Temporal dynamics of *Plasmodium falciparum* population in Metehara, east-central Ethiopia

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**Abstract**

**Background:** *Plasmodium falciparum* is the most serious, genetically most complex and fastest-evolving malaria parasite. Information on genetic diversity of this parasite would guide policy decision and malaria elimination endeavors. This study explored the temporal dynamics of *P. falciparum* population in two time points in Metehara, east-central Ethiopia.

**Methods:** The participants were quantitative real-time polymerase chain reaction-confirmed patients who were recruited for uncomplicated falciparum malaria therapeutic efficacy test in 2015 and 2019. Dry blood spot samples were analysed by the nested PCR to genotype *P. falciparum* merozoite surface protein (*msp1*, *msp2*) and glutamate-rich protein (*glurp*) genes.

**Results:** While *msp1*, *msp2* and *glurp* genotypes were successfully detected in 26(89.7%), 24(82.8%) and 14(48.3%) of 2015 samples (*n* = 29); the respective figures for 2019 (*n* = 41) were 31(68.3%), 39(95.1%), 25(61.0%). In 2015, the frequencies of K1, MAD20 and RO33 allelic families of *msp1*, and FC27 and IC/3D7 of *msp2* were 19(73.1%), 8(30.6%), 14(53.8%), 21(87.5%), 12(50.5%); and in 2019 it was 15(48.4%), 19(61.3%), 15(48.4%), 30(76.9%), 27(69.2%) respectively. MAD20 has shown dominance over both K1 and RO33 in 2019 compared to the proportion in 2015. Similarly, although FC27 remained dominant, there was shifting trend in the frequency of IC/3D7 from 50.5% in 2015 to 69.2% in 2019. The multiplicity of infection (MOI) and expected heterozygosity index (*He*) in 2015 and 2019 were respectively [1.43 ± 0.84] and [1.15 ± 0.91], 0.3 and 0.03 for *msp1*. However, there was no significant association between MOI and age or parasitaemia in both time points.

**Conclusion:** The lower genetic diversity in *P. falciparum* population in the two time points and overall declining trend as demonstrated by the lower MOI and *He* may suggest better progress in malaria control in Metehara. But, the driving force and selective advantage of switching to MAD20 dominance over the other two *msp1* allelic families, and the dynamics within *msp2* alleles needs further investigation.

**Keywords:** Merozoite surface proteins 1 and 2, Glutamate-rich protein, Genetic diversity, Multiplicity of infection, Heterozygosity, Allelic family, Gene, Allele, *Plasmodium falciparum*

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**Background**

Malaria remains among the leading deadliest diseases despite concerted efforts to eliminate it. Globally, 241 million malaria cases and 627,000 deaths occurred in 2020, which is more by 14 million cases and 69,000
death compared to what was reported in 2019 [1]. This rise was primarily attributed to the multifaceted COVID-19 disruption. In sub-Saharan Africa, the public health and socioeconomic burden of malaria is the heaviest [2]. Although 68% of the Ethiopian population still lives in malaria endemic areas, substantial progress was achieved and the health ministry set an ambitious plan of malaria elimination by 2030 [3]. A transition from high or moderate to low malaria transmission and eventual elimination and eradication requires continuous insight into the dynamics and genetic structure of the parasite population [4, 5].

Plasmodium falciparum, which is the gravest malaria parasite, is also the most challenging species because of its tremendous genetic plasticity [6] in terms of escaping diagnostics [7], therapeutics [8] and host immunity and thus vaccines [9]. It is genetically the most complex and most elastic Plasmodium species. Thus, continuous follow-up and dissection of the population of this parasite including systems biology approach has to be integrated into the routine malaria surveillance program and other research priorities to achieve elimination goals [10–12]. Multiplicity of infection (MOI) and expected heterozygosity (He) is considered useful surrogate marker of changes in malaria transmission intensity besides several non-genetic metrics [13].

In light of this, P. falciparum genetic diversity and population structure is commonly studied by genotyping block 2, block 3 and RII repeat regions of its merozoite surface protein (msp1, msp2) and glutamate-rich protein (glurp) genes, respectively [14]. Merozoite surface proteins (MSPs) encoded by msp genes, are both integral and peripheral membrane proteins on the surface of Plasmodium merozoites. MSP1 and MSP2 are the most abundant glycosylphosphatidylinositol-anchored proteins among the MSPs. These surface protein complexes are involved in multiple host–parasite interactions and are, therefore, of significant clinical and epidemiological relevance [15]. MSP1 which is 190-kDa is the most abundant MSP protein and has a major role in erythrocyte invasion, and is among leading blood-stage malaria vaccine candidates [16–19]. The msp1 gene contains 17 blocks of sequence flanked by conserved regions block 2, and is grouped commonly into three allelic families namely K1, MDA20 and RO33 although a new allele family, MR, is also reported to occur worldwide [20]. The msp2, on the other hand, is a glycoprotein encoding gene consisting 5 blocks where the central block is the most polymorphic. The msp2 alleles are grouped into two allelic families, FC27 and IC/3D7 [21]. The glurp gene has R0 (N-terminal nonrepetitive) and R2 (C-terminal repetitive) regions and encodes for a 220 kDa glutamate-rich protein (GLURP) antigen expressed throughout the lifecycle of the malaria parasites [22–24].

In contrast to the global efforts, the extent of P. falciparum genetic diversity is little investigated in Ethiopia. The present study was aimed at assessing the temporal changes in the genetic diversity of P. falciparum population circulating in Metehara using samples acquired in two time points, 2015 and 2019, in the backdrop of deployment of massive intervention strategies in the area and the country at large.

Methods
Study site
Samples used for this study were collected from Metehara, a sentinel site identified for routine monitoring of the therapeutic efficacy of artemether–lumefantrine in east-central Ethiopia (Fig. 1). Metehara is located at 8′33′N 39°16′E situated in the rift valley area at an elevation of 947 m some 128 km to southeast of Addis Ababa. The Awash River basin provides a favorable microhabitat to support malaria vector breeding. A sugar factory estate irrigation system which depends on the nearby Awash River for the cultivation of sugarcane, and Beska River are additional breeding sites for the malaria mosquitoes.

Study population and samples
Febrile patients having symptoms consistent with clinical malaria who were attending Metehara Health Centre were recruited; finger-prick blood samples were drawn and examined using malaria rapid detection kit (Car-eStart™ combo, Acces Bio, USA). Concurrently, smears were prepared and screened, and dried blood spots (DBS) were collected onto Whatman 903® filter paper (Schleicher & Schuell Bio Science, Keene, NH 03431, USA) for molecular analysis. The DBS were transported, by cold chain, to Addis Ababa and stored at − 20 °C at malaria research laboratory, Ethiopian Public Health Institute (EPHI), until analysed.

Parasite DNA extraction and infection confirmation
The molecular biology analysis of this work was done at Addis Ababa University, Aklilu Lemma Institute of Pathobiology malaria molecular laboratory. DNA was extracted from the DBS samples using the QIA amp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer’s protocol. The DNA was finally eluted with a final volume of 100 µl for each sample and stored at − 20 °C until it was used for the amplification reaction. Plasmodium falciparum mono-infection was confirmed by the quantitative real-time polymerase chain reaction (qRT-PCR) and parasitaemia quantified [25]. Primers and positive controls were obtained from MR4 (now BEI Resources, Manassas, VA, USA). All PCR
reaction mixtures were incubated in a thermal cycler (Perkin-Elmer Cetus PE 9600 iCycler thermal Cycler, Serial number 582BR006802 (Bio-Rad, Hercules, USA)). Primers used for genotyping *msp1* (block 2), *msp2* (block 3) and *glurp* (R2) are listed in Additional files 1, 2 and 3, respectively.

For genotyping the target genes, the established nested PCR method was used [26]. Two-rounds of PCR reactions were carried out in a final volume of 20 μl. In primary round reaction, 4 μl of gDNA, 10 μl GoTaq Green Master Mix (Promega), 0.5 μl (0.5 μM) of each primer and 5 μl nuclease-free water were used. In secondary rounds, 2 μl of PCR amplicon, 7 μl nuclease-free water was added to the master mix preparation for secondary amplifications reaction. Cycling conditions for both PCR reactions were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles for primary and 30 cycles for secondary reactions of denaturation at 95 °C for 1 min (94 °C for 1 min for the nested reaction) annealing at 58 °C for 2 min and extension at 72 °C for 2 min and; a final extension was carried out at 72 °C for 5 min. Positive (3D7) and DNA-free negative controls were included in each set of reactions.

The nested PCR products were resolved in 2% agarose gels (Caisson, Utah, USA), stained with ethidium bromide submerged in 0.5× TBE (Tris-borate EDTA) buffer electrophoresis at 120 V, 400 A for 45 min and visualized under UV trans-illumination and photographed (VersaDoc®, Bio-Rad, Hercules, USA) at 302 nm on gel documentation system. The sizes of DNA fragments were estimated by visual inspection using a 100 base pair (bp) DNA ladder marker (New England Biolabs. Inc, UK).

Presence of more than one genotype was taken as a polyclonal infection, while a single allele was considered as a monoclonal infection [27]. For *msp1* and *msp2* alleles, fragment sizes were within 20 bp interval, and for *glurp* 50 bp interval [28].

**Data analysis**

The statistical software SPSS (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used to conduct all statistical analyses. For each sample, MOI was scored as the maximum number of alleles observed when all loci were taken into account and the average MOI was calculated for each sub-population. The allelic frequency and mean MOI of the *msp1*, *msp2* and *glurp* genes were calculated using proportions of allele comparisons and the Chi-squared tests. The MOI was compared using the Student t-test to assess the relationship between MOI, parasite density and patient age, and the spearman’s rank correlation coefficient was calculated. *P*-value < 0.05 was selected as a threshold for statistically significant differences. As a measure for genetic diversity, the expected heterozygosity (*He*) was calculated.
using the formula $He = \left( \frac{n}{n-1} \right) \left( 1 - \sum p^2 \right)$ where ‘n’ is the number of isolates analysed and ‘p’ the frequency of each different allele at a locus [29].

Results

Demographic, parasitological and clinical data

Of the total participants in 2015 ($n = 33$), males were 17 (51.5%) with a male-to-female ratio of 1.1. Males in 2019 ($n = 50$) were 26 (52.0%) and the male-to-female ratio was also 1.1. While the mean (range) age of the participants in 2019 was 24 (3–60) year, in 2015 it was 25 (4–60). From these 83 total samples screened for $P. falciparum$ mono-infection, microscopy and RDT, respectively, could detect only 58 (69.9%) and 47 (56.6%) of the cases. The qPCR confirmed these and identified additional missed cases by microscopy/RDT raising the number of $P. falciparum$-positives to 70 (84.3%), 29 for 2015 samples and 41 for 2019. Therefore, the number of samples genotyped was 70. Asexual mean parasitaemia was 1593.00 (95% CI 992.10–2194.06) in 2015 and 3893.60 (95% CI 2286.05–5501.01) in 2019 (Table 1). The percentage of gametocytaemic patients was 3.01 and 2.06 in 2015 and 2019, respectively.

Frequencies of $msp1$, $msp2$ and $glurp$ allelic families

While $msp1$, $msp2$ and $glurp$ genotypes were successfully detected in 26 (89.7%), 24 (82.8%) and 14 (48.3%) of 2015 samples ($n = 29$), respectively; the respective figures for 2019 ($n = 41$) were 31 (75.6%), 39 (95.1%), 25 (61.0%). In 2015, the frequencies of K1, MAD20 and RO33 allelic families of $msp1$ were 19 (73.1%), 8 (30.6%) and 14 (53.8%), respectively. In 2019, the frequencies of the respective alleles were 15 (48.4%), 19 (61.3%) and 15 (48.4%). In 2015, the frequencies of FC27 and IC/3D7 allelic variants of $msp1$ were 87.5% and 50.5%, respectively. In 2019, the frequencies of the respective alleles were 76.9% and 69.2%, respectively (Table 2).

As far as $msp1$ allelic variants are concerned, among the samples analysed the frequency of K1 allele dropped from 73.1% in 2015 to 48.4% in 2019 while the frequency of MAD20 allele increased from 30.6% in 2015 to 61.3% in 2019. However, the frequency of RO33 allelic variant was 53.8% in 2015 and 50.5% in 2019. For $msp2$ allelic variants, the frequencies of FC27 and IC/3D7 alleles were 87.5% and 50.5% in 2015, respectively, and the corresponding figures in 2019 were 76.9% and 69.2%, respectively. In other words, the frequency of FC27 allele decreased from 87.5% in 2015 to 76.9% in 2019 while the frequency of IC/3D7 allele increased from 50.5% in 2015 to 69.2% in 2019. Higher proportion of $glurp$ was detected in 2019 compared to the baseline. However, for all the three genes the increase or decline of the allelic families or variants was not statistically significant.

MOI and $He$

The proportion of polyclonal infections for $msp1$ were 46.6% (12/26) in 2015 and 51.6% (16/31) in 2019, and for $msp2$ it was 37.5 (9/24) and 46.2 (18/39) respectively. Detailed monoclonal and polyclonal infections by the different allelic families in the two time points for the two genes are indicated in Tables 2, 3 and 4. The mean MOI in 2015 and 2019 were, respectively, $[1.43 \pm 0.84]$ and $[1.15 \pm 0.91]$ for $msp1$, $[1.18 \pm 0.67]$ and $[1.39 \pm 0.59]$ for

Table 1

Demographic, parasitological and clinical characteristics of study participants in 2015 and 2019 in Metehara, east-central Ethiopia

| Characteristics          | 2015 (N = 33) | 2019 (N = 50) |
|--------------------------|--------------|--------------|
| Male/female ratio        | 1.1          | 1.1          |
| Age (year), mean ± SD    | 25 ± 1.7     | 24 ± 1.7     |
| Age group                |              |              |
| < 5, no (%)              | 2(6.9)       | 6(12.0)      |
| 5–15, no (%)             | 7(21.2)      | 9(18.0)      |
| > 15, no (%)             | 24(82.8)     | 35(70.0)     |
| Feverish patients on day 0 (%) (axillary temperature > 37.5 °C) | 27(81.8) | 40(80.0) |
| Temperature (°C), mean (range) | 38.03(36.10–41.40) | 37.7(36.40–40.20) |
| Mean parasitaemia (parasites/µl) | 1593.00 (95% CI 992.10–2194.06) | 3893.60 (95% CI 2286.05–5501.01) |
| Gametocytaemic patients (%) | 3.01         | 2.06         |

SD: standard deviation, no: number, µl: micro liter, CI: confidence interval

Table 2

Frequency of monoclonal and polyclonal infections of alleles of $msp1$ and $msp2$ genes of $Plasmodium falciparum$ in 2015 and 2019 in Metehara, east-central Ethiopia

| Gene       | Allele | Year | 2015 | 2019 | Total |
|------------|--------|------|------|------|-------|
|            |        |      | 10   | 7    | 17    |
|            |        |      | 0    | 7    | 7     |
|            |        |      | 4    | 2    | 6     |
|            |        |      | 2    | 2    | 4     |
|            |        |      | 2    | 2    | 4     |
|            | K1     |      | 2    | 3    | 5     |
|            | MAD20  |      | 4    | 7    | 11    |
|            | RO33   |      | 3    | 3    | 6     |
|            | K1 + MAD20 |      | 4    | 7    | 11    |
|            | K1 + RO33 |      | 3    | 3    | 6     |
|            | MAD2 + RO33 |      | 3    | 3    | 6     |
|            | K1 + MAD20 + RO33 |      | 3    | 3    | 6     |
|            | IC/3D7 |      | 3    | 9    | 12    |
|            | FC27   |      | 12   | 12   | 24    |
|            | IC/3D7 + FC27 |      | 9    | 18   | 27    |
msp2, and [0.20 ± 0.42] and [0.61 ± 0.31] for glurp. Similarly, $He$ of msp1, msp2 and glurp in 2015 and 2019 were respectively 0.3, 0.03; 0.56, 0.57; and 0.55, 0.61 all with statistically significant variation. Neither parasite density (Spearman rank correlation 0.162, $p = 0.560$) nor participant age (Spearman rank correlation 0.118, $p = 0.670$) was significantly associated with either msp1 or msp2 MOI in both 2015 and 2019 samples.

**Allele fragment size**

Fragments sizes, in bp, for msp1 allelic families ranged for K1 (300–400), MAD20 (300–450) and RO33 (300–350); and that of msp2 allelic families FC27 and IC/3D7 was (400–800). Eight R2 repeat region fragments of glurp, designated I–VIII, with size range of 501–900 bp were identified (Table 5). Genotype II (551–600) was the most frequent variant seen in both 2015 (3(30.3%)) and 2019 (4(36.3%)).

**Discussion**

This study is the first of its kind to assess *P. falciparum* genetic diversity in two time points in a low malaria transmission area in Ethiopia. The genetic diversity of *P. falciparum* was relatively lower genetic diversity in 2019 than 2015 at least with respect to msp1 gene. Moreover, in terms of size polymorphism, the msp1 gene was higher in frequency than msp2 and glurp. The core findings of this study regarding the relative distributions of the allelic families of the three genes, MOI, $He$, and relationship between MOI and parasitaemia or patient age are similar to some studies and depart from others that have been done in various settings of Ethiopia, sub-Saharan Africa as well as globally.

*Plasmodium falciparum* genetic diversity may vary with malaria transmission intensity and ongoing control interventions, sample size, season of sample collection, and study design including participant clinical status, and socio-demographic characteristics. Some studies focused on febrile but uncomplicated patients other involved febrile and complicated cases, yet others worked on asymptomatic participants. There are several additional field and laboratory factors that limit comparison and contrast between various studies. Therefore, *P. falciparum* genetic diversity, MOI and overall population structure data interpretation and comparisons need caution.

### Table 3

Total percentage polyclonal infections for msp1, msp2 genes of *Plasmodium falciparum* in 2015 and 2019 in Metehara, east-central Ethiopia

| Gene | Polyclonal infection, no (%) |
|------|-----------------------------|
|      | 2015 | 2019 |
| msp1 | 12(38.7) | 15(48.4) |
| msp2 | 9(37.5) | 18(46.2) |

### Table 4

Multiplicity of infection and expected heterozygosity index for *Plasmodium falciparum* msp1, msp2 and glurp genes in Metehara, east-central Ethiopia

| Year | MOI (mean ± SD) | $He$ |
|------|-----------------|------|
|      | msp1 | msp2 | glurp | msp1 | msp2 | glurp |
| 2015 | 1.43 ± 0.84 | 1.18 ± 0.67 | 0.20 ± 0.42 | 0.30 | 0.56 | 0.55 |
| 2019 | 1.15 ± 0.91 | 1.39 ± 0.59 | 0.61 ± 0.31 | 0.03 | 0.57 | 0.61 |
| Overall | 1.67 | – | – | 0.48 | 0.70 | 0.58 |

$He$: heterozygosity index, MOI: multiplicity of infection, SD: standard deviation

### Table 5

Allelic size variants of msp1 and msp2 allelic families, and glurp R2 repeat region of *Plasmodium falciparum* population in 2015 and 2019 in Metehara, east-central Ethiopia

| Gene and allelic family | Variant fragment size (bp) | Frequency (%) |
|-------------------------|----------------------------|---------------|
|                        | 2015 | 2019 | 2015 | 2019 |
| msp1                   |     |      |      |      |
| K1                     | 300–400 | 19(73.1) | 15(48.4) |
| MAD20                  | 300–450 | 8(30.8) | 18(58.1) |
| RO33                   | 300–350 | 14(53.8) | 15(48.4) |
| msp2                   |     |      |      |      |
| ID/3D7                 | 400–800 | 12(50.0) | 27(69.2) |
| FC27                   | 400–800 | 21(87.5) | 30(76.9) |
| Glurp                  |     |      |      |      |
| I                      | 501–550 | 1(10.0) | 1(9.1) |
| II                     | 551–600 | 3(30.3) | 4(36.3) |
| III                    | 601–650 | 1(10.1) | 1(9.1) |
| IV                     | 651–700 | 1(10.1) | 1(9.1) |
| V                      | 701–750 | 1(10.1) | 1(9.1) |
| VI                     | 751–800 | 1(10.1) | 1(9.1) |
| VII                    | 801–850 | 1(10.1) | 1(9.1) |
| VIII                   | 851–900 | 1(10.1) | 1(9.1) |

bp: base pair
Previous studies from divergent settings in Ethiopia like in the southwest [30] and northwest [31–33] using variable sample size (88–118) collected during peak malaria transmission season, recorded high level (59–78%) P. falciparum polyclonal infections with respect to the target genes of this study. It was only in a semi-arid area in the northeast of Ethiopia that P. falciparum polyclonal infection was reported below 50% [34]. The overall mean MOI in these studies that reported high polyclonal infections and based on uncomplicated malaria patients ranged 1.2–2.8 and He 0.20–0.82. None of these same four studies documented significant relationship between MOI and patient age or parasitaemia. While allelic family K1 of msp1 was dominant in the southwestern and two northwestern sites, MAD20 was higher from one northwestern setting. Concerning msp2 allelic families, in three of the above sites the frequency of IC/3D7 was higher although marginally in the northwest. In the rest two sites FC27 was predominant.

In another study in the southwest Ethiopia [35], K1 was the predominant msp1 allelic family followed by MAD20 and RO33, and msp2 allelic family FC27 had higher frequency compared to IC/3D7 with 80% polyclonal infection and overall mean MOI of 3.2. The study reported He of 0.43 for msp1 and 0.85 for msp2 demonstrating high levels of genetic diversity and mixed-strain infections. However, it did not observe significant association between MOI and age or parasite density.

A study in east-central Ethiopia detected more frequent MAD20 allelic family of msp1 followed by K1 and RO33 [36] and overall MOI 1.67 and He 0.48 indicating slightly low P. falciparum genetic diversity. Similarly, the distribution of IC/3D7 and FC27 allelic families of msp2 was nearly comparable with polyclonal infection 40.5%, MOI of 1.4 and He 0.49 in the east-central part of the country [37]. This is considered intermediate P. falciparum genetic diversity. The current study, which is along the same rift valley with this previous studies, has similar MAD20 dominance suggesting possible P. falciparum strain connectivity. Similarly lower P. falciparum genetic diversity was noticed in the western edge of Ethiopia where MAD20 of msp1 and FC27 of msp2 reported to predominate [38].

Dominance of the msp1 allelic family MAD20 among uncomplicated malaria cases was recorded in neighboring Sudan although the distributions of FC27 and IC1/3D7 msp2 allelic families were approximately the same across disease severity [39]. The authors observed no statistically significant differences in MOI between different age groups although the majority of P. falciparum isolates from uncomplicated and severe malaria patients consisted of multiple genotypes.

In other sub-Saharan Africa countries like Congo Brazzaville [40], K1 allelic family tended to predominate followed by MAD20 and the distribution of msp2 allelic families 3D7 and FC27 was nearly equal. The study revealed overall MOI of 2.64 with 86% polyclonal infections and no correlation between MOI age or parasite density. Moreover, a study [41] in selected sub-Saharan Africa countries with varying levels of endemicity, namely Malawi, Tanzania, Uganda, Burkina Faso and São Tomé found highly polymorphic on P. falciparum msp1, msp2 and glurp markers with low allele frequencies but very high He values (0.68–0.99).

In South Africa [42], RO33 allele of msp1 (84.8%), 3D7 allele of msp2 (90.8%) were dominant in 2006–2007, but in the same setting in 2013–2016 polyclonal infection rates of msp1 ranged from 76.7 to 29.1% and msp2 from 62.4 to 28.3% with significant decline of allelic types in 2013–2016. Similarly, the MOIs for both msp1 and msp2 were higher in isolates in 2006–2007 than those in 2013–2016 showing dramatic reduction in P. falciparum genetic diversity. On the other hand, K1 of msp1 and FC27 of msp2 were the principal allelic families detected in Madagascar [43] although the proportions of both allelic families of both genes varied significantly between sites although genetic diversity was similar between sites and that parasite flow within sites was limited.

In West Africa African countries such as Burkina Faso, msp1 allelic family K1 was predominant followed by MAD20 and R033 [44]. In msp2, the 3D7 allelic family was the most frequently detected with 93.1% compared to FC27 with 41.3%. High genetic diversity and allelic frequencies of both msp1 and msp2 were seen with overall mean MOI of 1.95. A latter study in Burkina Faso that genotyped P. falciparum msp1 and msp2 demonstrated a negative correlation between MOI and host age and parasite density, suggesting within-host competition among co-infecting genetically distinct P. falciparum variants [45]. Moreover, the authors reported the presence of each allelic family of the msp1 and msp2 genes year round with no significant monthly fluctuation.

In Cameroon [46], msp1 K1 allelic family was the most abundant and the proportion of polyclonal infection was 60%. In Southern district of Brazzaville [47], K1 allelic family was predominant and 3D7 family was the most prevalent in the msp2 gene. Overall, the mean MOI was 2.2 and 83% of the cases were polyclonal infections. No statistically significant correlation was seen between MOI and patient age but there was with parasite density. *Plasmodium falciparum* clinical isolates had high diversity and mainly of multiple clones. The basis for the positive association between parasite densities and MOI was discussed by the authors.
From Equatorial Guinea [48], MAD20 allelic family was very predominant (96.07%) followed by K1; and concerning msp2, the FC27 allelic family was the most frequently detected with (97.69%). A study [49] that examined the genetic diversity of *P. falciparum* within a household in north-central Nigeria, reported remarkable degree of genetic diversity and polyclonal infections. Further study that analysed *P. falciparum* isolates from Nigeria and Senegal found K1 and IC/3D7 to be the most frequent msp1 and msp2 allelic families in both countries [50]. MOI was over 1 showing the widespread presence of polyclonal infections in both countries although more commonly in Senegal than in Nigeria.

An earlier study in Senegal [51] documented very large *P. falciparum* genetic diversity. In Senegal [52], for msp1 gene, K1 allelic family was predominant and for msp2 gene, IC/3D7 allelic family was the most represented. Polyclonal isolates found were 36% and 31% for msp1 and msp2 respectively having an overall mean MOI 2.56 showing low to intermediate genetic diversity (He = 0.394–0.637). A recent study that employed multiplexed amplicon deep sequencing in two sites in Senegal [53] found K1 and IC/3D7 allelic families to be the most predominant allelic families of msp1 and msp2 genes in both sites. The mean MOI for both genes was 3.07 and 1.76 revealing a high genetic diversity.

The genetic diversity and relative distribution of allelic frequencies of *P. falciparum* msp1 and msp2 or glurp genes in Southeast Asia is not much different from findings in Sub-Saharan Africa. For instance, from northern India, extensive diversity was found in msp1 alleles with predominantly RO33 alleles and K1 the least [54]. From Malaysia, the msp1 and msp2 allelic families that were predominant were K1 and FC27, respectively [55]. The glurp genotype VI of the R2 was predominant. In the same study; MAD20, 3D7 and glurp genotype IV allelic families of the three genes were predominant in a different setting. A study from Indonesia [56] revealed the higher proportion of MAD20 followed by K1 and RO33 for msp1 and 3D7 for msp2.

In a study conducted at China–Myanmar border region from 2006 to 2011 [57], the msp1 gene MAD20 family was dominant, followed by the K1 and RO33 families. For the msp2 gene, the most frequent allele was the FC27 family. A significant positive correlation between the MOI and parasite density was found in the msp1 gene. High rate of multiple genotypes (96.5%) with overall MOI of 3.21 was observed in *P. falciparum* isolates from along the Thai-Myanmar borders [58]. The findings were different from site to site and K1 and MAD20 were predominant. The study revealed that 3D7 was the most dominant msp2 allelic family and glurp gene was the least diverse. In Laos, the frequency of K1 was the highest followed by MAD20 and RO33 with high level of polyclonal infections although the MOI did not exceed 2.0 [59]. The authors did not get parasite density or age statistically significantly related to MOI. In Vietnam, one study [60] demonstrated that for msp1, MAD20 was the most prevalent followed by K1 allelic family, with no sample testing positive for RO33. For msp2, 3D7 allelic family was predominant. The MOI of msp1 and msp2 was 2.6 and 1.1, respectively, and the overall mean MOI was 3.7 according to the study.

Pertaining South American countries, a study from Brazil [61] that compared the temporal dynamics of glurp gene of *P. falciparum* in 1993 and 2008 found no differences between the two time points with regard to the frequencies of the fragment variants of the R0 and R2 regions of the gene. The authors found that polyclonal infections were less common and there was overall limited polymorphic variation of glurp. Similarly, the genetic diversity and allele frequencies of msp1, msp2 and glurp genes were evaluated and detected few polyclonal infections and low genetic diversity among locally transmitted *P. falciparum* isolates in Panamá [62].

In summary, the decrease in the frequency of K1 allelic variants from 73.1% in 2015 to 48.4% in 2019 in the current study may indicate the susceptibility of the variants carrying this allele to the ongoing intervention strategies in the country as opposed to MAD20 allelic variant that increased in frequency from 30.6% in 2015 to 61.3% in 2019, suggesting possible positive selection pressure favoring this allele. On the other hand, RO33 allelic variants of msp1 were slightly decreased from 53.8% in 2015 to 48.4% in 2019 showing probable modest susceptibility to the ongoing malaria intervention strategies. By the same token, msp2 allelic variant FC27 decreased in frequency from 87.5% in 2015 to 76.9% in 2019 while the frequency of IC/3D7 increased from 50.5% in 2015 to 69.2% in 2019. This may suggest that malaria intervention strategies in the study area have impacted on the frequency of parasite strains carrying this allelic variant as opposed to IC/3D7 allelic variant that seems to be positively selected to populate in the study area despite the ongoing intervention strategies.

**Conclusions**

This study found limited genetic diversity of *P. falciparum* isolates from the study area with most infections being monoclonal. This may be explained by the low prevalence of infection in the setting. However, there is a need for further studies in the current and similar as well as different transmission settings in the country using larger sample size in multiple seasons, and employing other more robust population genetic markers such as microsatellites.
and amplicon deep sequencing data in addition to allele fragment length polymorphisms. The dynamics in the relative allelic family distribution of the MAD20 of msp1 and IC/3D7 of msp2 requires a longitudinally assessment.

Abbreviations

He: Expected heterozygosity; MOI: Multiplicity of infection; MSP1: Merozoite surface protein 1; MSP2: Merozoite surface protein 2; GLURP: Glutamate-rich protein; msp1: Merozoite surface protein 1 gene; msp2: Merozoite surface protein 2 gene; glurp: Glutamate-rich protein gene; qRT-PCR: Quantitative real-time polymerase chain reaction; TBE: Tris-borate EDTA.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-022-04277-5.

Additional file 1. Primers used for msp1 genotyping.
Additional file 2. Primers used for msp2 genotyping.
Additional file 3. Primers used for glurp genotyping.

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Author contributions

AGR was fully involved in all phases of the study including laboratory work, data analysis, interpretation, and drafting of the manuscript. AL was involved in statistical analysis of the data. HM and AA participated in study design and field data collection and revised the manuscript. LG and HH conceived the research idea, designed the study, supervised AGR and critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset supporting the conclusion of this article is included within the article and the primers used to genotype the three genes are presented in attached Additional files 1, 2 and 3.

Declarations

Ethics approval and consent to participate

The study was ethically approved by the Institutional Review Board (IRB) of College of Natural and Computational Sciences, Addis Ababa University and EPHI Ethics and Scientific Committee. Written informed consent/assent was obtained from participants or parents/guardians for minors. Malaria positive cases were treated as per the national treatment guideline [63].

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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