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Assessment of Allelic Distribution and Multiplicity of Infection in the Three Northern Local Government Area, Taraba State, Nigeria

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Abstract

All the three allele types for MSP1- K1, MAD20 and RO33 and of MSP2- 3D7 and FC27 were identified in Zing and Lau, except in Jalingo where RO33 of the MSP1 family was not amplified. In Jalingo, mono-infection was observed in K1 with the highest frequency of 28 (25%) and MAD20 3 (2.7%). RO33 mono-infection was not seen. Mixed infection was seen in K1+MAD20, 13 (11.6%), K1+RO33, 12 (10.7%) and K1+MAD20+RO33, 15 (13.4%). In Zing, unlike Jalingo K1 has the lowest allele frequency 3 (2.6%) for mono-infections, MAD20 23 (19.8%) and RO33 26 (22.4%) with the highest frequency. Mixed infections include K1+MAD20 4 (3.4%), K1+RO33 1 (0.9%), MAD20+RO33 11 (9.5%) and K1+MAD20+RO33 17 (14.7%). In Lau, 10 (9.2%) alleles were found for K1, 20 (18.3%) for MAD20 and 4 (3.7%) RO33 mono-infections. Mixed infections include 32 (29.4%) K1+MAD20 with highest frequency, 2 (1.8%) k1+RO33, 4 (3.7%) MAD20+RO33 and 2 (1.8%) K1+MAD20+RO33. For the MSP2 family, 24 (21.4%) 3D7, 11 (9.8%) FC27 and 33 (29.5%) mixed infection of 3D7+FC27 in Jalingo. In Zing, 28

(24.1%) 3D7, 15 (12.9%) FC27 and 42 (36.2%) mixed infection of 3D7+FC27. Also, in Lau, 30 (27.5%) 3D7, 12 (11.0%) FC27 and 38 (34.9%) mixed infection of 3D7+FC27. The allelic diversity of *P. falciparum* MSP1 and MSP2 is mostly due to meiotic recombination events involving genetically distinct parasite clones that infect the same mosquito vector, and hence, human host. Therefore, the proportion of mixed infections and the number of clones per individual is one of the prerequisites to generate new genotypes and to increase the diversity of the parasitic population. Multiple clonal infections with different genotypes of *P. falciparum* were identified among the *P. falciparum* isolates in the study locations with a moderately high Multiplicity of Infection (MOI). Haematological and biochemical tools are recommended as an adjunct tool in the management of malaria infection especially in underdeveloped countries like Nigeria.

Keywords: Allelic Diversity, Multiplicity, Infection, Malaria, Parasites, Northern Taraba State, Nigeria

INTRODUCTION

Several species of intracellular protozoa of the genus *Plasmodium* cause malaria in humans. Among them are *Plasmodium ovale*, *Plasmodium vivax, Plasmodium falciparum*, and *Plasmodium malariae*. (Joshi *et al*., 2008; WHO, 2019) and more recently, *P. knowlesi* (World Malaria Report, 2018). *P. falciparum* and *P. vivax* cause the most serious forms of the disease (World Health Organization, 2010; Elhassan *et al*., 2009; Idonije *et al*., 2011; WHO, 2022). Humans contract diseases from the bite of female Anopheles mosquitoes, which spread the disease around the world. These disease cuasing organisms have a complicated life history that requires a vertebrate host for the asexual cycle and female *Anopheles* mosquitoes for completion of the sexual cycle. Malaria poses a threat to public health with 80 to 90% of morbidity and mortality occurring in Africa, afflicting both young and old (Federal Ministry of Health, 2005; Adeneye *et al*., 2007; Kamau *et al*., 2015). In addition, reports showed that malaria could be transmitted by transfusion of infected blood (Ali & Kadaru, 2005; WHO, 2010), sharing needles (Tracy and Webster, 2001) and congenital transmission (Ezechukwu *et al*., 2004).

In 2020, there were 241 million cases of malaria, compared to 227 million cases in 2019, according to the most recent World Malaria Report. An estimated 627 000 people

died from malaria in 2020, an increase of 69 000 over the year before. A recent modification in WHO's technique for measuring malaria mortality accounts for one third of these deaths (22,000), while about two thirds of these deaths (47 000) were caused by interruptions during the COVID-19 pandemic (irrespective of COVID-19 disruptions). 32 sub-Saharan African nations, which account for almost 93% of all malaria fatalities worldwide, were subjected to the new cause-of-death methodology (World Malaria Report, 2021). Applying the methodology revealed that malaria has taken a considerably higher toll on African children every year since 2000 than previously thought (WHO, 2022). The WHO African Region continues to carry a disproportionately high share of the global malaria burden. In 2020, the Africa region was home to 95% of all malaria cases and 96% of deaths. About 80% of all malaria deaths in the region were in children under the age of five. Just over half of all malaria deaths globally occurred in four African countries: Mozambique (3.8%), the Democratic Republic of the Congo (13.2%), Nigeria (31.9%), and the United Republic of Tanzania (4.1%) (WH0, 2022).

Approximately 24% of Nigerians reside in low transmission zones, while 76% of the country's population lives in high transmission areas. Malaria is contagious across the country. The transmission season can last all year round in the south and is about 3 months or less in the northern part of the country.

With its elevated morbidity and mortality, malaria continues to be one of the most common parasite diseases in the tropics. In sub-Saharan Africa, *Plasmodium falciparum* is the most common malarial parasite; outside of Africa, *Plasmodium vivax* is the most common parasite and accounts for over 30% of cases in South-East Asia (World Malaria Report, 2018). According to Khuraiya *et al.* (2016), there is typically a significant morbidity and mortality rate in instances of severe malaria as well as those with changed haematological and biochemical parameters, which typically result in frequent malaria sequelae. However, infection with *P. falciparum* represented more than 90% of the global malaria mortality and thus it is still a relevant global threat to public health (Snow, 2015). Furthermore, an estimated of 781,000 deaths and 225 million cases of *falciparum* malaria were recorded annually (WHO, 2010). Moreover, *P. falciparum* infection is frequently correlated to cerebral malaria and mortality that promote neurological sequelaes (Schiess *et al.*, 2020). Although clinical presentations are common in malaria diagnosis particularly in poor countries, however, those symptoms of fever are reasonable for malaria diagnosis due to lack of specificity as it is common with other illnesses making clinical diagnosis more confront

(Erhart *et al.*, 2004). Furthermore, this results in an overuse of antimalarial medications, decreasing their specificity and lowering the standard of treatment provided to patients (Reyburn *et al.*, 2007). While, microscopic diagnosis continues to be the gold standard, most economical technique for malaria diagnosis, but its sensitivity and specificity for parasite detection are still weak particularly in endemic areas in Africa, as this technique needs a qualified technical expertise, consumes more time and deficient for a satisfactory health care issues (Reyburn *et al.*, 2007; WHO, 2009; Maina *et al.*, 2010).

Haematological derangements are some of the most common complications in malaria and they play a major role in malaria pathology. The extent of these alterations varies with the level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity. Severe anaemia is the predominant severe malaria syndrome peaking in the first two years of life and is attributed to *P. falciparum* (Waitumbi *et al.*, 2000 A successful course of treatment for malaria patients, particularly non-immune youngsters, depends on a timely and precise diagnosis (Miana *et al.*, 2010).

Malaria causes a variety of haematological alterations, which differ depending on the Plasmodium species, age, gender, and a number of other variables. In individuals with malaria, anemia is a significant pathology that exacerbates the disease situation in the infected hosts when it coexists with other related haematological changes (Abdulkareem *et al.*, 2017). Anaemia, thrombocytopenia, leucocytosis, leukopenia, moderate atypical lymphocytosis, monocytosis, eosinophilia, and neutrophilia are among the haematological abnormalities that have been linked to malaria (Das *et al.*, 2017; D'souza, 2018). According to Kurtzhals *et al.* (1998), there was a positive correlation between the malaria patients' levels of inflammatory biomarkers and eosinophilia, suggesting that inflammatory processes or T cell activation may be involved in the generation of eosinophils during acute sickness. It has been shown that thrombocytopenia is a frequent finding in both falciparum and vivax malaria. Additionally, it has been noted that the degree of illness is closely correlated with leukopenia and leukocytosis, lymphopenia, monocytosis, and neutrophilia (Tobó´n-Castaņo *et al.*, 2015). Since severe falciparum malaria reduces platelet survival, the degree of thrombocytopenia is thought to be significant to the disease's prognosis (D'souza, 2018).

P. falciparum invades red blood cells (RBCs) to form intra-erythrocytic trophozoite stages, which devour almost 70% of the hemoglobin in red blood cells to produce a crystalline form of hemomagglutinin (Tilley *et al.*, 2011). Thus, hemoglobin (Hgb) levels in individuals with *P. falciparum* infection decrease, leading to anemia, particularly in severe cases (Sakzabre *et al.*, 2020). As a result, malaria infections disrupt the physiology of the hematopoietic system, which in turn modifies hematological parameters that could be linked to malaria immunity, hemoglobinopathy, nutritional condition, and demographic factors (Awoke & Arota, 2019). While thrombocytopenia was more significant and frequent in *P. falciparum* malaria patients from Pakistan, Khan *et al.* (2018) found that there were global hematological abnormalities during malaria infections. Additionally, (Awoke and Arota, 2019) found that malaria patients had considerably lower mean values of Hgb, Hct, platelets, WBCs, RBCs, and lymphocytes than did non-infected individuals. Due to ideal breeding conditions, such as high humidity, high temperatures, high rainfall, and lots of stagnant water, malaria primarily affects tropical locations. This is because these variables facilitate the life cycle of mosquitoes, which transmits the diseases (WHO, 2018).

Physicochemical properties of the blood are constant but may undergo slight variations under normal physiological conditions. However, the relative constancy in the internal environment of the blood system exhibits wide and profound perturbation and distortions under clinically defined pathophysiologic states. Among these ailments include cancer, genetic flaws, starvation, parasite infestations, and so forth. Studies have revealed that haematological and biochemical alterations occur in malaria infected blood and there are common complications associated with this disease. Alterations in physicochemical parameters of *P. falciparum* infested blood may vary with level of malaria endemicity, presence of haemoglobinopathies, nutritional status, demographic factors and level of malaria immunity (Erhart *et al.*, 2004). Consequently, a practitioner can develop a dependable diagnosis and effective treatment interventions when they are aware of changes in blood parameters associated with malaria infection.

High bilirubin, elevated liver enzymes, and elevated creatinine are examples of biochemical abnormalities seen in malaria patients, all of which exacerbate the disease already present. According to Al-Salahy *et al.* (2016), there is a correlation between acute malaria infection and elevated serum levels of bilirubin and liver enzymes, suggesting that the infection is linked to acute liver injury. In patients with malaria, renal problems such as elevated blood urea and impaired creatinine clearance are directly linked to high levels of

parasitaemia (Sharma *et al.*, 2012). These aberrant blood and metabolic profiles are a major factor in the high morbidity and death rates, as well as in the development of deadly consequences.

During the hepatic stage of the malaria parasite's life cycle, when malaria sporozoites transform into merozoites, the liver is a crucial organ. One frequent side effect that typically follows a malaria infection is liver damage. Studies have shown that persons with malaria may experience a sudden elevation in liver enzyme levels, which may be a sign of liver malfunction (Onyesom and Onyemakonor, 2011). This could be as a result of the invasion of liver cells by the sporozoite during malaria parasite life cycle. The changes caused in the hepatic cell by sporozoite can lead to the leakage of parenchymal and membranous enzymes of the liver into the circulatory system, which can be responsible for the increase in liver enzymes (Chidoka *et al.*, 2013). Liver is one of the important organs of the body. It plays a vital role for the proper function of the body. Liver enzyme tests are broadly defined as tests useful in the evaluation and treatment of patients with hepatic dysfunction. They also help to monitor therapy and assess prognosis. In clinical diagnosis, plasma activities of ASAT, ALAT and ALAP are useful diagnostic markers of liver diseases. As a result of high level of complications and death of children due to malaria infection, there is need to evaluate the extent of hepatic dysfunctions in malaria cases so that there will be proper management of malaria infection and its associated complications (Ogbadoyi and Tsado, 2009).

METHODS

Study Area

Taraba state is the second largest state in terms of landmass in Nigeria. It is located in the Southern part of northeastern Nigeria along the eastern borderland between Nigeria and Cameroon. The state lies roughly between latitude 6° 25'N and 9° 30'N and between longitude 9°30'E and 11° 45'E. It is bordered on the west by Nasarawa and Plateau States, to the north by Bauchi and Gombe States and by Adamawa State to the northeast. It also shares its southwestern boundary with Benue State. Taraba State is bounded on the south and southeast by the Republic of Cameroon (an international boundary). The state covers a land area of about 60,291 km2 with a population of about 2,300,736 people according to the 2006 census. Taraba state is made up of 16 Local Government Areas (LGAs) and 3

Senatorial districts (northern Taraba, southern Taraba, and Taraba central). Northern Taraba consists of six LGAs, Ardo Kola, KarimLamido, Lau, Jalingo, Yorro and Zing.

Study Sites

The study was conducted in three centres of three Local Government Areas selected randomly in Taraba State which include Federal Medical Centre in Jalingo, General Hospital in Lau and General Hospital, Zing. The residents of the Local Governments are predominantly Mumuye, Yandang, with few minor tribes viz Jenjo, Fulani and Hausa among others and they are mostly farmers. The climate in the area can be described as tsub-humid type with two distinct (wet and dry seasons). It has an average rainfall of 7 months annually with total range between 1,200mm and 2000mm in the months of April and October. The temperature is relatively high throughout the year averaging 28-32^{o}C with occasional peak at 44.0 ^{o}C between March and April.

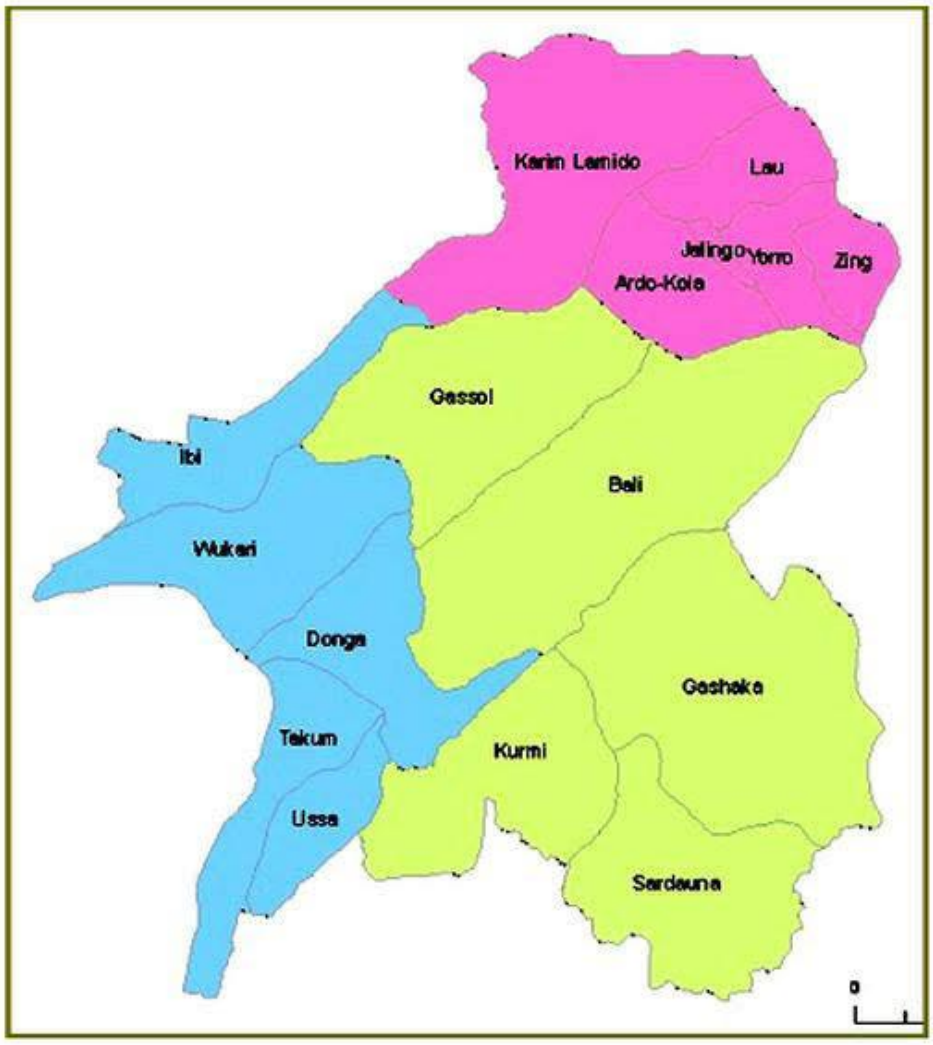


Figure 1: Map of Taraba State showing the study area

Study population and Selection of subjects

A total of 1,500 patients who were diagnosed for the presence of *Plasmodium species*, by the routine laboratory methods, that is, giemsa stained blood film examination, as per WHO protocol (WHO, 2018) and Immunochromatography was included in this study. A total of 500 participants was selected randomly from each centre in the three Local Government Areas chosen for the study.

Inclusion criteria

Only subjects who consent to the study were included. Only individuals with *P. falciparum* infection were included as test subjects

Exclusion criteria

Subjects who refuse to give consent were excluded. Subjects who are infected with or who have co-infection with other types of *Plasmodium* species were excluded. Subjects with underlying medical condition(s) inimical to biochemical or haematological indices including liver and kidney problems were excluded as well. Children below the age of 1 year were excluded as well.

Research Design

This is a prospective cross-sectional study involving subjects with *Plasmodium falciparum* (*P. falciparmn*) malaria infection serving as tests while *P. falciparum* negative subjects serving as controls.

Data Collection

Sample collected was given a coded number without the patient's name and labelled appropriately taking note of the centre of collection, Local Government Area, gender, age group, marital status, educational status etc. Data were collected for a period of 12 months concurrently in the three study sites.

Sample Handling and Disposals

All the samples collected for this research work was handled discretely by professional Laboratory Technicians or scientist who determine the infection status of participants. After testing, the samples were incinerated with the aid of Kerosene. The Data collected was kept confidential and only use for research purpose only. The test result of each individual was made known to them following request.

Sample Size Estimation

The sample size was determined from a z-test formula for the calculation of sample size.

Sample size (n)

$$\frac{(Z_{1-a})^2 \, x \, P(1-P)}{d^2}$$ ----------Equation 1

Where n = minimum sample size,

Z_{1-a} value of standard normal deviation which is at 95% confidence level has been found to be 1.96.

P=best estimate of the population prevalence obtained from the literature review.

d=proportion of sample error in a given population.

At prevalence rate of *Plasmodium* malaria of 19.2.0% (Yakubu *et al.*, 2019), using 5% precision

at 95% confidence level, the minimum sample size n for this study is calculated as follows:

Where Z=1.96,

P=19.2%, 0.192 d= 5%, 0.05

Therefore, n= (1.96) x 0.192 x (1-0.192)

0.05^2

n=238

The minimum sample size is 238

Added attrition was = 110% of 238

Total sample size=500 x 3 =1500

Questionnaire

A structured questionnaire was administered for all consenting participants to capture their demographic characteristics which included sex, age, marital status, educational level, whether or not they are on any form of condition they are managing.

Validation of Questionnaire

Content validity was carried out by experts in the subject matter by reviewing the questionnaire to ensure that it covers all relevant topics comprehensively. The

questionnaire was also compared with existing validated questionnaire and literature to ensure it covers all aspects. A pilot study was conducted with a small sample of the target population to check for clarity and relevance.

Reliability Test

Cronbach's Alpha test was used to measure the internal consistency of the questionnaire. The reliability was retested by administering the questionnaire to the same group of people at two different points in time and the correlation between the two sets was calculated.

Informed Consent

Written informed consent for participation in the study was sought from the patients in accordance with standards for human experimentation and the Helsinki Declaration.

Ethical Approval

The Ethical approval was sought from the Taraba State Ministry of Health Ethical Committee and presented to the health facilities where the blood samples were collected in compliance with the Declaration on the Right of the Patient (Foster *et al.*, 2001). Before enrolment for the study, the patients/subjects involved signed an informed consent form. Guardian/Parent sign the consent form on behalf of participants below the age of 18 years old.

Collection and preparation of blood specimen

All blood samples were collected by a phlebotomist or Laboratory Technician and screened by a Laboratory Scientist or Technician. With sterile aseptic precaution, about 5mL of venous blood was collected from the cubital vein of adult and/or by pricking the hand or leg in children. Three milliliters (3mL) of the blood samples were transferred into plain bottles to allow for coagulation, whereas the remaining 2mL was transferred into ethylenediaminetetraacetic acid (EDTA) bottles for malaria parasite tests and haematological studies. The coagulated blood samples were centrifuge at 3000 rpm for 10 minutes, the serum transferred into Bijou bottle and stored frozen until required for biochemical analyses (Onyesom *et al.*, 2010). The researcher in Collaborations with the laboratory Technicians or Scientist were responsible for safety issues during sample collection. Participants who reacted negatively or whose skins were damaged during sample collections were referred to a doctor in the facility and the cost implications was handled by the researcher. The researcher did not provide medications for malaria positive participants

but were referred to a physician for proper treatment. The participants were made aware and given a typed or written test result regardless whether positive or negative.

Preparation of thick and thin blood films

Thick and thin blood films were prepared on the same slide for each subject and stained using 10% Giemsa stain as described by Cheesebrough (2006). Using a completely clean grease free microscope slide a small drop of blood was added to the centre of the slide and a large drop about 15mm to the one end of the slide. Using a smooth edge slide spreader, the thin blood film was spread immediately without delay, the large drop was spread to make a thick film to cover an area of about 15 x 15mm. the slide was allowed to air dry. The thin film was fixed with absolute methanol for two minutes without touching the thick films.

Giemsa Staining Technique

The giemsa stain was diluted to 10% solution (90ml buffered distilled water pH 7.0 to 10ml Giemsa stain in a measuring cylinder and mixed). The slides were placed on a staining rack. The diluted stain was flooded on the slides and allowed to stain for 45-60 minutes. The slides were washed using buffered distilled water pH 7.0. The back of the slides were wiped off with cotton wool and placed in a clean rack. A drop of oil immersion was applied to an area of the film. An area that was well stained and not too thick was selected and examined for malaria parasites and malaria pigments and was confirmed by examining the thin blood films (WHO, 2020).

Parasite Density Test

Measurement of parasite density of peripheral blood smear was done via Giemsa stained techniques. The films were examined microscopically using x100 objective under oil immersion (Cheesbrough, 2006) as reported by Sumbele *et al.* (2010). Level of parasitaemia was in microliter (µl) of blood thick film preparation (Erhart *et al.*, 2004). According to WHO (2005), level of parasitaemia were graded as low+ (1 to 999/µl), moderate++ (1000 to 9999/µl) and severe++++ (>200,000/µl).

Molecular Genotyping of Parasite

All molecular analysis were performed at the Malaria Genomic Research & Training Center (MGRTC) of the Department of Biochemistry and Nutrition of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria.

DNA extraction

Blood sample from patients with uncomplicated malaria was spotted on Whatman 3mm filter paper to make Dried Blood Spot (DBS). The DBS was punctured into a 1.5ml eppendoff tube. Parasite DNA was extracted from the punctured DBS using the phenol/chloroform method (Sambrook *et al.*, 1989). Briefly, the punctured DBS were treated using proteinase K. Then, the phenol extraction/ethanol precipitation method was used to obtain the parasite DNA. This DNA was resuspended in 50 μL of buffer, therefore, 1 μL of the DNA template corresponded to 3 μL of whole blood (Cuhna *et al.*, 2009). The extracted DNA was stored in -20°C until it was used.

DNA amplification

To genotype the parasite, the merozoite surface proteins 1, *msp1* and *msp2* allelic families were targeted for amplification in a nested PCR technique according to Snounou and Beck (1998). Three loci, i.e K1, MAD20 and RO33 were independently amplified from the *msp1* allele while two loci, i.e 3D7 and FC27 were amplified from *msp2*. The *msp1* gene was amplified in a primary PCR reaction, while the K1, MAD20 and RO33 were amplified in a secondary PCR step. Likewise, the *msp2* allele was amplified in a primary PCR step, while the 3D7 and FC27 were amplified in a secondary PCR reaction. The primer sequences are shown in the table below.

Statistical analysis

The data obtained was analysed using SPSS V28. Chi-square was used to compare the prevalence of malaria infection between age, sex, occupation and educational status of the subjects. The Odd Ratio (OR) was determined using logistic regression to find the relation between the malaria infection and the haematological parameters. The general linear model was used for differentiation of haematological parameters of patients examined likewise biochemical parameters. T-test was also used to determine relationship between haematological and biochemical parameters of malaria parasitized and non-parasitized subjects. Statistical significance was set at 0.05.

RESULTS

Allelic distribution and Multiplicity of Infection (MOI) of the MSP1 and MSP2 families from Study sites

Allele typing analysis displayed the high polymorphic nature of *P. falciparum* in the different study sites with respect to MSP1 and MSP2. All the three allele types for MSP1- K1, MAD20 and RO33 and of MSP2- 3D7 and FC27 were identified in Zing and Lau, except in Jalingo where RO33 of the MSP1 family was not amplified. In Jalingo, mono-infection was observed in K1 with the highest frequency of 28 (25%) and MAD20 3 (2.7%). RO33 mono-infection was not seen. Mixed infection was seen in K1+MAD20, 13 (11.6%), K1+RO33, 12 (10.7%) and K1+MAD20+RO33, 15 (13.4%). In Zing, unlike Jalingo K1 has the lowest allele frequency 3 (2.6%) for mono-infections, MAD20 23 (19.8%) and RO33 26 (22.4%) with the highest frequency. Mixed infections include K1+MAD20 4 (3.4%), K1+RO33 1 (0.9%), MAD20+RO33 11 (9.5%) and K1+MAD20+RO33 17 (14.7%). In Lau, 10 (9.2%) alleles were found for K1, 20 (18.3%) for MAD20 and 4 (3.7%) RO33 mono-infections. Mixed infections include 32 (29.4%) K1+MAD20 with highest frequency, 2 (1.8%) k1+RO33, 4 (3.7%) MAD20+RO33 and 2 (1.8%) K1+MAD20+RO33. For the MSP2 family, 24 (21.4%) 3D7, 11 (9.8%) FC27 and 33 (29.5%) mixed infection of 3D7+FC27 in Jalingo. In Zing, 28 (24.1%) 3D7, 15 (12.9%) FC27 and 42 (36.2%) mixed infection of 3D7+FC27. Also, in Lau, 30 (27.5%) 3D7, 12 (11.0%) FC27 and 38 (34.9%) mixed infection of 3D7+FC27.

Table 1: Allelic distribution and Multiplicity of Infection (MOI) of the MSP1 and MSP2 families from Jalingo

FAMILY	HAPLOTYPE	FREQUENCY	MOI
	K1	28	
	MAD20	3	
	RO33	0	
	K1+MAD20	13	
	K1+RO33	12	
	MAD20+RO33	0	
	K1+MAD20+RO33	15	1.77
MSP1	TOTAL	71	
	3D7	24	
	FC27	11	
	3D7+FC27	33	1.49
MSP2	TOTAL	68	

Table 2: Allelic distribution and Multiplicity of Infection (MOI) of the MSP1 and MSP2 families from Zing

FAMILY	HAPLOTYPE	FREQUENCY	MOI
	K1	3	
	MAD20	23	
	RO33	26	
	K1+MAD20	4	
	K1+RO33	1	
	MAD20+RO33	11	
	K1+MAD20+RO33	17	1.59
MSP1	TOTAL	85	
	3D7	28	
	FC27	15	
	3D7+FC27	42	1.49
MSP2	TOTAL	85	

Table 3: Allelic distribution and Multiplicity of Infection (MOI) of the MSP1 and MSP2 families from Lau

FAMILY	HAPLOTYPE	FREQUENCY	MOI
	K1	10	
	MAD20	20	
	RO33	4	
	K1+MAD20	32	
	K1+RO33	2	
	MAD20+RO33	4	
	K1+MAD20+RO33	2	1.57
MSP1	TOTAL	74	
	3D7	30	
	FC27	12	
	3D7+FC27	38	1.48
MSP2	TOTAL	80	

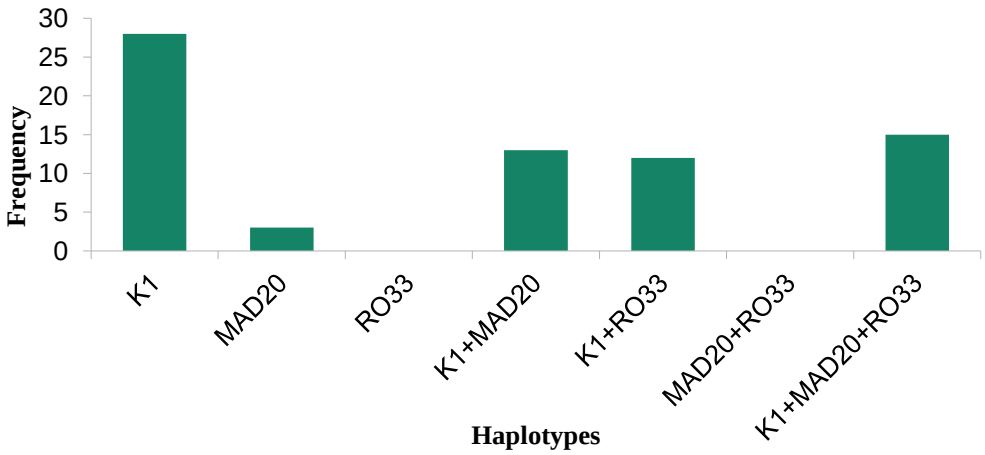


Figure 2: Allelic distribution of MSP1 famiy in Jalingo

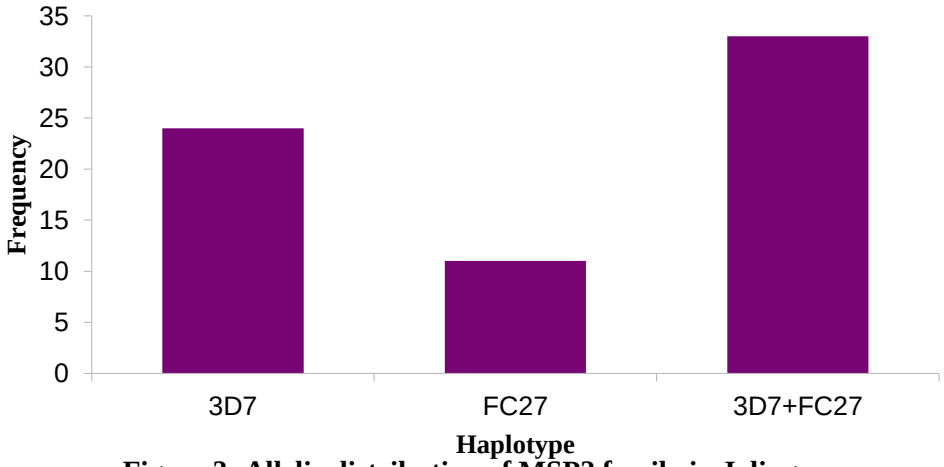


Figure 3: Allelic distribution of MSP2 family in Jalingo

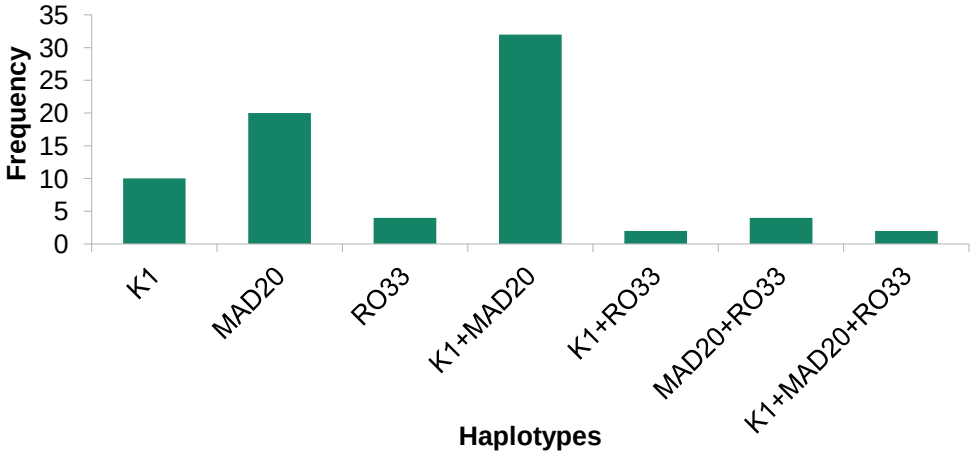


Figure 4: Allelic distribution of MSP1 family in Lau

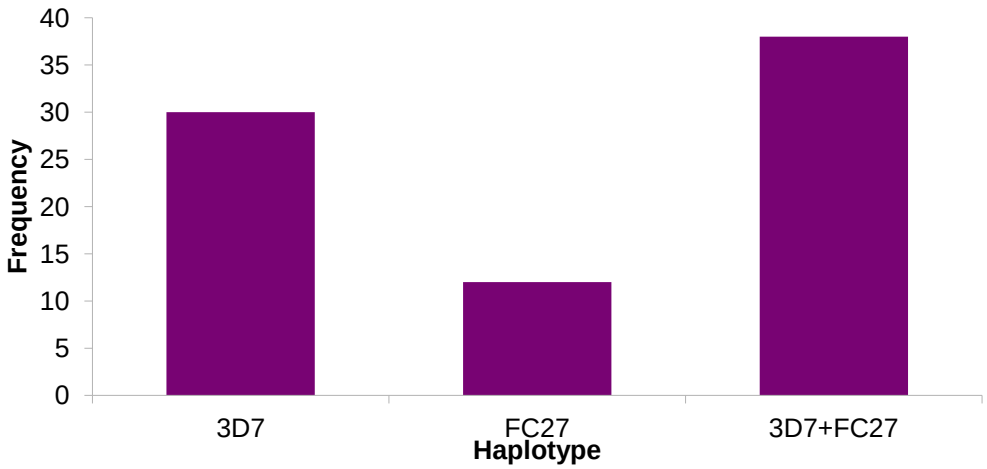
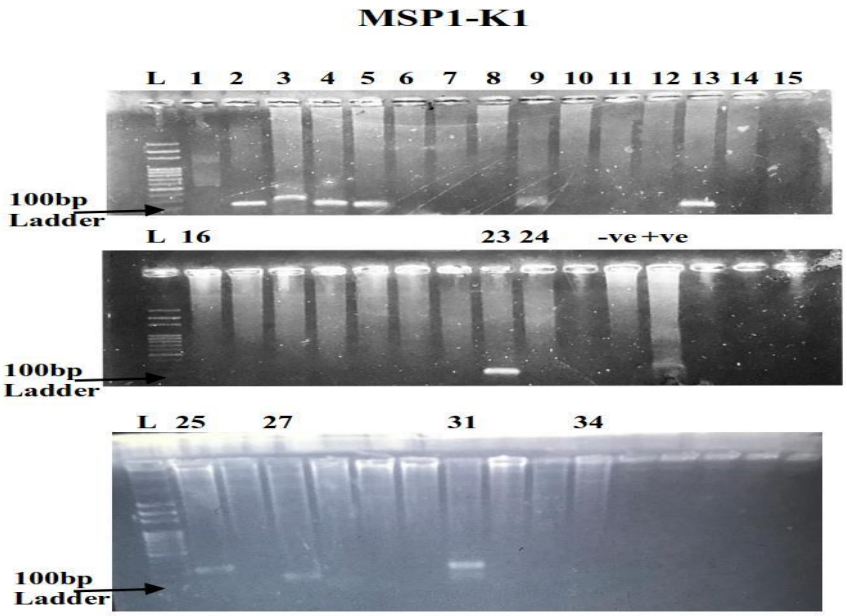


Figure 5: Allelic disribution of MSP2 family in Lau



1-12 is A1-A12; 13-24 is B1-B12;
25-34 is C1-C10

Plate I: DNA bands of *Plasmodium falciparum* Msp1-K1 on gel

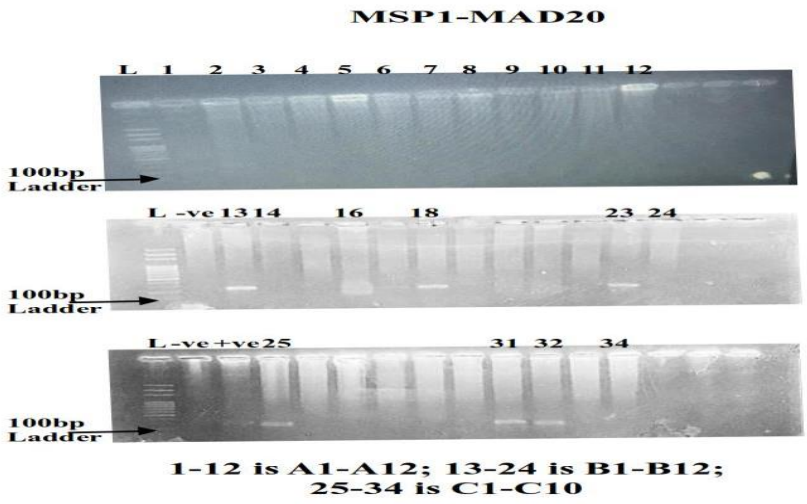


Plate II: DNA bands of *Plasmodium falciparum* Msp1-MAD20 on gel

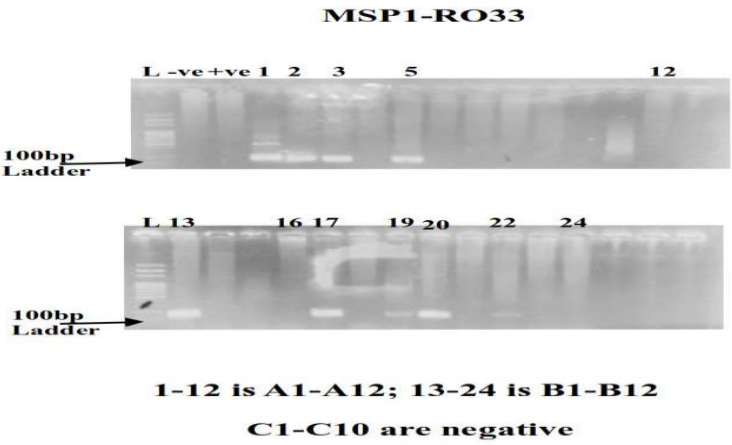


Plate III: DNA bands of *Plasmodium falciparum* Msp1-Ro33 on gel

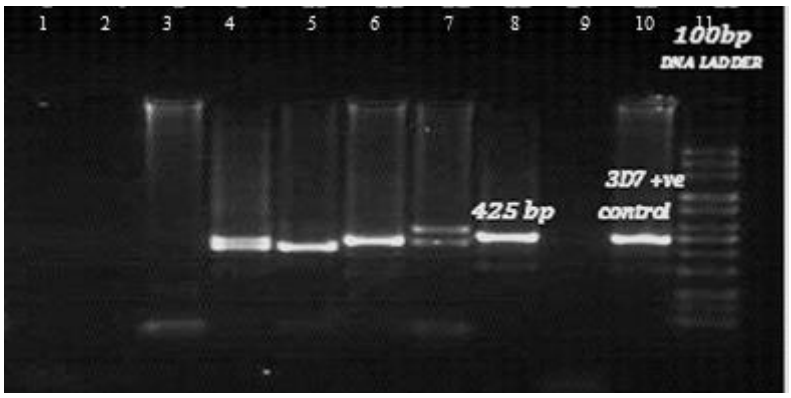


Plate 1V: Agarose gel electrophoregram of MSP2/ FC27 family resolved on 1.2% gel

Lane 1, 2, 3= Negative samples;

Lanes 4,5, 6,8 ==infection with single FC27 clone of *P. falciparum* (425bp, 450 & 500bp);

Lane 7 == infection with 2 FC27 clones of *P. falciparum*

Lane 9 == No template control;

Lane 10= *P. falciparum* 3D7 positive control, 500bp (FC27clone).

Lane 11 == 100bp to 3kb DNA Ladder

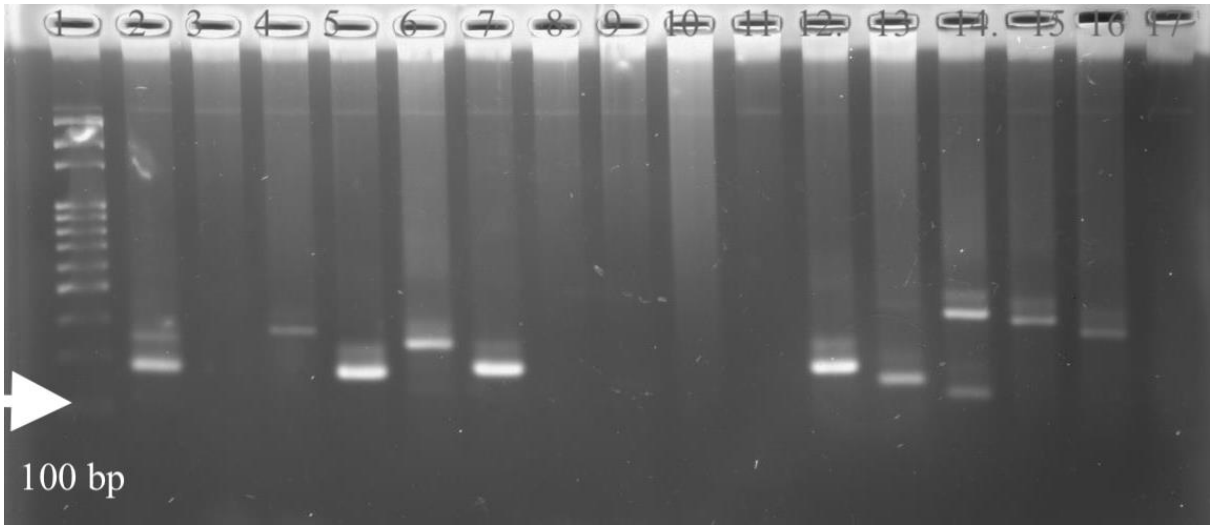


Plate V**: Agarose gel electrophoregram of MSP2 / 3D7 family resolved on 1.2% gel**

Lane 2 & 14= two infections of 3D7 clone (325bp, 400bp)

Lanes 4,5,6,12, 13,14 & 15 == infection with 1(one) 3D7 clone (275bp &325bp);

Lane 3 ,8-11& 17 == negative for 3D7 clone,

Lane 16 == *P. falciparum* 3D7 positive control (375 bp 3D7 clone);

Lane 1==100bp DNA Ladder. Arrow= Direction of run

DISCUSSION

Genetic diversity of *Plasmodium falciparum* associated with patients attending health facilities in Northern Taraba State in relation to Merozoite Surface Protein (MSP) Allelic Genotyping

Allele typing analysis displayed the high polymorphic nature of *P. falciparum* in the different study sites with respect to MSP1 and MSP2. PCR amplification of the 18s-rRNA gene of

the samples showed that 75.9% samples were positive from samples collected from Jalingo, 73.3% samples were also positive from Zing and 75.2% samples from Lau. A total of 74.8% samples were positive from the three sites all together. MSP1 amplification was successful for 83.5% while 80.2% samples were positive for the MSP2 allelic families of samples positive from Jalingo. From Lau, 90.2% of samples positive for MSP1and 97.6% samples were positive for MSP2. Meanwhile, all the samples positive for 18s-rRNA from Zing were all 100% successfully amplified for MSP1 and MSP2 which is in line with the report of Deitsch and Chitnis (2012) and Beeson *et al.* (2016).

For the samples from Jalingo, K1 has the high number of alleles for the MSP1 allelic families, with majority of the alleles 41.2% existing as mono-infection, which is consistent with infections reported in South-Western Nigeria **by** Olasehinde *et al.* (2012**).** The rest of the infections exist as mixed infection with MAD20 (19.1%), with RO33 (17.6%) and as K1+MAD20+RO33 (22.1%). MAD20 mono-infection only exist in only 4.2% of the infections. RO33 mono-infection was not found among the infections from this location.

Unlike what was noticed for the MSP1 genotyping of Jalingo samples where K1 has the highest frequency, samples from Zing had the highest frequency of RO33 mono-infection (30.6%) which corroborates to the study of Funwei *et al.* (2018). Following RO33 is MAD20 (27.1%) and K1 has the frequency of mono-infections (3.5%). The remaining alleles exist as mixed infection of K1+MAD20 (4.7%), K1+RO33 (1.2%), MAD20+RO33 (12.9%) and as K1+MAD20+RO33 (20%).

Different from what was observed in the samples from Jalingo and Zing for MSP1 genotyping, samples from Lau had highest allelic frequency and mono-infection of MAD20 (27%), then K1 (13.5%) and least RO33 infections (5.4%) as reported in Minna, Nigeria **by** Usman-Yamman *et al.* (2021**).** Mixed infections were seen K1+MAD20 (43.2%), K1+RO33 (2.7%), MAD20+RO33 (5.4%) and K1+MAD20+RO33 (2.7%)

Both 3D7 and FC27 alleles were successfully amplified for the MSP2 family from the three sites. 3D7 has the highest frequency and majority of the alleles exists as mixed infection, which replicates the infection pattern found in Cameroon as reported by Basco *et al.* (2004**).**

The allelic diversity of *P. falciparum* MSP1 and MSP2 is mostly due to meiotic recombination events involving genetically distinct parasite clones that infect the same mosquito vector, and hence, human host. Therefore, the proportion of mixed infections

and the number of clones per individual is one of the prerequisites to generate new genotypes and to increase the diversity of the parasitic population. Multiple clonal infections with different genotypes of *P. falciparum* were identified among the *P. falciparum* isolates in the study locations with a moderately high Multiplicity of Infection (MOI). Several studies have found that the genetic diversity of this parasite varies depending on a variety of parameters, including the degree of infection, the age of the infected persons, and their geographic location (Kiwanuka, 2009; Ashley *et al.*, 2014; Mohammed *et al.*, 2019; Ajogbasile *et al.*, 2021; Usman-Yamman *et al.*, 2021). Ashley *et al.* (2018) reported that in malaria-endemic countries like Nigeria, where transmission rates are higher, diversity is usually greater.

CONCLUSION

A significant alteration was observed in the blood and biochemical parameters of the *Plasmodium falciparum* infection cases. This indicated the existence of a strong statistical association between the abnormal haematological and biochemical parameters and the occurrence of complications in a malaria cases. Multiplicity of infection (MOI) values among the *P. Falciparum* population in the study, reflecting both the high endemic level and malaria transmission on northern senatorial zone of Taraba State, Nigeria. These data provide valuable information for surveillance of *P. falciparum* infection and assessing the appropriateness of the current malaria control strategies in the endemic area. Results of this study suggest that the conventional diagnostic methods for the detection of malaria parasite (microscopy and RDT) are not adequate when accurate epidemiological data are needed to monitor, control and develop malaria elimination interventions. PCR permitted for the accurate diagnosis, confirming it as a valuable tool for epidemiological surveys, mass screening, and assessment of interventions for malaria elimination. This research will be helpful for future treatment strategies that would be tailored to the specific needs of Taraba and Nigeria's malaria- endemic populations.

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