Advances
2022; 3(1): 25-33
http://www.sciencepublishinggroup.com/j/advances
doi: 10.11648/j.advances.20220301.15

Absence of Biomakers of Resistance in K13 Propeller Gene of *Plasmodium falciparum* from Gombe L.G.A of Gombe State, Nigeria

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To cite this article:

Ismail Muhammad, Pukuma Micah Sale, Augustine Linda Midala. Absence of Biomakers of Resistance in k13 Propeller Gene of *plasmodium falciparum* from Gombe L.G.A of Gombe State, Nigeria. *Advances*. Vol. 3, No. 1, 2022, pp. 25-33.
doi: 10.11648/j.advances.20220301.15

Received: February 6, 2022; **Accepted**: February 24, 2022; **Published**: March 9, 2022

Abstract: Malaria is still one of the life threatening parasitic disease in Sub-Saharan Africa. The causative agent of the disease always provide a means of avoiding the action of most commonly recommended drugs like Artemisinin based Combination Therapy (ACTs) through development of resistance. The aim of this surveillance study was to investigate the status of some biomarkers of Artemisisnin resistance in K13 Propeller gene of *Plasmodium falciparum* from Gombe L.G.A. Nigeria. 200 blood samples were collected from consented study subjects and analysed using Microscopy, RDT and PCR. DNA was extracted using Quick-DNA™ Miniprep (No. D4069), Purity and Concentration of the DNA was determined using Nanodrop Spectrophotometer. 57 true positive samples were selected and used for molecular analysis. Nested PCR was used to amplify required codon (M442V, N554S, A569S and A578S) portion of K13 the gene. Both Primary and Secondary PCR were carried out in 25µl containing DNA template 5µl, distilled water 6.5µl, 0.5µl each of the forward and reverse primer (F5'GGGAATCTGGTGGTAAACAGC3' and R5'CGGAGTGACCAAATCTGGGA3'for primary PCR, F5'GCCTTGTTGAAAGAAGCAGA3' and GCCAAGCTGCCATTCATTTG3' for Nested PCR) and 12.5µl Master mix. Thermocyclic were set as 95°C for 2minute (initial denaturation), followed by 35 cycles at 95°C for 45seconds denaturation, 57°C for 20s, Annealing 60°C for 150s extension and final extension at 60°C for 10min, while for secondary PCR was 95°C for 1min, followed by 35 cycles at 95°C for 30s, 55°C for 20s, 60°C for 60s and final extension at 60°C for 10minute. The PCR products were subjected to electrophoresis in 2% agarose and stained with ethidium bromide. The amplicons were purified and sequenced, afterwhich the sequenced products were subjected to BLAST software. All the fifty seven sequenced amplicon were found to be wild type. All isolate of *Plasmodium falciparum* used in the study were sensitive to ACTs from Further research should be carried out using large sample size and also targeting other bio makers of artemisisnin resistance associated with K13.

Keywords: Gombe, K13 Propeller Gene, Resistance, Artemisisnin, *Plasmodium falciparum*

1. Introduction

Malaria is an old disease that continues to cause significant human morbidity and mortality, as well as being a life-threatening parasitic disease, particularly in Africa. Infected female Anopheles mosquitoes act as vectors for malaria transmission during blood meals from humans. The disease is caused by four *Plasmodium* parasites (*P.vivax, P.malariae, P.ovale, and P.falciparum)* [24]. *Plasmodium falciparum* is the most common of these parasites and is responsible for the majority of severe sickness and fatalities in Sub-Saharan Africa. *P. vivax* and *P. ovale* have hypnozoites, which are dormant liver stages that can resurface months or years after initial infection to cause the sickness [32]. Malaria symptoms range from a mild fever with chills, headache, and vomiting to life-threatening complications such as severe anemia, respiratory difficulty due to metabolic acidosis, or cerebral

malaria, which can lead to death [1, 15]. Pregnant women and children under the ages of five are most vulnerable to the disease [20] and is the leading cause of morbidity and mortality among them, this is partly due to weak immunity [29]. Maternal anemia, placental malaria, mortality, stillbirth, early birth, intra-uterine growth restriction, and low birth weight are only a few of the disease's negative repercussions for pregnant women and their new-borns [17].

Each year, about 225 million clinical cases of malaria are registered worldwide [14] with the disease causing approximately 405,000 deaths. Approximately 93% of these instances occur in Africa's Sub-Saharan area, with Nigeria accounting for 25% of these occurrences [2]. Malaria is endemic in Nigeria, which means that the entire population is at risk, and over half of the population contracts the disease at least once a year. In the southern half of the country, disease transmission occurs all year, whereas in the north, it is more seasonal [4]. Malaria has severe social and economic consequences: it affects Nigeria's GDP by around 1% yearly and is the biggest cause of absenteeism [27]. One major concern about Malaria situation in African countries, particularly Nigeria, is that the situation will worsen as a result of the ongoing pandemic, which has compromised malaria treatment and prevention measures [33] by diverting the world's attention to the development, production, and distribution of the Covid-19 vaccine.

Treatment and prevention of malaria depend on either synthetic antimalarial drugs form different classes of recommended drugs such as Quionoline (eg Chlororquine), Antifolate (Sulphadoxine/Pyramethamine), Artemisinin or herbal traditional antimalarial. These drugs usually work by reducing the parasites' virulence or killing them [4]. One of the primary drawbacks of these medications, particularly synthetic antimalarials, is the generation of *Plasmodium falciparum* parasites that are resistant to all known antimalarial drugs [28]. In malaria-endemic nations like Nigeria, anti-malarial drug resistance is a major public health issue [11]. In order to combat the parasite's continuous rise in antimalarial drug resistance, the World Health Organization (WHO) recommended the use of Artemisinin-based Combination Treatments (ACTs) in 2001, with six different ACTs recommended worldwide: Artesunate + Amodiaquine (AS + AQ), Artemether + Lumefantrine (AL), Artesunate + Sulfadoxine-Pyrimethamine (AS + SP), Artesun [7, 9, 39]. ACTs entails combining and using two synthetic antimalarial drugs (Artemisinin or its derivatives), one with a fast onset but short half-life and the other with a slower onset but longer half-life [9, 16]. Artemisinins are extremely fast-acting, killing both immature ring forms and mature blood-stage parasites [34]. During the first three days of treatment, the Artemisinin compound reduces the parasites load (reduction of parasite biomass), while the companion drug eliminates the remaining parasites [42].

In numerous malaria-endemic countries, different combinations of ACTs form an important part of malaria treatment programs [7, 25]. After proving the failure of other drugs such as Chloroquine and Sulphadoxine/Pyrimethamine by Drug Therapeutic Efficacy Testing (DTET) due to the emergence of resistance, Nigeria officially accepted ACTs as a recommended drug for first-line treatment of uncomplicated malaria in 2005 [11, 41]. like other antimalarials, *Plasmodium falciparum*, develops resistance to this most recently indicated drugs (ACTs) [6]. Clinical resistance to Artemisinin, as well as mutations in the K13 propeller gene, which are significantly linked to artemisinin resistance, were first discovered in western Cambodia, (specifically Thailand–Cambodia border) in 2008 [8, 10, 40], because of this the region is regarded as the primary epicentre of drug-resistant *P. falciparum* malaria [30]. Since then, Artemesinin resistance has been documented in five countries in the Greater Mekong Sub region, including Cambodia, Laos, Thailand, Myanmar, and Vietnam [12, 31, 36].

Artemisinin resistance's evolution and global expansion would be a significant public health disaster, especially in Africa, where ACTs are heavily employed in the treatment of approximately 90% of *P. falciparum* malaria cases in children under the age of five worldwide [9]. In the instance of artemisinin resistance, mutations in several of *Plasmodium*'s adverse drug reaction genes, especially the K13 propeller gene, cause resistance [37]. Nine non-synonymous mutations in the k13 gene of *Plasmodium falciparum* have been validated, including F446I, N458Y, M476I, Y493H, R5397T, I543T, P553L, R561H, and C580Y, as well as eleven other potential markers [5]. To date, thirteen independent K13 mutations have been shown to be associated with clinical resistance, with evidence of independent emergence of the same mutation in different geographic areas [22]. In sub-Saharan Africa, increasing frequencies of non-synonymous mutations on the K13 gene have been reported throughout the continent. Increased frequency of non-synonymous mutations on the K13 gene have been documented across Sub-Saharan Africa. The efficacy of first- and second-line antimalarial drugs should be evaluated on a frequent basis, according to WHO, in order to detect and prevent the spread of resistant parasite populations early [38]. As a result, the aim of this surveillance study was to determine the presence of biomarkers of resistance in the K13 propeller gene of *Plasmodium falciparum* in Gombe local government area, Gombe state, Nigeria.

2. Methodology

2.1. Study Area

The study was conducted in Gombe Local Government Area, (Figure 1) Gombe State, Nigeria. The Local Government lies between 11°14′07″E and 11°4′42″E, and Latitudes 10°16′48″N and 10°17′24″N with a total land mass 52km^2. Gombe Local government has a projected population figure of 367,500 people (3.3% annual change) (National Population Commission, 2006). The vegetation of the local government is typical of that of Gombe State which is Sudan Savannah and experience two distinct season, dry season

which normally commences from November- March and rainy season from April- October with mean annual rainfall of 863.2mm. Agriculture is the major occupation in the region (mostly Peasant farmers) while some engage in business and few are civil servant. Malaria infection is endemic in the local government with *Plasmodium falcifarum* accounting for the majority of the reported cases and transmission is all-year round in the local government with it peak during rainy season (August-October).

2.2. Ethical Consideration and Consent of the Subject

Before commencement of the research, the research proposal was submitted to Gombe State Ministry of Health for approval. After which the approval was communicated via a later MOH/ADM/621/VOL. I/222 dated 21st February 2020. Verbal and or written consent of the subjects were sought after briefing them on the research and the need for them to participate. In a situation whereby the subjects were not of legal age (matured enough), consent of his/her parents/guardians were sought. All the subjects were assured that, all information collected will be strictly used for the purpose of the research only and will be treated with high level of confidentiality. In addition, quality control and quality assurance was assured when handling and treating each of the samples.

2.3. Study Subjects and Inclusion Criteria

Human beings of all age group and gender who willingly and voluntarily agreed to participate in the study were used as the study subject. Three (3) recruitments centres were selected; these are Gombe town maternity (gidan Magani), Sunnah clinic and Idi children and Women Hospital Gombe, where a total of 200 volunteers actively participated in the study.

Only patients who reported themselves to the selected hospitals with (presumed to be malaria positive) symptoms of malaria (fever) or history of fever in last 24hours and referred by a physician for screening of malaria infection and in addition they have not used any anti-malarial drugs 60 days prior to the data collection. All subjects having multiple infections were not recruited in the research; only subjects with *Plasmodium falciparum* mono-infection were recruited. For molecular analysis only true positive samples with a very good DNA concentration (200ng and above) and high level of purity (A260/280 of 1.8) were used.

2.4. Blood Sample Collection and Analysis

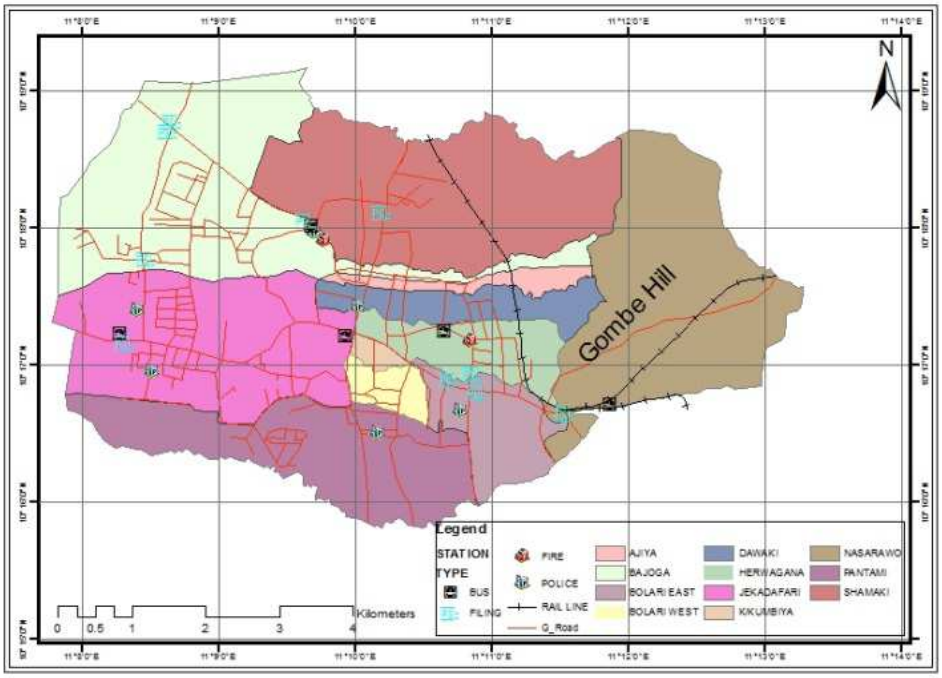
Vein puncture technique was used to collect Venus blood and analysed using Rapid Diagnostic Test (RDT) and Microscopy (Stained with Giemsa). All those samples found to be positive for malaria (*Plasmodium falcifarum)* either by RDT or Microscopy or both were selected and preserved on Whatman paper, and allowed to air dried and stored at room temperature for molecular analysis.

2.5. DNA Extraction

The DNA was extracted using Quick-DNA™ Miniprep Plus Kit with catalog No. D4069 from Zymo research, following manfacturer's instruction with slight modification.

2.6. Purity and Concentration Determination

Nanodrop Spectrophotometer was used to determine the concentration and purity of the extracted DNA.



Source: GIS Laboratory, Geography department, Gombe State University

Figure 1. *Map of Gombe Local Government Area.*

2.7. Primers and Confirmation of Plasmodium Specie

All Validated Primers were supplied from Inqaba biotec^{TM} Africa's genomic company. For the molecular confirmation of *Plasmodium falciparum* 18S Portion of small sub unit ribosomal RNA gene was amplified in Classic DW-K960 thermal cycler using the following set of primers: F5'AACAGACGGGTAGTCATGATTGAG3' and R5'GTATCTGATCGTCTTCACTCCC3'. The reaction was carried in 25µl reaction mixture containing 5µl of the extracted DNA as the template, 1µl of primer (0.5µl each of Forward and Reverse primer), 6.5µl distilled water and 12.5µl of the PCR Master mix (Containig dNTPs, $Mgcl_2$ and T*aq* DNA Polymerase). The gene was amplified by setting an initial denaturation at 95°C for 15minutes then followed by forty (40) cycles of denaturation at 94°C for 45 seconds while annelling at 60°C for 90seconds and extension at 72°C for 1minute. The final extension was carried out at 72°C for 5minutes. Distilled water was used as negative control for all Polymerase Chain Reaction (PCR) in this research.

2.8. Amplifications of the k13 Genes

For the amplification of *K13* propeller gene nested PCR was used where F5'GGGAATCTGGTGGTAAACAGC3' and R5'CGGAGTGACCAAATCTGGGA3' were used for the primary PCR. The reaction was carried out in a 25µl containing DNA template 5µl, distilled water 6.5µl, 0.5µl each of the forward and reverse primer and 12.5µl Master mix. For the primary PCR the thermocycling conditions were 95°C for 2minute (initial denaturation), followed by 35 cycles at 95°C for 45seconds denaturation, 57°C for 20 second, Annealing 60°C for 150seconds extension and final extension at 60°C for 10minute. For the nested PCR, F5'GCCTTGTTGAAAGAAGCAGA3' and GCCAAGCTGCCATTCATTTG3' were the primers used. In this 5µl of the PCR product of the primary PCR was used as a template for the secondary PCR. Like the primary PCR, the reaction was carried out in a 25µl reaction mixture, but the template used was 5µl of the PCR product of the primary PCR. The cyclic conditions were 95°C for 1minute (initial denaturation), followed by 35 cycles at 95°C for 30seconds denaturation, 55°C for 20second annealing, 60°C for 60seconds extension and final extension at 60°C for 10minute as shown in table 1 below.

The product of the secondary PCR produced a band size of 849bp which correspond to the codon position of 427-709 of k13 propeller gene of *Plasmodium falciparum.*

Table 1. *Primers and PCR cyclic conditions.*

PCR type	Primer	Primer Sequence	Initial denaturation	denaturation	Annealing	Extension	Final extension	No. of cycles
Normal PCR	18S	F5'AACAGACGGGTAGTCATGATTGAG3' R5'GTATCTGATCGTCTTCACTCCC3'	95°C 15min.	94°C 45s	60°C 90s	72°C 60s	72°C 5min.	40
Primary PCR	K13N1	F5'GGGAATCTGGTGGTAAACAGC3' R5'CGGAGTGACCAAATCTGGGA3	95°C 2min.	95°C 45s	57°C 20s,	60°C, 150s	60°C 10min.	35
Nested PCR	K13N2	F5'GCCTTGTTGAAAGAAGCAGA3' GCCAAGCTGCCATTCATTTG3'	95°C 1min.	95°C 30s	55°C 20s	60°C 60s	60°C 10m.	35

2.9. Electrophoresis

All PCR product obtained were subjected to electrophoresis on 2% agarose gel and stained with ethidium bromide. All gels were allowed to run for a period of 1hour at 100mA, after which the gel was visualised using UV transilluminator.

2.10. Sequencing of the PCR Product and BLAST

The entire amplified PCR products were sent to Cambrige Genomic Services, UK for purification and sequencing. After which all sequenced amplicons were subjected to Basic Local Alignment Search Tool (BLAST) in order to determine the position of mutation and the nature of the mutation.

3. Result

3.1. Demographic and Clinical Characteristic of the Study Subjects

A total of 200 study subjects were used, comprised of 114 (57.0%) and 86 (43.0%) male and female respectively. The age of the subjects ranges from 5-55years with the mean of 28.60±10.6. The mean ambient body temperature of the subjects ranges from 33-43°C with the mean of 37.77±1.92. For the molecular analysis, the concentration of the DNA sample extracted ranges from 100.10-600.2ng/µl of the sample, and the mean concentration was 300.55±1.03. For purity, the mean value of A260/280 was 1.72±0.55 and it ranges from 0.7-5.11 as shown in table 2 below.

Table 2. *Demographic and clinical characteristic of the study subject and basic characteristics of the DNA sample.*

Characteristics	Mean	Range	Male	Female
Age	28.60±10.60	5-55 Years	114 (57.0%)	86 (43.0%)
Body Temperature	37.77±1.92	33-43°C		
DNA Concentration	300.57±10.03	100.10-600.2.ng/l		
A260/280	1.72±0.55	0.7-5.11		

3.2. Result of the Blood Analysis

Out of the 200 blood sample collected, 167 (83.5%) sample were positive by microscopy, 132 (79.04%) and 105 (62.87%) were positive by Rapid diagnostic test and Polymerase chain reaction respectively as shown in the figure 2 below. 06 (3.59%) and 13 (7.78%) were invalid when tested with RDT and PCR respectively. In addition 80 (40%) of the collected blood samples were true positive (that is confirmed positive by the three techniques (Microscopy, RDT and PCR) and also 57 (71.25%) of the true positive samples met the inclusion criteria for the actual molecular analysis.

3.3. Single Nucleotide Polymorphisms (SNPs) at Codon Position M442V, N554S, A569S and A578S of K13 Gene of Plasmodium falciparum

All the fifty seven (57) isolate of K13 Propeller gene used in this study when subjected to BLAST revealed no any mutation at the selected codon position, as it revealed 100% similarity (wild type) to already deposited K13 gene of *Plasmodium falciparum* from the gene bank, as shown in table 3 and figure 3 below.

Table 3. *Prevalence of SNPs at codon M442V, N554S, A569S and A578S of K13 gene of Plasmodium falciparum.*

Codons	M442V	N554S	A569S	A578S
Wild genotype	ATG	AAT	GCA	GCT
Amino acid	Methionine	Asparagine	Alanine	Alanine
No. of Isolate with wild type	57 (100%)	57 (100%)	57 (100%)	57 (100%)
Mutant genotype	GTG	AGT	TCA	TCT
Amino acid	Valine	Serine	Serine	Alanine
No. of Isolate with mutant alleles	00 (0.00%)	00 (0.00%)	00 (0.00%)	00 (0.00%)

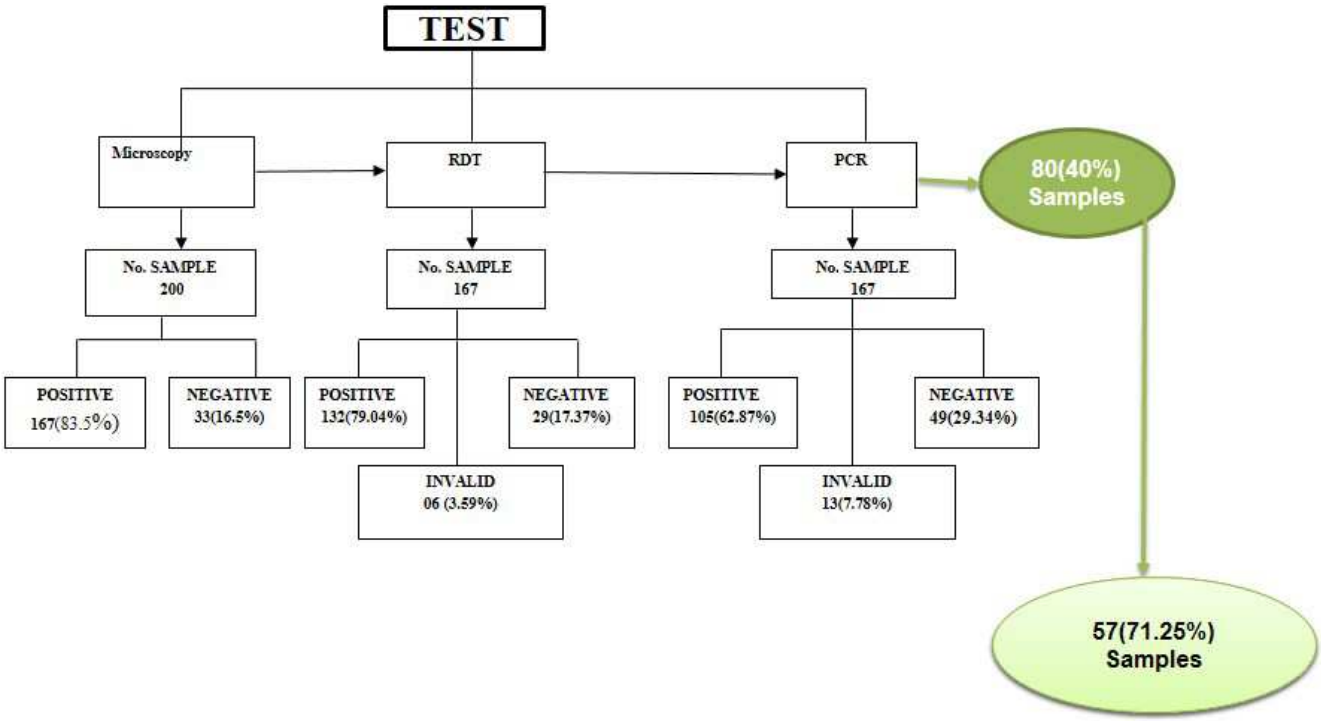


Figure 2. *Blood analysis using the three techniques (Microscopy, RDT and PCR).*

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Plasmodium falciparum isolate MM_22 kelch13 propeller domain protein (kelch13) gene, partial cds

Sequence ID: MT263362.1 Length: 849 Number of Matches: 1

Range 1: 1 to 849 GenBank Graphics ▼Next Match ▲Previous Match

Score	Expect	Identities	Gaps	Strand
1568 bits(849)	0.0	849/849(100%)	0/849(0%)	Plus/Minus

```
Query  1    GCCAAGCTGCCATTCATTTGTATCTGGTGAAAAGAAATGACATGAATTTAGAACTTCGCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  849  GCCAAGCTGCCATTCATTTGTATCTGGTGAAAAGAAATGACATGAATTTAGAACTTCGCC  790

Query  61   ATTTTCTCCTCCTGTAATTATATAAGAATCTGACAATGTGGCAGCTCCAAAATTCAtttt  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  789  ATTTTCTCCTCCTGTAATTATATAAGAATCTGACAATGTGGCAGCTCCAAAATTCATTTT  730

Query  121  tttCTCTGGTACACCATTTAGAAATTGCCATCTTTTATTAAATGGTTGATATTGTTCAAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  729  TTTCTCTGGTACACCATTTAGAAATTGCCATCTTTTATTAAATGGTTGATATTGTTCAAC  670

Query  181  GGAATCTAATATGTTATGTTCATTATCAATACCTCCAACAACATATATTTGATTAAGGTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  669  GGAATCTAATATGTTATGTTCATTATCAATACCTCCAACAACATATATTTGATTAAGGTA  610

Query  241  ATTAAAAGCTGCTCCTGAACTTCTAGCTTCTAATAAGGCATATGGAAATTGTTCCCATTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  609  ATTAAAAGCTGCTCCTGAACTTCTAGCTTCTAATAAGGCATATGGAAATTGTTCCCATTT  550

Query  301  ATTCATTTTTTCTTCATATACTTCAATAGAATTTAATCTCTCACCATTAGTTCCACCAAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549  ATTCATTTTTTCTTCATATACTTCAATAGAATTTAATCTCTCACCATTAGTTCCACCAAT  490

Query  361  GACATAAATTTTATTATCAAAAGCAACACACATAGCTGATGATCTAGGGGTATTCAAAGG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  489  GACATAAATTTTATTATCAAAAGCAACACACATAGCTGATGATCTAGGGGTATTCAAAGG  430

Query  421  TGCCACCTCTACCCATGCTTTCATACGATGATCATATGCTTCTACATTCGGTATAATAGA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429  TGCCACCTCTACCCATGCTTTCATACGATGATCATATGCTTCTACATTCGGTATAATAGA  370

Query  481  AGAGCCATCATATCCCCCAATACAATAAATTCTACCATTTGACGTAACACCACAATTATT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369  AGAGCCATCATATCCCCCAATACAATAAATTCTACCATTTGACGTAACACCACAATTATT  310

Query  541  TCTTCTAGGTATATTTAAATTACTTGAAACATACCATACATCTCTTAAACGATCATACAC  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309  TCTTCTAGGTATATTTAAATTACTTGAAACATACCATACATCTCTTAAACGATCATACAC  250

Query  601  CTCAGTTTCAAATAAAGCCTTTATAATCATAGTTATTACCACCAAAAACGTATAAGAAATT  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249  CTCAGTTTCAAATAAAGCCTTTATAATCATAGTTATTACCACCAAAAACGTATAAGAAATT  190

Query  661  ATTCAATACAGCACTTCCAAAATAAGCTTTTTTGGTAGACATAGGTGTACACATACGCCA  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189  ATTCAATACAGCACTTCCAAAATAAGCTTTTTTGGTAGACATAGGTGTACACATACGCCA  130

Query  721  GCATTGTTGACTAATATCTAATAATTCCATCGAATTTAAATATTCTACACCATCAAATCC  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129  GCATTGTTGACTAATATCTAATAATTCCATCGAATTTAAATATTCTACACCATCAAATCC  70

Query  781  ACCTATACAAAATACTAATGGGAATGGTAAAAATTTAATACCATAAAATTCTGCTTCTTT  840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  69   ACCTATACAAAATACTAATGGGAATGGTAAAAATTTAATACCATAAAATTCTGCTTCTTT  10

Query  841  CAACAAGGC  849
            |||||||||
Sbjct  9    CAACAAGGC  1
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Figure 3. *BLAST result of K13 Propeller gene of Plasmodium falaciparum.*

4. Discussion

In this study three different techniques were used to analyse the blood samples for the presence of *Plasmodium falciparum*. Though these techniques produced different results, but still confirmed the endemicity of malaria in the study area (Gombe). These differences obtained might attributed to the differences in the sensitivities and specificity of the different techniques and other intrinsic factors peculiar to each of the techniques like DNA concentration and its purity in the case of PCR. Prevalence of 83.5% recorded in

this study by the 'gold standard' technique (Microscopy) is by far higher than 44.9% reported by Mac et al., [19] from Abuja, Nigeria. The sequence region (1736373-1737122) of the secondary PCR (Nest II) of K13 Propeller gene of *Plasmodium falcifarum* obtained in this study is 100% identical to the portion of K13 propeller gene of *Plasmodium falciparum* already deposited in the gene bank by Abubakar et al., [2] with accession number MT263362. Similarly, other K13 gene deposited with the following accession numbers MK877455, MK877430, MT263359 and MT263356 showed 100% complementarity with the portion of the gene used in this study.

Although ACTs remain largely effective in endemic areas, there are fears that resistance to artemisinin derivatives may spread from Asia to Africa. It is also possible that resistant parasite mutants may evolve independently in these areas. Several authorities have reported several other variants of K13 propeller gene that are associated with drug resistance specifically ACTs in *Plasmodium falcifarum.* For example Ocan et al., [26] reported the following variant N87K, G112E, E130G, T149S, K189T, K189N, R223K, R255K, D281V, R254L, I376V, and E252Q as molecular markers associated with ACT resistance, But C580Y variant is validated candidate associated with delayed parasite clearance [23]. The findings of this present study revealed zero prevalence of single nucleotide polymorphism in selected variant (M442V, N554S, A569S, A578S) of K13 propeller gene. This result is contrary to the findings of Yobi et al., [43] who reported a prevalence of 1.0% of non synomous mutation from Congo. Similarly, He et al., [13] and Mathieu et al., [21] respectively reported a prevalence of 52.78% from China-Myanmar border and 8.8% from Amazonia all from different variants of K13 Propeller gene. Nevertheless it is similar to finding of Taylor et al., [35] who reported absence of molecular makers associated with artemisinin resitance across and within sub-Saharan African parasite populations.

Nevertheless absence of mutations from the selected variants (M442V, N554S, A569S, A578S) in this study, may not translate in to total and complete absence of molecular makers for artemesinin resisatance in the study area. This is because K13 propeller gene has over one hundred markers that are associated with resistance in K13 Propeller gene [18]. Therefore, the absence mutation from K13 gene in this present study might be attributed to the limited number of the variants targeted in this study, as there are many variants responsible for development of resistance. Though, Studies carried out to detect mutations in the kelch13 gene to date have found none of the major mutations associated with delayed parasite clearance in isolates from Nigeria [3].

5. Conclusion

Malaria is endemic in the Local Government as each of the three techniques (Microscopy, RDT and PCR) revealed more than 60% malaria prevalence. This study revealed high sensitivity of the parasite to Artemisin as all the *Plasmodium falciparum* isolate used in the study lack active Biomakers of artemisinin resistance at the selected codon positions, as such any artemisin-treatmenent failure in the L.G.A. may not be attributed to artemisisn resistance. Therefore, ACTs may continue to be relevant and use as the best candidate drug for malaria treatment and management in Gombe, as the parasite has not yet develop resistance to the combined drugs based on the molecular makers used in this study.

6. Recommendation

The use of ACTs should be maintained in Gombe Local Government Area using different recommended combination, as well as alternating the combination within the recommended ACTs combination for episode of malaria attack, as the parasite did not show any prevalence of bio markers in the selected codon of the gene in the LGA. Further research should be carried out using large sample size and also targeting other bio makers of artemisisnin resistance associated with K13.

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