  and  $6.97 \times 10^9/L$  for males and females respectively; females having slightly higher value, with no statistically significant difference. The result in Table 4 shows the effect of malaria on white blood cells. The prevalence of low WBC was 33 (71.74%), normal WBC 54 (61.36%) and high WBC was 14 (87.5%), although, with no statistical significance ( $P < 0.05$ ) (Table 4). The mean platelet counts for malaria-positive individuals were  $190.98 \times 10^9/L$  and  $163.4 \times 10^9/L$  for males and females respectively (Table 3). However, the platelet counts recorded for malaria-negative individuals were  $228.66 \times 10^9/L$  and  $233.1 \times 10^9/L$  for males and females respectively, both within normal range. There was a marked reduction in platelet counts in malaria-positive females compared to the males. The platelet counts in the study subjects reveal 75 (74.26%) low counts (thrombocytopenia) with a statistical significance at  $P < 0.05$ , indicating the effect of malaria on blood platelets count (Table 4).

### 4. DISCUSSION

In this study we investigated the profile of blood cells in malaria patients in order to determine haematological changes associated with malaria in semi-urban population. The study area falls within malaria-endemic zone and a prevalence of 67.33% was recorded in the study population. This infection prevalence is high and is consistent with the previous studies from Kano State with high malaria prevalence of 62.5% in patients attending two hospitals in Kano Metropolis, and 51.7% in pregnant women attending antenatal clinic [2]. Dawaki et al. [2] reported high malaria prevalence among children in Kebbi (64.0%), Awka (59.6%), and Abuja (58.0%). In this study fever was the most frequent symptom of malaria reported by the study subjects. Greenwood et al. [10] reported that in endemic areas 30-50% of fever cases are due to malaria. Although very few number of individuals presented with convulsion, indicating a severe malaria, most of them falling within 1-10year age-group, such cases should be given special attention as majority tend to be fatal consequences in children. The World Health Organisation [11] and Aina et al. [12] reported that most deaths due to malaria occur among children living in Africa where the disease accounts for approximately 27% of all childhood deaths, and children under five years are the most vulnerable because of weak immunity.

**Table 1. Age- and gender- specific frequency of reported symptoms presumptive of malaria in the study population**

Category	Number examined	Malaria symptoms						
		Fever	Headache	Convulsion	Vomiting	Chills	Body pain	
Gender	Male	87(58.0)	68(78.16)	62(71.29)	2(2.30)	20(22.99)	58(66.67)	41(47.13)
	Female	63(42.0)	51(80.95)	47(74.60)	4(6.35)	31(49.21)	47(74.60)	31(49.20)
	Total	150(100)	119(79.3)	109(72.67)	6(4.00)	51(34.00)	95(63.33)	72(48.00)
Age-group*	1-10	20(13.33)	14(70.00)	14(70.00)	5(25.0)	7(35.00)	16(75.00)	11(55.00)
	11-20	21(14.0)	21(100.0)	12(57.14)	1(4.76)	9(42.86)	15(71.43)	13(61.90)
	21-30	30(20.0)	26(86.67)	21(70.0)	0(0.00)	13(43.33)	26(86.67)	19(63.33)
	31-40	34(22.67)	28(82.35)	26(76.47)	0(0.00)	13(38.24)	25(73.53)	11(32.35)
	41-50	17(11.33)	11(64.71)	11(64.71)	0(0.00)	3(17.65)	6(35.29)	9(52.84)
	51-60	16(10.67)	11(68.75)	13(81.25)	0(0.00)	4(25.00)	2(12.50)	3(18.75)
	≥61	12(8.00)	8(66.67)	12(100.0)	0(0.00)	2(16.67)	5(41.67)	6(50.00)
	Total	150(100)	119(79.3)	109(72.67)	6(4.00)	51(34.00)	95(63.33)	72(48.00)

\*Age in years; # Numbers in parenthesis are percentages

**Table 2. Socio-demographics and malaria prevalence in the study population**

Characteristics	Category	Number examined	Number infected	Percentage prevalence	$\chi^2$	P value
Gender	Male	87(58.0)	58	66.70	0.042	0.08
	Female	63(42.0)	43	68.25		
	Total	150(100)	101	67.33		
Age-group (years)	1-10	20	11	55.00	54.59	0.06
	11-20	21	17	80.95		
	21-30	30	18	60.00		
	31-40	34	25	73.53		
	41-50	17	13	76.47		
	51-60	16	10	62.50		
	≥61	12	7	58.33		
Mean age±SD	31.89±18.58 (Range: 1-79 years)					
Marital status	Single	82(54.67)	57	69.51	0.33	0.59
	Married	64(42.67)	41	64.06		
	Divorced	4(2.67)	3	75.00		
Occupation	Unemployed	39	29	74.36	1.72	0.21
	Apprentice	6	2	33.33		
	Civil servants	11	6	54.54		
	Artisans	9	6	66.66		
	Farmers	31	15	48.39		
	Traders	21	11	52.38		
	Students	36	27	75.00		
	Others	7	5	71.43		
	None	37	29	78.38		
Education level	<i>Almajiri</i> * school	19	13	68.42	1.95	0.20
	Primary school	61	43	70.50		
	Secondary school	25	13	52.00		
	College/University	8	3	37.50		

\**Almajiri*: usually a male pupil/student receiving Arabic non-formal education  
P <0.05 is considered statistically significant

**Table 3. Mean haematological parameters in malaria and non-malaria cases relative to sex**

Parameters*	Male		Female		t-test	P value
	Malaria +ve	Malaria -ve	Malaria +ve	Malaria -ve		
PCV (%)	33.67±6.77	39.72 ±7.24	30.37 ±6.54	35.95 ±6.36	0.859	0.240
Hb (g/dL)	11.26 ±2.26	13.23 ±2.39	10.1 ±2.14	11.81 ±2.14	0.989	0.213
WBC (×10 <sup>9</sup> /L)	6.87 ±4.02	6.76 ±3.68	6.97 ±4.7	6.26 ±2.29	0.557	0.338
Platelets (×10 <sup>9</sup> /L)	190.98 ±129.1	228.66 ±102.5	163.4 ±99.38	233.1 ±76.7	0.292	0.399

\*Normal range: PCV: 37-47% (Female), 42-50% (Male); Hb: 12.0-16.0g/dL (Female), 14.0-18.0 g/dL (Male); WBC: 4.0-11.0×10<sup>9</sup>/L; Platelets: 200-350×10<sup>9</sup>/L Source: ABIM<sup>10</sup>; Waugh and Grant<sup>11</sup>  
*P* <0.05 is considered statistically significant

**Table 4. Effect of malaria prevalence on blood cell parameters (n=101)**

Parameter*	Value	Condition	Number(%)	$\chi^2$	P value
PCV	Normal	-	20(19.8)	35.21	0.00
	Low	Anaemia	81(80.2)		
	High	Erythrocytosis	0(0.00)		
Hb	Normal	-	24(23.76)	22.17	0.00
	Low	Anaemia	77(76.24)		
	High	Erythrocytosis	0(0.00)		
WBCs	Normal	-	54(53.47)	4.79	0.09
	Low	Leucopenia	33(32.67)		
	High	Leucocytosis	14(13.86)		
Platelets	Normal	-	22(21.78)	34.58	0.00
	Low	Thrombocytopenia	75(74.26)		
	High	Thrombocytosis	4(3.96)		

\* PCV: packed cell volume; Hb: haemoglobin concentration; WBCs: white blood cells  
*P* <0.05 is considered statistically significant

This study further indicates lack of influence of age of the subjects on the prevalence of malaria, an observation corroborating the report of WHO [13] that most adults living in malaria endemic areas have partial immunity and are at risk of chronic or repeated infections. Similarly, there was no gender difference in malaria prevalence, suggesting an equal predisposition of male and female individuals to mosquito bite and susceptibility to Plasmodium infection. However, in another research reported by Dawaki et al. [2] a prevalence rate of 60.6% for falciparum malaria was recorded which differed significantly by age-group, but not by gender. Moreover, we found no association between malaria prevalence and educational level, marital status and occupation of the study subjects. These sociodemographic factors were similarly reported in several studies [14,15] not influencing malaria prevalence. Changes in blood cells profile in malaria infections are well recognized, but specific changes may vary with level of malaria, background haemoglobinopathies, nutritional status, demographic factors and malaria immunity [16]. Haematological changes are among the most common complications in malaria as the changes involve the major cell lines such as red blood cells, white blood cells, and platelets [6]. In this study significant changes were observed in packed cell volume, haemoglobin concentration and blood platelet counts. The packed cell volume showed that 84.38% of the malaria positive patients were mild anaemia (males:33.67±6.77;females:30.37±6.54), a finding which is concordant with earlier reports [17,18]. Moreover, haemoglobin concentration values showed that 81.08% of malaria-positive patients have a mild anaemia, with mean haemoglobin concentrations of 11.26±2.26 (male

and 10.1±2.14 (female), with statistical significance at *P*<0.05. These values showed a high occurrence of anaemia in malaria patients than in patients tested negative for malaria. This, in addition to other factors, can affect their immunity state and coincided with the findings of Hill et al. [19] who reported that haematological changes related to malaria are subject to variation depending on the level of disease endemicity, nutritional status, genetic factors, sociodemographic conditions, ethnicity and immunity. According to the World Health Organisation Scientific Group 1, the levels of haemoglobin below which anaemia is likely to occur for a population living at sea level are: 11 g/dL for children aged six months to six years, 12 g/dL for children aged between 6 and 14 years, 13 g/dL for adult males, 12 g/dL for non-pregnant adult females and 11g/dL for adult pregnant females [20]. The pathogenesis of anaemia in malaria is extremely complex, multifactorial and is thought to result from a combination of haemolysis of parasitized and innocent unparasitized red blood cells, accelerated removal of both parasitized and non-parasitized red blood cells, depressed and ineffective erythropoiesis due to tumour necrosis factor alpha, anaemia of chronic disease, and splenic phagocytosis or pooling [21,22,23]. Menendez et al. [24] reported that malaria-related anaemia is associated with many factors which involve increased destruction and reduced production of red blood cells. Malarial anaemia is usually normocytic and normochromic [22]. However, anaemia associated with malaria can also be microcytic and hypochromic due to the high frequencies of haemoglobinopathies and iron deficiency in endemic countries [22]. During malaria infection, there are soluble derivatives



released by the parasite that induce bone marrow dysfunction. These derivatives are therefore implicated in the pathogenesis of malarial anaemia [25]. At least for *P. vivax* infection, the decrease in haemoglobin concentrations was attributed to the activity of the parasites.

This work recorded an insignificant change in white blood cell counts in both malaria-positive and malaria-negative patients, irrespective of sex and age-group. This finding was in agreement with Maina et al. [6] who reported a normal count of white blood cells in malaria cases. Similarly, alterations in the white blood cell counts are less reported and have been associated with factors such as severity, *Plasmodium* species, concurrent infections, and treatment response [26]. On the contrary, leucopenia has been reported in several studies [17,27] as a common finding in malaria, and McKenzie et al. [27] ascribed the leucopenia to the localisation of leucocytes away from the peripheral circulation, splenic sequestration and other marginal pools rather than actual depletion or stasis. Descriptions of leucocytosis and leucopenia in malaria patients have been observed [28] but studies have not shown a specific leucocyte profile alteration in malaria. It has been found that WBC counts were low in malaria patients and that there was a trend towards lower WBC counts in patients infected with either *P. falciparum* or *P. vivax* [29].

The low platelet counts recorded in this study was in agreement with the findings of several studies [22,30] which described thrombocytopenia as a strong predictor of malaria. In this study, 74.26% of patients with low platelet counts are positive for malaria, in line with the reports of high prevalence of thrombocytopenia in malaria by other investigators; 53.0% and 58.97% [22,31]. Moreover, Jamison et al. [32] observed a reduced platelet count in acute malaria cases. Although there was a significant association between malaria and thrombocytopenia it is premature to ascribe this pathological change to malaria as there may be other underlying factors which have not been considered in this study. Pain et al. [33] suggested that the mechanism of thrombocytopenia in malaria is probably the consequence of several factors including immune factors and the destruction or sequestration of platelets. In acute malaria infection platelets are found to be hypersensitive and there is increase in plasma concentrations of

platelet-derived chemokines such as  $\beta$ -thromboglobulin ( $\beta$ -TG) and platelet factor 4 (PF-4/CXCL4), coupled with increased production of thromboxane A<sub>2</sub> and prostacyclin [34]. This study did not consider other factors that might affect blood indices such as incidence of chronic haematuria, haemorrhage, sickle cell status as well as nutritional status of the study population.

## 5. CONCLUSION

Changes in haematological parameters due to malaria are a common pathologic complication in subjects living in malaria-endemic communities. Although white blood cell counts were not significantly affected, red cell indices indicate a development of mild anaemia with high percentage of study population having thrombocytopenia as a result of low platelet counts. Malaria is therefore a serious infection having a negative impact on blood cells contributing to the pathophysiology of malaria.

## 6. RECOMMENDATIONS

Further research need to be conducted for details on differential blood cell counts, with particular reference to the role of malaria in the development of pathologic thrombocytopenia in the study population, taking into cognizance blood cell profiling to evaluate species-specific haematological changes due to malaria. Improved nutrition through consumption of fruits and vegetables by malaria patients should be encouraged through public enlightenment programme in order to replenish the low blood cell counts for restoration of health normalcy.

## CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the management of the Gaya General Hospital, after submission of letter of introduction, which contained the objectives of the study, from the Department of Biology, Kano University of Science and Technology, Wudil. The subjects involved in the study were first briefed on the objectives of the study and those who consented were registered for the study. The parents or guardians of non-adults gave their consent on behalf of their wards. The subjects were assured of confidentiality of the information they provided, which would remain anonymous, and would be used strictly for the purpose of the study.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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