

# Detection of High Frequency of MAD20 Allelic Variants of Plasmodium Falciparum Merozoite Surface Protein 1 Gene from Adama and its Surroundings, Oromia, Ethiopia

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## Research

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# Abstract

**Background:** One of the major challenges in developing an effective vaccine against asexual stages of *Plasmodium falciparum* is genetic polymorphism within parasite population. Understanding the genetic polymorphism like block 2 region of merozoite surface protein (*msp-1*) genes of *P. falciparum* enlighten mechanisms underlining disease pathology, identification of the parasite clone profile from the isolates, transmission intensity and potential deficiencies of the ongoing malaria control and elimination effort in the locality. Detailed understanding of local genetic polymorphism is an input to pave the way for better management, control and elimination of malaria. The aim of this study was to detect the most frequent allelic variant of the merozoite surface protein (*msp-1*) gene of *P. falciparum* clinical isolates from selected health facilities in Adama town and its surroundings, Oromia, Ethiopia.

**Methods:** A total of 139 clinical isolates were successfully amplified for *msp-1* gene using specific sets of primer. Nested PCR amplification conducted, using specific primers targeting K1, MAD20, and R033 alleles followed by gel electrophoresis for fragment analysis. Based on the detection of a PCR fragment, infections were classified as monoclonal or multiple infections.

**Result:** 19 different size polymorphism of *msp-1* gene were identified in the study, with 67(48 %) MAD20, 18 (13 %) K-1 and 18 (13 %) R033 allelic family. Whereas, the multiple infections were 21(15 %), 8(5.8 %), 4(2.9 %), 3(2.2 %) for MAD20+K-1, MAD20+R033, K-1+ R033, and MAD20+K-1, R033, respectively. The overall Multiplicity of Infection (MOI) was 1.3 and the expected heterozygosity ( $H_e$ ) was 0.58 indicating intermediate *falciparum* malaria transmission.

**Conclusion:** The status of *msp-1* allele size polymorphism, MOI and  $H_e$  observed in the study revealed an intermediate genetic diversity of *P. falciparum* clinical isolates, indicating that the ongoing malaria control and elimination effort should be intensified to effectively monitor the potential malaria resurgence in the study area. Moreover, deriving force that led to high predominance of MAD20 allelic variant revealed in such malaria declining region demands further research.

## Background

Despite an enormous efforts to control and eventually eliminate malaria, studies reveal that it's still a major public health problem specifically in sub Saharan Africa (SSA) where more than 90% of the disease burden prevails (WHO, 2020; Arie & Gay, 2019; WHO, 2017). About 68% of Ethiopian population is inhabiting in 75% of the countries land mass that is malarious, where *P. falciparum* and *P. vivax* accounts for 70 and 30%, respectively (Solomon et al., 2020). Studies revealed that, multiple factors greatly affected malaria control and elimination efforts. To mention few; the emergence of insecticide resistance by the mosquito vectors, increased population density, global warming, poverty, the lack of effective vaccines, the emergence and spread of drug resistant strains (Mwingira et al., 2011; Sonko et al., 2014). In addition, genetic diversity of *P. falciparum*, the main causative agent of

severe malaria, is the major hurdle. The driving force for the occurrence of such genetic diversity could be; considerable chromosomal variation mainly in the sub telomeric regions, genetic recombination between different parasite clones during meiosis in the mosquito (Gardner et al., 2002), and the sub telomeric deletions (Scherf et al., 1992) largely contributes. The presence of exceptionally high Adenine and thymine (AT-rich 80.6%) content of falciparum genome (Sexton et al., 2019) also significantly contribute for such variation. In addition, the existence of approximately 60 var genes that are expressed one at a time (Gardner et al., 2002, Smith et al., 2013), thereby ensuring only one *P. falciparum* Erythrocyte membrane protein (PfEMP-1) variant exposed to the immune system switching in var transcription provide a base for antigenic variation and immune scape during chronic infections (Miller et al., 2002). These are the major factors for the frequent emergence of falciparum variants, which ultimately jeopardize the effectiveness of vaccine research for the control and elimination of malaria.

Genetic diversity is a prominent feature of *P. falciparum* infection indicating its evolutionary fitness (Takala et al., 2010), as high genetic diversity is an indicator of the intensity of transmission (Soe et al., 2017), and potential challenges in malaria control programs (Mohammed et al., 2018). Studies have shown that malaria reduction as the result of intensified control efforts is accompanied by reduced genetic diversity of the parasite populations (Park et al., 2013). Widespread use of specific antimalarial drugs can also alter the genetic diversity because of selective pressure on specific parasite strains.

Molecular characterization of falciparum malaria enables us to investigate the genetic diversity of infection with consideration of various factors such as disease phenotype, age and host immunity (Mahdi Abdel Hamid et al., 2016). Genetic diversity of *P. falciparum* is usually determined through genotyping of the polymorphic regions block 2 of *msp1* (Mohammed et al., 2018; Bakhiet et al., 2015; Snounou & Singh, 2002). *msp1* is one of the major *P. falciparum* blood-stage malaria vaccine targets (Holm & Bentley, 1999). They are involved in erythrocyte invasion (Holder, 2019) and are targeted by the immune responses (Woehlbier et al., 2006). *msp1* is a 190 KDa surface protein encoded by the *msp1* gene located on chromosome 9 and contains 17 blocks of sequences flanked by conserved regions (Hamid et al., 2013), (Snounou & Singh, 2002), (Smythe et al., 1991). The precise functional role of *msp1* during invasion has not been fully evaluated, and its macromolecular characterization have been incomplete (Lin et al., 2016).

*Msp-1* markers are useful to investigate genetic diversity, multiplicity of infections (MOI) and parasite carriage. Polymorphism in *msp1* and *msp2* frequently reported from different parts of the world. Of the 17 blocks of *msp1*, block 2 is the most polymorphic region characterized into three allelic families namely (K1, MDA20 and R033). Based on variation in length and sequence diversity, this region is a commonly targeted part in determining genetic diversity and multiplicity of infection (MOI) in clinical isolates of *P. falciparum*.

In this regard, even though genetic diversity of *P. falciparum* is extensively studied in different parts of the world, limited data were yet available from south western and north western part of Ethiopia. The aim of this study was to broadly assess genetic diversity with main emphasis on the detection of the

predominant allelic family of block 2 region of msp-1 gene of *P. falciparum* clinical isolates from uncomplicated symptomatic patients from three districts in central Ethiopia, and examine its association with parasite density, disease severity and other patient characteristics in the study area.

## Materials And Methods

### Study sites

Health facility based cross sectional study conducted at Adama, Modjo, Wonji, Awash Malkasa and Olanciti towns from September 2019 to August 2020. These sample collection sites include Adama city administration, Adama district which include Wonji and Awash Malkasa, Modjo town capital of Lume district, Olanciti the capital of Boset district. The location of these study sites is as shown in (Fig. 1). For patient data and sample collection, we purposively selected health facilities from each site depending on their patient caseload, physical location and the availability of qualified and experienced medical laboratory technologist participated in similar research work.

Adama is the major town next to the main capital in central Ethiopia. It is found at about 99 km south east of Addis Ababa. Modjo located at the distance of 16 km north-west of Adama, Wonji situated 8km south of Adama, Awash Malkasa situated 15km south- east of Adama and Olanciti located at about 23km north- east of Adama

The study sites are located in the Great Rift Valley region having rain fall pattern that is heavy from mid - June to mid-September followed by major malaria transmission season and shorter rainy period in March accounting for minor malaria transmission (Golassa & White, 2017). The catchment population of the study site estimated to reach 800,000 inhabitants.

Awash Malkasa, and Olanciti)

Adama and its surrounding area is a well-known malarious site in central Ethiopia. The major factors that account for malaria endemicity in the region are; its physical location in the Great Rift Valley region of east Africa, latitudinal location of the area, which is less than 2000 meter above sea level. The rainfall patterns, average annual temperature that varies from 16–32°C that is favorable for the breeding of *Anopheles Arabiensis* (the predominant malaria vector in the region), and various micro ecological factors that favor mosquito breeding (File et al., 2019)

## Clinical Data And Blood Sample Collection And Processing

A total of 171 microscopically confirmed *P. falciparum* infected patients were included in this study. Patient data and blood sample collected from patients (aged 1 to 66 years). The inclusion criteria for the study was uncomplicated malaria patient with the history of fever onset since the within 24 hours. Blood sample collection through finger prick for Dry Blood Spot (DBS) preparation and clinical data collection

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held from September 2019 to August 2020 by trained medical laboratory technologist from the catchment area of those selected health facilities in the study area. After consent of patient or guardian, spotted blood on Whatman TM 3MM filter paper allowed to air dry in dust free area. The dried Blood Spot (DBS) placed in a zip-lock bag with silica gel temporarily stored at 0–4 degree celcius to prevent DNA degradation. For longer time storage of DBS in deep freeze (-20 °C), we used Adama regional laboratory.

## Microscopy And Parasite Count

Microscopic examinations conducted for both thick and thin blood smear prepared for malaria microscopy protocol. All slides examined by two independent laboratory technologists to determine species identification for *Plasmodium falciparum* and its parasite density. In case of discordance, the slides read by a third laboratory technician. In addition, parasite density estimated by counting and recording the asexual stage of the parasite per 200 White Blood Cells (WBC) in thick film. Moreover, when the sexual form (gametocytes) seen, the slides were excluded from the count. The parasite density of the asexual stage estimated by counting the number of WBC by field examined by assuming that 8000 WBC were present in 1µl of blood. The parasite density per microliter (µl) of blood was calculated by using the

following formula: Parasite Density per microliter (µl) of blood = Number of Parasite counted  $\times \left( \frac{8000}{200} \right)$ ,

(Mahdi Abdel Hamid et al., 2016, WHO, 2010). For comparison with ranked order variables, parasitemia were categorized in to five levels; : L1 (< 50 parasite/µl blood), L2 (50–499 parasite / µl blood), L3 (500–4999 parasite / µl blood), L4 (5000–49999 parasite/ µl blood), and L5 ( $\geq$  50,000 parasite/ µl blood) (Diouf et al., 2019).

## Extraction Of The Parasite Dna

Extraction of parasite genomic DNA and genotyping the polymorphic region msp-1 were conducted at malaria research laboratory, Akililu Lema Pathobiology Institute, Addis Ababa University (AAU). Genomic DNA of *P. falciparum* extracted from approximately 200µl of frozen blood sample spotted on Whatman 3 filter paper for nested PCR amplification. 0.5% Tween® 20 (Sigma-Aldrich, USA) was used to lyse RBC; tracked by treatment with 6% chelex ® 100 (Sigma-Aldrich, USA) and heat treatment in water bath at 96°C following the optimized standard operating procedure (SOP) to free the parasites DNA (Snounou & Singh, 2002).

### PCR amplification for genotyping of msp-1 gene and gel electrophoresis

Nested PCR amplification targeting the unique sequence of 18 srRNA gene was held by using specific primer pairs for molecular detection of *P. falciparum* from the isolates (Das et al., 1995). In the present study we used, the polymorphic region of the confirmed *P. falciparum* msp-1 gene (block 2) as a genetic marker for the genotyping of parasite populations. The primers and PCR conditions used during this

Loading [MathJax]/jax/output/CommonHTML/jax.js usly described works (Snounou & Singh, 2002, Farooq et al.,

2009, Chen et al., 2018). Briefly, all reactions carried out in a final volume of 20µl. In the first round (N1) reaction containing 0.5 µl of each primer, 5x FIREPol® .Master Mix (MM), 11 µl of nuclease free water aliquot to 16µl to which 4µl of DNA template was added. In nested (N2) reaction, we used 2µl of the amplicon product. The PCR amplification profile for both N1 and N2 reactions includes; initial denaturation at 95°C at 3 minutes, denaturation at 94°C for 1 minute, annealing 58°C for 1 minute, elongation 72°C for 2 minutes and final elongation at 5minutes (Snounou & Singh, 2002; Farooq et al., 2009; Chen et al., 2018). The PCR reaction mixture incubated in a thermal cycler (VWR), Schmidt, Germany. To monitor the quality the protocol allele specific positive control 3D7 and DNA free negative control were included in each reaction. Separation of the PCR product was performed on 2% agarose gel electrophoresis stained with ethidium bromide (Snounou & Singh, 2002). Stained agarose gels visualized under Benchtop 2UV trans-illuminator (UVP), USA and photographed to estimate band size in relation to 50bp DNA ladder (Invitrogen, by thermal Fisher-scientific). Infections considered as monoclonal when a single PCR fragment was detected on each locus and polyclonal when more than one fragment identified on a locus. Polymorphism in each allele family analyzed by assuming that one band represented one amplified DNA fragment derived from a single copy of *P. falciparum* msp-1. Multiplicity of infection (MOI) defined as the average number of detected *P. falciparum* genotypes per infected patient. Allele for each family were considered the same when the fragment size is less than 20bp (Mohammed et al., 2015).

## Data Analysis

Data analyzed, after entering and processing it by using Statistical Package for Social Sciences (SPSS version 20). To examine endemicity or potential importation of the msp- 1 allelic variants; confirmed malaria patient due to *P. falciparum* were categorized in to those having travel history or not in the preceding 30 days to other places where malaria is endemic. Multiplicity of Infection (MOI) for *P. falciparum* calculated as a total number of parasite genotypes for the same gene and the number of PCR positive isolates. Descriptive analysis performed to compare the distribution of different allele families in relation to patient data.

To test the correlation of two variables, we used, Pearson correlation test. Pearson Chi square test also conducted for statistical comparison of categorical variables.  $P < 0.05$  is used to test the level of statistical significance to accept or reject the hypothesis.

The expected heterogeneity ( $He$ ) calculated by the formula;

$$He = \left( \frac{n}{n-1} \right) \left( 1 - \sum p^2 \right), \quad (1)$$

Where “n” stands for the number of the isolates analyzed and “p” represents the frequency of each different allele at a locus.

# Ethical Consideration

Ethical approval of the study was obtained from Institutional Ethical Review Board of ASTU, certificate reference number RECSOANS/BIO/01/2019 and approval of Oromia Regional State Health Bureau. In addition, written informed consent obtained from parents or guardian prior to recruitment.

## Results

### Socio-demographic and Parasitological data

MSP-1 allelic diversity and frequency successfully analyzed for 139 samples out of 171 microscopically confirmed malaria patients due to *P. falciparum*. From the patient enrolled for this study; 120 (70%) were males. The age of the study participant ranged from 1 to 66. Mean  $\pm$  SD ( $27.0 \pm 13.6^*$ ) years. Asexual parasite density ranged from 64 – 104,320 parasites/  $\mu$ l with a geometric mean of 5,654 parasites/ $\mu$ l. Of all study participants 83 (60%) were from urban inhabitants, and only 15 (11%) were having travel history to malarious area. Of all the study subjects, by occupation 67% *P. falciparum* malaria cases were detected from students, daily laborers and farmers (Table 1).

Table 1. Socio-demographic characteristics and parasitological data of the study population at Adama and its surroundings (n = 139)

Patient characteristics	Value
Mean age (year)	27.0 ± 13.6*(SD)
Age range (year)	1- 66
Sex ratio (Male/Female)	95/44
Residence (urban/ Rural)	83/56
Travel history to malarious area	15 (11%)
Educational level	
Not attended formal education	18(13%)
Attended primary school	71(51%)
Attended secondary school and above	50(37%)
Occupation	
Farmer	26 (19%)
Housewife	14 (10%)
Daily laborer	33 (24%)
Government employee	14 (10%)
NGO employee	2 (1.4%)
Business man	6 (4.3%)
Student	44 (32%)
Geometric mean of parasitic density (P/μl) of blood	5,654.0
Parasite density range (P/μl) of blood	64 – 104,320.0
Parasitaemia level	9 (6.4%)
1. μl of blood)	
(500-4999 P/μl of blood)	45.3%)
(5000-49,999 P/μl of blood)	63 (45.3%)
( ≥50,000 P/μl of blood)	4 (2.9%)

### Geometric mean of the parasite density across different age groups

The association between geometric mean of the parasite density of *P. falciparum* patients of different  
 Loading [MathJax]/jax/output/CommonHTML/jax.js children (5-14 years) carry disproportionate burden of the

infection (Figure 2). However, the correlation between parasite density with patient's age is not statistically significant (Pearson's correlation = 0.12, P = 0.6).

### Allele typing and diversity profile across different age groups

From all age groups, 74% of the isolates had monoclonal infections (Figure 3A). The prevalence of multiple infection slightly increases with age group (Figure 3B). However, no significant correlation exists between parasite density and multiple infection (Pearson's correlation = -0.07,  $X^2 = 0.6$ ), and age of the patient with parasite density (Pearson's correlation = 0.12,  $X^2 = 0.6$ ) (Figure 2). Similarly, no significant variation in msp-1 allelic families with age ( $X^2 = 0.5$ ), sex ( $X^2 = 0.56$ ), residence ( $X^2 = 0.2$ ), travel history ( $X^2 = 0.9$ ), educational level ( $X^2 = 0.8$ ) and occupation ( $X^2 = 0.5$ ) (Table 1).

### Allelic Polymorphism of block 2 region of msp-1 gene and their level of severity, spatial and seasonal features

From the total of 139 successfully genotyped samples by nested PCR; the frequency of msp-1 allelic families detected in monoclonal isolates were 48%, 13%, 13% for MAD20, K1 & RO33, respectively, and the remaining 24% were diclonal (MAD20 +K-1, MAD20 +RO33, K-1+RO33) and 2% triclinal (MAD20+ K1+ RO33) infections. From all *P. falciparum* msp-1 gene amplified by nested PCR for block 2 region, 19 different alleles were identified of which 8 alleles were MAD20 (160-280 bp), 6 alleles were K-1(100-270 bp), and 5 alleles of RO33 type (100-200 bp). The overall MOI was 1.3, with the expected heterozygosity of 0.58 (Table 2).

Table 2. Genetic diversity and genotype multiplicity of *P. falciparum* clinical isolates from Symptomatic uncomplicated malaria patients in Adama and its surroundings (n =139)

Msp-1 alleles (n= 139)	Frequency %	Allele size (bp)	Number of alleles	Overall MOI	<i>He</i>
K-1	18 (12.9)	100-270	6	1.3	0.39
MAD20	67 (48.2)	160-280	8		
RO33	18(12.9)	100-200	5		
K-1 + MAD20	21(15.1)				
K-1 +RO33	4(2.9)				
MAD20 + RO33	8(5.8)				
K-1 + MAD20 +RO33	3(2.2)				
Total	139				

Of the total multiclonal infections 29(80%) were detected during the major malaria season (September to December) and the rest were from the isolates of minor malaria season in the region (Golassa & White, 2017). No statistically significant variation in the seasonal distribution of polyclonal infection ( $X^2 = 0.8$ ) in the study area. Moreover, 33 (92%) patients with polyclonal infection were having no travel history to other malaria endemic places. Thus, no statistical significant variation in the distribution of allelic variants in relation to patient's travel history in the study area ( $X^2 = 0.9$ ) (Table 3).

Table 3. The relationship between polyclonal infections, its seasonality and travel history of malaria patient due to *P. falciparum* in Adama and its surroundings (n = 139).

Allelic type	Season		Chi-square ( $X^2$ )	Travel history		Chi-square ( $X^2$ )
	Major	Minor		Yes	No	
MAD20+K-1	16	5	0.8	2	18	0.9
MAD20+RO33	8	0		1	7	
K-1+RO33	3	1		0	4	
MAD20+K-1+RO33	2	1		0	3	
Total	29	7		3	33	

In this study, of all *P. falciparum* isolates; 83 (60%) were from urban locality, and the rest are from rural area (Table 4). Allelic variants of msp-1 did not show significant variation between urban and rural areas; and seasonal variations were not statistically significant ( $X^2 = 0.23$ ) and ( $X^2 = 0.57$ ), respectively.

Table 4. Rural, urban and seasonal variations in the distribution of *P. falciparum* msp-1 block 2 region allelic variants in Adama and its surroundings (n =139)

Analysis of the spatial feature of msp-1 allelic variants and MOI from the study sites has shown that 65 (47%), 18 (13%), 17 (12%), 18 (13%), 21(15%) isolates were from Adama, Modjo, Wonji, Malkasa, and Olanciti sites, respectively. The spatial variation of the distribution of msp-1 allelic variant across sample collection sites was significantly related ( $P = 0.000$ ) (Figure 3), showing heterogeneity in their distribution (Figure 4).

## Discussion

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Msp-1 block 2 allele types	Number of positive alleles	Rural	Urban	$\chi^2$	Major malaria season	Minor malaria season	$\chi^2$
MAD20	67	25	42	0.23	46	21	0.57
K-1	18	11	7		13	5	
RO33	18	10	8		15	3	
MAD20+K-1	21	5	16		16	5	
MAD20+RO33	8	3	5		8	0	
K-1+RO33	4	3	1		3	1	
MAD20+K-1+RO33	3	1	2		2	1	

In Ethiopia, even though enormous efforts have been made at national and local levels to control and eventually eliminate malaria, limited molecular data exists on genetic polymorphism of *P. falciparum*, the most predominant and virulent malaria parasite in the region. The present study aimed to assess genetic polymorphism of *P. falciparum* clinical isolates from symptomatic patients based on block 2 region msp-1 genotypes and multiplicity of infection. This is the first study that widely investigated the status of *P. falciparum* genetic diversity from three districts of the study areas in central Ethiopia, and examined the spatial and seasonality of such polymorphism in relation to parasite density and other patient characteristics.

The study revealed that, geometric mean of parasite density abruptly rose up in school age children (SAC) and relatively stable afterwards (Fig. 2). In addition, no statistically significant correlation existed between parasite density and age of the patients (Pearson's correlation = 0.12, P = 0.6). Even though a number of factors may contribute to the fluctuation of parasitaemia level overtime in symptomatic patients, the geometric mean of microscopically detectable parasitemia levels could be used to explain the finding of this study (Shekalaghe et al., 2005). The major factor that mainly contributed for higher parasitaemia level in SAC is delayed acquisition of protective immunity during this immunological transition age making this age group more vulnerable to malaria infection than adults (Makenga et al., 2020).

In the present study we found that, multiple infections gradually rose up with age group (Fig. 2B), although the variation was not statistically significant ( $\chi^2 = 0.5$ ). This finding is in congruent with the report from Burkina Faso (Soulama et al., 2009), and Tanzania (Pinkevych et al., 2014), where they explained that episode of infection in children is commonly for very short duration and the duration of episode of infection increases with age contributing to the multiple infections. Other reports suggested that multiple infections vary with parasite density, immunity status, the overall prevalence of infection in the population and transmission intensity as reviewed by (Eldh et al., 2020, Pacheco et al., 2016, Kiwuwa et al., 2013). Other studies have shown an inverse association. Therefore, the relationship between

malaria patient age, level of parasitemia, number of clones of infection, transmission intensity and status of immunity to malaria parasite needs further investigation.

In the study we found that, no significant correlation existed between multiple clone infections of *P. falciparum* with seasonal variation of malaria incidence and travel history of patients (Table 3). In favor this finding, report from south western Ethiopia (Getachew et al., 2015), has shown that no correlation or negative correlation was found between the proportion of multi-clonal infections and parasite prevalence. On the other hand, report from Indonesia (Noviyanti et al., 2015), and Papua New Guinea (Fola et al., 2017), shows the presence of positive correlation between the rate of polyclonal infections and annual parasite incidence. The predominance of policlonality (92%) in those patients having no travel history depicts real features of malaria epidemiology with respect to the genetic marker of *msp-1* gene.

In this study, we found that, 26% of the isolates having multiple genotype infection. The overall MOI of 1.3 and the expected heterozygosity of (0.39) (Table 2). This finding differ from the report from north western Ethiopia and south western Ethiopia where Mohammed et al and (Abamecha et al., 2020) reported 75% and 80% frequency of multi-clonal infections, and 1.8 MOI (1.8) with *He* (0.79), 2.0 MOI and 0.43 *He*, respectively. This shows that malaria transmission in our study area exhibits slightly lower genetic diversity, compared with north western and south western Ethiopia. This could be due to the ongoing intensified scale up of interventions, differences in local epidemiology, demographic and environmental conditions that might have resulted in observed reduced genetic diversity pattern in Adama and its surroundings. In the present study, from 171 collected sample from field sites 139 (81%) successfully amplified for *msp-1* gene; revealing 19 different length polymorphism of *msp-1* allelic variants; 8 MAD20 (160 -330bp), 6 K-1 (100–270)bp, and 5 RO33 (100 -220bp). This shows the level of size polymorphism of *msp-1* alleles in our study area. However, the number of alleles identified may have been under estimated due to a number of limitations like sensitivity of PCR technique used, inability to differentiate minor fragments, the possible existence of similar size fragments and the same size fragment having different amino acid motifs (Abamecha et al., 2020, (Peakall & Smouse, 2012)

Size polymorphism of *msp-1* allelic variant identified in the present study is slightly higher than the report from Chewaka district of south western Ethiopia (Abamecha et al.) and Humera of north western Ethiopia (Mohammed et al., 2018). And less diverse than Kolla Shele district of south western part of Ethiopia (Mohammed et al., 2015), and more or less similar to the report from Equatorial Guinea (Chen et al., 2018), Bobo-Dioulasso of Burkina Faso (Somé et al., 2018). The major factor that may account for such variation could be; the scope of study sites covered and local malaria transmission patterns might have contributed. Gel analysis of the present study revealed that; from 139 *msp-1* amplicon 103 (74%) were monoclonal infection, whereas the remaining 36 (26%) were poly-allelic type, with 15% for (MAD20 + K-1), 5.7% for (MAD20 + RO33), 2.8% for (K-1 + RO33), and 2.1% were MAD20 + K-1 + RO33 type. The proportion of monoclonal infection was 48% MAD20, 13% K-1 and 13% RO33 (Table 2). This finding differ from the report from south western Ethiopia (Mohammed et al., 2015) and (Abamecha *et al.*), where they reported that K-1 was the most prevalent allelic family. Similarly, report from Cameroon, Gambia, Nigeria and

Loading [MathJax]/jax/output/CommonHTML/jax.js t was least predominant (Metoh et al., 2020, Zakeri et al.,

2005). On the other hand, in agreement with the report from north western part of Ethiopia (Mohammed et al.), Sudan by (Mahdi Abdel Hamid et al., 2016), and Equatorial Guinea (Chen et al., 2018) of the three msp-1 gene allelic families MAD20 was the predominant allelic type. Although the deriving forces for such variation needs further investigation; the difference in micro-ecological factors and the local transmission intensity (Yavo et al., 2016, Färnert et al., 2008), could play a significant role. Moreover, evolutionary process like genetic drift resulting uneven reproduction of the parasite lineages, types and rate of mutations, inbreeding, and the contribution of allelic variants in reproductive success are some of the factors that might have contributed for such variation (Escalante, 2020). In addition, in this study when the spatial feature of the distribution of msp-1 gene allelic variant in urban and rural areas (Table 4), were examined no statistically significant ( $P = 0.2$ ) variation was revealed. This finding could be taken as an evidence to show similarity of malaria epidemiology and the possible crossbreeding of the parasite populations between urban and rural settings in the study area, demanding similar intervention endeavors. Similarly, no statistically significant variation of multi-clonal infection of msp-1 gene with parasite density ( $P = 0.6$ ), and seasonality (major and minor malaria season) ( $P = 0.8$ ). This could be due to the characteristic feature of low transmission settings in such malaria endemic regions (Adjah et al., 2018, Mohammed et al., 2017). On the other hand, study sites based distribution of allelic variant has shown a highly significant variation ( $P = 0.000$ ), (Fig. 3). This could be due to the difference in local micro ecology of the areas, intensity of local transmission pattern, and differences in the age of the study population (Somé et al., 2018, Mwingira et al., 2011), and the relative potential differences and challenges on the ongoing malaria control and elimination endeavors in those sites.

## Conclusion

As malaria prevalence get pushed back due to various the scale up of intervention efforts, information on its genetic diversity with main emphasis on the relative predominance of specific allele is paramount. The study revealed that slightly lower genetic diversity of *P. falciparum* clinical isolates found in the study area. However, disproportionately high frequent MAD20 variant form detected. The driving force for such selective advantage for this allele under declining malaria prevalence in our study area demand further investigation. Moreover, declining malaria transmission setting like the one demonstrated in the present study and the like demands a unique evaluation of malaria transmission dynamics for targeted interventions. Thus, this information will serve as a baseline molecular evidence for further research on areas having similar malaria epidemiology to make the control and elimination efforts of malaria effective.

## Abbreviations

Bp

Base pair; msp-1: Merozoite surface protein-1; MOI: Multiplicity of infection; PCR: Polymerase chain reaction; SAC: School age children.

# Declarations

## Ethics approval and consent to participate

The research and ethical committee of Adama Science and Technology University reviewed and approved the study protocol, as verified through certificate reference number RECSOANS/BIO/01/2019. Oromia Health Bureau also approved the study protocol.

## Consent for publication

- Not applicable

## Availability of data and materials

All relevant data is included in manuscript, and the datasets analyzed in the study are available from the corresponding author on reasonable request. Additional data uploaded with main document.

## Competing interests

- The authors declare that they have no competing of interest

## Funding

- No funding was obtained for this study

## Authors' contributions

TF designed, conducted and analyzed the study, drafted and wrote the manuscript. TC assisted the molecular laboratory work. GS Organized the molecular laboratory work. HD designed the study and finally reviewed and approved the manuscript. LG conceived the idea, designed the study, supervised the molecular laboratory work, and reviewed and approved the manuscript.

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## References

1. Abamecha A, Abamecha A, Abamecha A, El-Abid H, Yilma D, Addisu W, Ibenthal A, Bayih AG, Noedl H, Yewhalaw D, Yewhalaw D, Moumni M, Abdissa A, Abdissa A. Genetic diversity and genotype multiplicity of Plasmodium falciparum infection in patients with uncomplicated malaria in Chewaka district, Ethiopia. *Malaria Journal*. 2020;19(1):1–9. <https://doi.org/10.1186/s12936-020-03278-6>.
2. Adjah J, Fiadzoe B, Ayanful-Torgby R, Amoah LE. Seasonal variations in Plasmodium falciparum genetic diversity and multiplicity of infection in asymptomatic children living in southern Ghana. *BMC Infect Dis*. 2018;18(1):1–10. <https://doi.org/10.1186/s12879-018-3350-z>.
3. Arie F, Gay F. (2013). *Malaria Control and Elimination*.
4. Bakhiet AMA, Abdel-muhsin AA, Elzaki SG, Al-hashami Z, Albarwani HS, Alqamashoui BA, Al-hamidhi S, Idris MA, Elagib AA, Beja-pereira A. Acta Tropica Plasmodium falciparum population structure in Sudan post artemisinin-based combination therapy. *Acta Trop*. 2015;148:97–104. <https://doi.org/10.1016/j.actatropica.2015.04.013>.
5. Chen JT, Li J, Zha GC, Huang G, Huang ZX, Xie D, De, Zhou X, Mo HT, Eyi JUM, Matesa RA, Obono MMO, Li S, Liu XZ, Lin M. Genetic diversity and allele frequencies of Plasmodium falciparum msp1 and msp2 in parasite isolates from Bioko Island, Equatorial Guinea. *Malaria Journal*. 2018;17(1):1–9. <https://doi.org/10.1186/s12936-018-2611-z>.
6. Das A, Holloway B, Collins WE, Shama VP, Ghosh SK, Sinha S, Hasnain SE, Talwar GP, Lal AA. Species-specific 18S rRNA gene amplification for the detection of P. Falciparum and P. Vivax malaria parasites. *Mol Cell Probes*. 1995;9(3):161–5. <https://doi.org/10.1006/mcpr.1995.0025>.
7. Diouf B, Diop F, Dieye Y, Loucoubar C, Dia I, Faye J, Sembène M, Perraut R, Niang M, Toure-Balde A. Association of high Plasmodium falciparum parasite densities with polyclonal microscopic infections in asymptomatic children from Toubacouta, Senegal. *Malaria Journal*. 2019;18(1):1–8. <https://doi.org/10.1186/s12936-019-2684-3>.
8. Eldh M, Hammar U, Arnot D, Beck HP, Garcia A, Liljander A, Mercereau-Puijalon O, Migot-Nabias F, Mueller I, Ntoumi F, Ross A, Smith T, Sondén K, Vafa Homann M, Yman V, Felger I, Färnert A. Multiplicity of Asymptomatic Plasmodium falciparum Infections and Risk of Clinical Malaria: A Systematic Review and Pooled Analysis of Individual Participant Data. *J Infect Dis*. 2020;221(5):775–85. <https://doi.org/10.1093/infdis/jiz510>.
9. Escalante AA. (2020). *HHS Public Access*. 7(4), 1–26. <https://doi.org/10.1128/microbiolspec.AME-0010-2019.MALARIA>.
10. Färnert A, Lebbad M, Faraja L, Rooth I. Extensive dynamics of Plasmodium falciparum densities, stages and genotyping profiles. *Malaria Journal*. 2008;7:5–9. <https://doi.org/10.1186/1475-2875-7-241>.

11. Farooq U, Malla N, Dubey ML. (2009). *falciparum field isolates from north and north-western India. June*, 109–116.
12. File T, Dinka H, Golassa L. (2019). A retrospective analysis on the transmission of Plasmodium falciparum and Plasmodium vivax: The case of Adama City, East Shoa Zone, Oromia, Ethiopia. *Malaria Journal*, 18(1). <https://doi.org/10.1186/s12936-019-2827-6>.
13. Fola AA, Harrison GLA, Hazairin MH, Barnadas C, Hetzel MW, Iga J, Siba PM, Mueller I, Barry AE. Higher complexity of infection and genetic diversity of plasmodium vivax than plasmodium falciparum across all malaria transmission zones of Papua New Guinea. *Am J Trop Med Hyg*. 2017;96(3):630–41. <https://doi.org/10.4269/ajtmh.16-0716>.
14. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, ... Barrell B. Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature*. 2002;419(6906):498–511. <https://doi.org/10.1038/nature01097>.
15. Getachew S, To S, Trimarsanto H, Thriemer K, Clark TG, Petros B, Aseffa A, Price RN, Auburn S. Variation in complexity of infection and transmission stability between neighbouring populations of Plasmodium vivax in Southern Ethiopia. *PLoS ONE*. 2015;10(10):1–15. <https://doi.org/10.1371/journal.pone.0140780>.
16. Golassa L, White MT. Population-level estimates of the proportion of Plasmodium vivax blood-stage infections attributable to relapses among febrile patients attending Adama Malaria Diagnostic Centre, East Shoa Zone, Oromia, Ethiopia. *Malaria Journal*. 2017;16(1):1–11. <https://doi.org/10.1186/s12936-017-1944-3>.
17. Hamid MMA, Mohammed SB, Hassan IM, El. (2013). *Genetic Diversity of Plasmodium falciparum Field Isolates in Central Sudan Inferred by PCR Genotyping of Merozoite Surface Protein 1 and 2*. 5(2), 95–101. <https://doi.org/10.4103/1947-2714.107524>.
18. Holder AA. (2019). *The carboxy-terminus of merozoite surface protein 1: structure, specific antibodies and immunity to malaria*. 2009, 1445–1456. <https://doi.org/10.1017/S0031182009990515>.
19. Holm I, Bentley GA. (1999). *The Crystal Structure of C-Terminal Merozoite ° Resolution, Surface Protein 1 at 1. 8 Å a Highly Protective Malaria Vaccine Candidate*. 3, 457–464.
20. Kiwuwa MS, Ribacke U, Moll K, Byarugaba J, Lundblom K, Färnert A, Fred K, Wahlgren M. Genetic diversity of Plasmodium falciparum infections in mild and severe malaria of children from Kampala, Uganda. *Parasitol Res*. 2013;112(4):1691–700. <https://doi.org/10.1007/s00436-013-3325-3>.
21. Learner P. I. (n.d.). *MALARIA*.
22. Lin CS, Uboldi AD, Epp C, Bujard H, Tsuboi T, Czabotar PE, Cowman XAF. (2016). *Multiple Plasmodium falciparum Merozoite Surface Protein 1 Complexes Mediate Merozoite Binding to Human*. 291(14), 7703–7715. <https://doi.org/10.1074/jbc.M115.698282>.
23. Mahdi Abdel Hamid M, Elamin AF, Albsheer MMA, Abdalla AAA, Mahgoub NS, Mustafa SO, Muneer

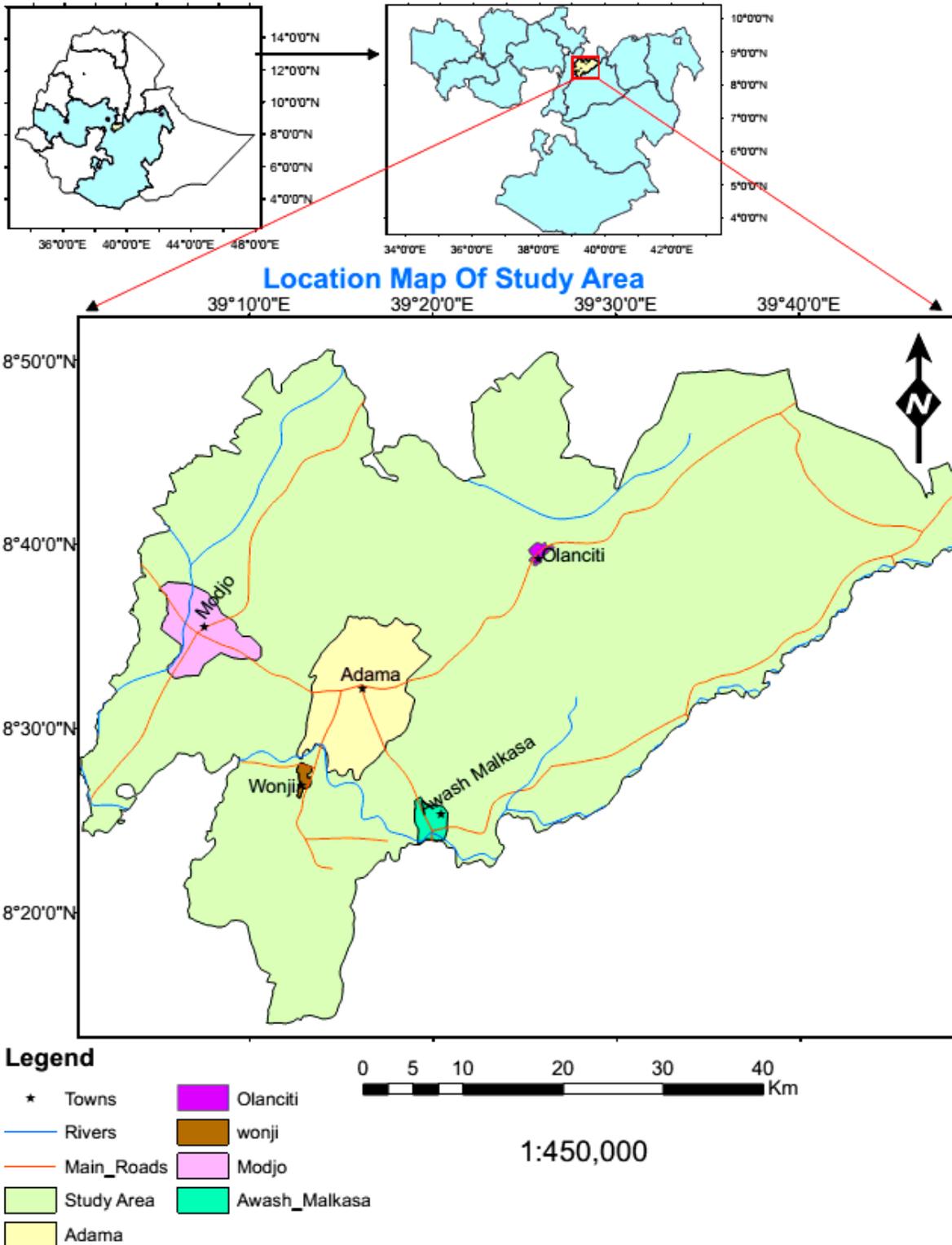
- patients with uncomplicated and severe malaria in Gezira State, Sudan. *Parasites Vectors*. 2016;9(1):1–8. <https://doi.org/10.1186/s13071-016-1641-z>.
24. Makenga G, Menon S, Baraka V, Minja DTR, Nakato S, Delgado-Ratto C, Francis F, Lusingu JPA, Van Geertruyden JP. Prevalence of malaria parasitaemia in school-aged children and pregnant women in endemic settings of sub-Saharan Africa: A systematic review and meta-analysis. *Parasite Epidemiology Control*. 2020;11:e00188. <https://doi.org/10.1016/j.parepi.2020.e00188>.
  25. Metoh TN, Chen JH, Fon-Gah P, Zhou X, Moyou-Somo R, Zhou XN. Genetic diversity of *Plasmodium falciparum* and genetic profile in children affected by uncomplicated malaria in Cameroon. *Malaria Journal*. 2020;19(1):1–15. <https://doi.org/10.1186/s12936-020-03161-4>.
  26. Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature*. 2002;415(6872):673–9. <https://doi.org/10.1038/415673a>.
  27. Mohammed H, Kassa M, Assefa A, Tadesse M, Kebede A. Genetic polymorphism of Merozoite Surface Protein-2 (MSP-2) in *Plasmodium falciparum* isolates from Pawe District, North West Ethiopia. *PLoS ONE*. 2017;12(5):1–11. <https://doi.org/10.1371/journal.pone.0177559>.
  28. Mohammed H, Kassa M, Mekete K, Assefa A, Taye G, Commons RJ. Genetic diversity of the msp-1, msp-2, and glurp genes of *Plasmodium falciparum* isolates in Northwest Ethiopia 11 Medical and Health Sciences 1108 Medical Microbiology. *Malaria Journal*. 2018;17(1):1–8. <https://doi.org/10.1186/s12936-018-2540-x>.
  29. Mohammed H, Mindaye T, Belayneh M, Kassa M, Assefa A, Tadesse M. (2015). *Genetic diversity of Plasmodium falciparum isolates based on MSP-1 and MSP-2 genes from Kolla-Shele area, Arbaminch Zuria District, southwest Ethiopia*. 1–7. <https://doi.org/10.1186/s12936-015-0604-8>.
  30. Mwingira F, Nkwengulila G, Schoepflin S, Sumari D, Beck H, Snounou G, Felger I, Olliaro P, Mugittu K. allele frequency and diversity in sub-Saharan Africa. *Malaria Journal*. 2011;10(1):79. <https://doi.org/10.1186/1475-2875-10-79>.
  31. Noviyanti R, Coutrier F, Utami RAS, Trimarsanto H, Tirta YK, Trianty L, Kusuma A, Sutanto I, Kosasih A, Kusriastuti R, Hawley WA, Laihad F, Lobo N, Marfurt J, Clark TG, Price RN, Auburn S. Contrasting Transmission Dynamics of Co-endemic *Plasmodium vivax* and *P. falciparum*: Implications for Malaria Control and Elimination. *PLoS Neglected Tropical Diseases*. 2015;9(5):1–19. <https://doi.org/10.1371/journal.pntd.0003739>.
  32. Pacheco MA, Lopez-Perez M, Vallejo AF, Herrera S, Arévalo-Herrera M, Escalante AA. Multiplicity of Infection and Disease Severity in *Plasmodium vivax*. *PLoS Neglected Tropical Diseases*. 2016;10(1):1–18. <https://doi.org/10.1371/journal.pntd.0004355>.
  33. Park DC, Neafsey DE, Daniels R, Chang H, Se PD, Schaffner SF, Hamilton EJ, Lukens AK, Tyne D, Van Mboup S, Sabeti PC, Ndiaye D, Wirth DF, Hartl DL, Volkman SK. Genetic Surveillance Detects Both Clonal Epidemic Transmission of Malaria following Enhanced Intervention in Senegal. 2013;8(4):4–10. <https://doi.org/10.1371/journal.pone.0060780>.
  34. Peakall R, Smouse PE. GenALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research on updates. *Bioinformatics*. 2012;28(19):2537–9.

<https://doi.org/10.1093/bioinformatics/bts460>.

35. Pinkevych M, Petravic J, Bereczky S, Rooth I, Färnert A, Davenport MP. Understanding the Relationship Between Plasmodium falciparum Growth Rate and Multiplicity of Infection. *J Infect Dis*. 2014;211(7):1121–7. <https://doi.org/10.1093/infdis/jiu561>.
36. Scherf A, Carter R, Petersen C, Alano P, Nelson R, Aikawa M, Mattei D, Pereira da Silva L, Leech J. Gene inactivation of Pf11-1 of Plasmodium falciparum by chromosome breakage and healing: identification of a gametocyte-specific protein with a potential role in gametogenesis. *The EMBO Journal*. 1992;11(6):2293–301. <https://doi.org/10.1002/j.1460-2075.1992.tb05288.x>.
37. Sexton AE, Doerig C, Creek DJ, Carvalho TG. Post-Genomic Approaches to Understanding Malaria Parasite Biology: Linking Genes to Biological Functions. *ACS Infectious Diseases*. 2019;5(8):1269–78. <https://doi.org/10.1021/acsinfecdis.9b00093>.
38. Shekalaghe S, Alifrangis M, Mwanziva C, Enevold A, Mwakalinga S, Mkali H, Kavishe R, Manjurano A, Sauerwein R, Drakeley C, Bousema T. (2005). *Low density parasitaemia, red blood cell polymorphisms and Plasmodium falciparum specific immune responses in a low endemic area in northern Tanzania*. 1, 1–7. <https://doi.org/10.1186/1471-2334-9-69>.
39. Smith JD, Rowe JA, Higgins MK, Lavstsen T. Malaria's deadly grip: Cytoadhesion of Plasmodium falciparum-infected erythrocytes. *Cell Microbiol*. 2013;15(12):1976–83. <https://doi.org/10.1111/cmi.12183>.
40. Smythe JA, Coppel RL, Day KP, Martint RK, Oduolat AMJ, Kemp DJ, Anders RF. (1991). *Structural diversity in the Plasmodium falciparum merozoite surface antigen 2*. 88(March), 1751–1755.
41. Snounou G, Singh B. (2002). *Nested PCR analysis of Plasmodium parasites*. May 2014. <https://doi.org/10.1385/1-59259-271-6>.
42. Soe TN, Wu Y, Tun MW, Xu X, Hu Y, Ruan Y, Ye A, Win N, Nyunt MH, Cho N, Mon N, Han KT, Aye KM, Morris J, Su P. (2017). *Genetic diversity of Plasmodium falciparum populations in southeast and western Myanmar*. 1–6. <https://doi.org/10.1186/s13071-017-2254-x>.
43. Solomon A, Kahase D, Alemayehu M. Trend of malaria prevalence in Wolkite health center: An implication towards the elimination of malaria in Ethiopia by 2030. *Malaria Journal*. 2020;19(1):1–8. <https://doi.org/10.1186/s12936-020-03182-z>.
44. Somé AF, Bazié T, Zongo I, Yerbanga RS, Nikiéma F, Neya C, Taho LK, Ouédraogo JB. Plasmodium falciparum msp1 and msp2 genetic diversity and allele frequencies in parasites isolated from symptomatic malaria patients in Bobo-Dioulasso, Burkina Faso. *Parasites Vectors*. 2018;11(1):1–8. <https://doi.org/10.1186/s13071-018-2895-4>.
45. Sonko ST, Jaiteh M, Jafali J, Jarju LBS, Alessandro UD, Camara A. (2014). *Does socio-economic status explain the differentials in malaria parasite prevalence ? Evidence from The Gambia*. 1–12.
46. Soulama I, Nébié I, Ouédraogo A, Gansane A, Diarra A, Tiono AB, Bougouma EC, Konaté AT, Kabré GB, Taylor WR, Sirima SB. Plasmodium falciparum genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in burkina Faso. *Malaria Journal*. 2009;8(1):1–8.

47. Takala SL, Coulibaly D, Thera MA, Batchelor AH, Cummings MP, Escalante AA, Ouattara A, Traoré K, Djimdé AA, Doumbo OK, Plowe CV. (2010). *Malaria: Implications for Vaccine Development*. 1(2), 1–21. <https://doi.org/10.1126/scitranslmed.3000257>.Extreme.
48. WHO. (2020). World Malaria Report 2020. World Health Organization. In *World Health*.
49. Woehlbier U, Epp C, Kauth CW, Lutz R, Long CA, Coulibaly B, Kouyate B, Arevalo-herrera M. (2006). *Analysis of Antibodies Directed against Merozoite Surface Protein 1 of the Human Malaria Parasite Plasmodium falciparum*. 74(2), 1313–1322. <https://doi.org/10.1128/IAI.74.2.1313>.
50. *World malaria report 2017*. (2017).
51. Yavo W, Konaté A, Mawili-Mboumba DP, Kassi FK, Mbuyi T, Angora ML, Menan EK, E. I. H., & Bouyou-Akotet MK. (2016). Genetic polymorphism of msp 1 and msp 2 in Plasmodium falciparum isolates from Côte d'Ivoire versus Gabon. *Journal of Parasitology Research*, 2016. <https://doi.org/10.1155/2016/3074803>.
52. Zakeri S, Bereczky S, Naimi P, Gil JP, Djadid ND, Färnert A, Snounou G, Björkman A. Multiple genotypes of the merozoite surface proteins 1 and 2 in Plasmodium falciparum infections in a hypoendemic area in Iran. *Tropical Medicine International Health*. 2005;10(10):1060–4. <https://doi.org/10.1111/j.1365-3156.2005.01477.x>.

## Figures



**Figure 1**

Map of the study area, showing sample collection sites (Adama, Modjo, Wonji, Awash Malkasa, and Olanciti) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or

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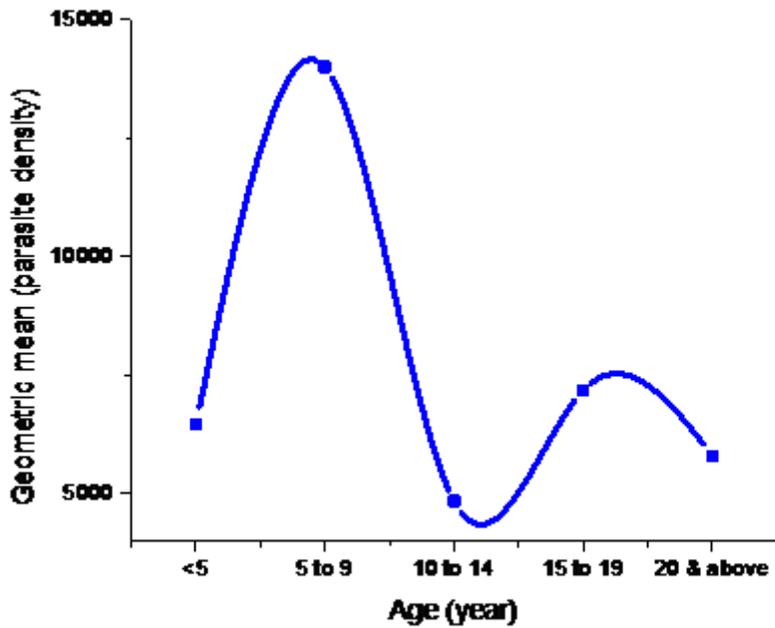


Figure 2

Relationship between geometric mean of the parasite density of *P. falciparum* patients with age groups in Adama and its surroundings (n= 139).

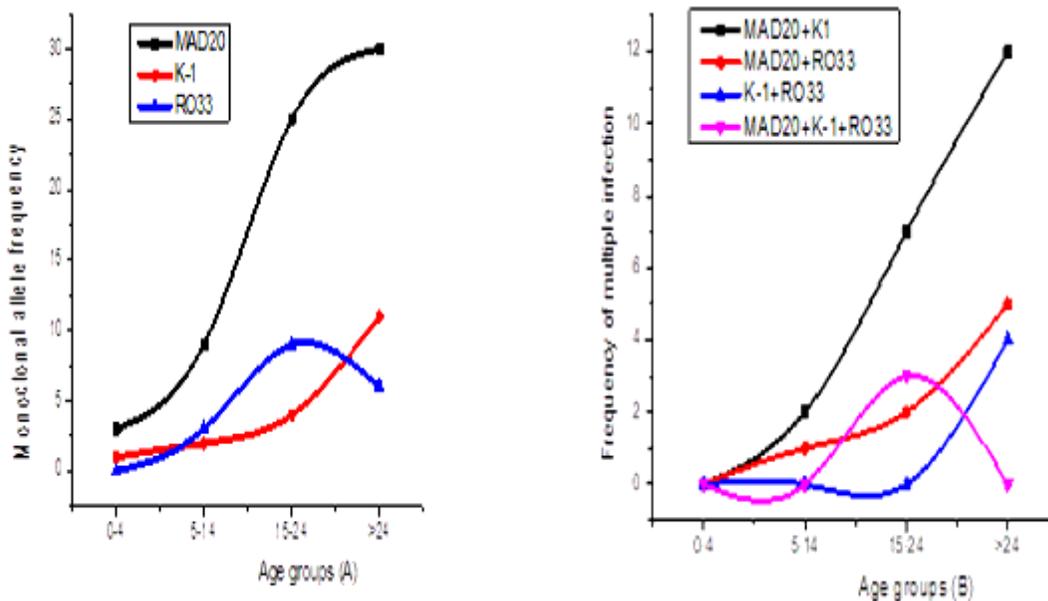


Figure 3

The frequency of monoclonal (A) and polyclonal (B) allele typing of *msp-1* gene across different age groups of malaria patients due to *P. falciparum* in Adama and its surroundings (n = 139)

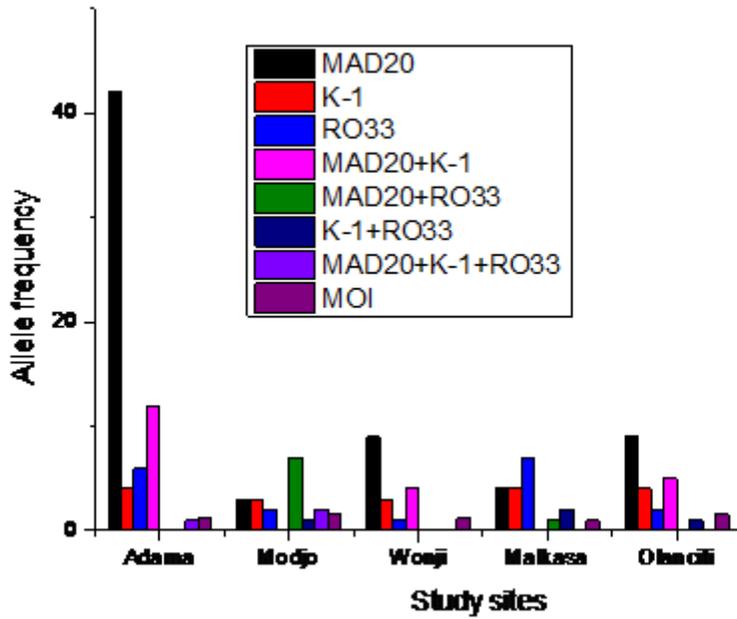


Figure 4

Distribution *P. falciparum* msp-1 gene allelic families isolated in Adama and its surroundings (n= 139)

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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