Associations of Single Nucleotide Polymorphisms in IL-18 Gene with *Plasmodium falciparum*-Associated Malaria

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Introduction: Interleukin-18 (IL-18) is a pro-inflammatory cytokine, reported to be involved in the initial immune responses against malaria. Genetic variations in the host are an important factor that influences the etiology of malaria at several disease levels. Polymorphisms within the IL-18 gene are associated with susceptibility and clinical outcome of several diseases.

Methods: We genotyped single nucleotide polymorphisms (SNPs) in IL-18 of patients infected with *Plasmodium falciparum* with varying extent of parasitemia and different age groups.

Results: SNPs rs5744292 (OR = 70.446; 95% CI = 4.318–1149.323; p<0.0001) and rs544354 (OR = 1.498; 95% CI = 1.088–2.063; p=0.013) were found to be significantly associated with parasitemia in *P. falciparum*-infected patients when compared with healthy control subjects. SNP rs5744292 (OR = 7.597; 95% CI = 1.028–56.156; p=0.019) was associated with increased parasite density in infected patients. SNPs rs544354 (OR = 0.407; 95% CI = 0.204–0.812; p = 0.009) and rs360714 (OR of 0.256; 95% CI=0.119–0.554; p = 0.001) were significantly associated with parasite density in an age-dependent manner, with the risk alleles present more frequently among the younger (1–9 years) patients. Several haplotypes were found to have a significant association with parasitemia. In-vitro expression analysis using luciferase reporter assay showed that SNPs rs1946518 and rs187238 in the IL-18 gene, SNPs rs544354 (OR = 1.498; 95% CI = 1.088–2.063; p=0.013) were found to be significantly associated with increased transcriptional activity.

Conclusion: Our results suggest that polymorphisms within the IL-18 gene are associated with the susceptibility to *P. falciparum* infection and related parasitemia among groups with different parasite density and across various age groups.

Keywords: *Plasmodium falciparum*, IL-18 gene, SNPs

Introduction

Malaria is a parasitic disease caused by the unicellular protozoan of the genus *Plasmodium* and is spread mainly through infected female Anopheles mosquitoes. Despite several control programs, malaria continues to be a major health concern worldwide, particularly in low and middle-income countries. The WHO estimates there were 228 million cases of malaria globally resulting in 405,000 deaths in 2018.¹ Malaria is presently widespread along the tropical and sub-tropical belt, in certain areas of the Americas, many parts of Asia, and much of Africa, particularly sub-Saharan Africa.² Malaria has been known to be endemic in the lowlands of ...
Saudi Arabia, and three species of *Anopheles* mosquitoes have been identified in this region, namely *An. arabiensis*, *An. Sergentii* and *An. Gambiae*. Among the different species of *Plasmodium* infecting humans, *Plasmodium falciparum* is the most prevalent in African countries and Saudi Arabia and it is linked to most of the malaria-related deaths worldwide.\(^5,6\)

Large variation has been observed in the clinical presentation of the *P. falciparum* infection, from asymptomatic individuals to patients with severe cerebral malaria and other fatal forms of the infection. The individual variations in the clinical manifestation and progression to severe forms of *P. falciparum* infection have been reported to be influenced by several factors such as age, genetic polymorphisms in the host, genotype of the parasite, and the environmental/social conditions.\(^7,8\) Numerous studies have explored genetic influence in the pathomechanism and identified several host genes that can significantly influence the diverse clinical outcomes of malaria such as hemoglobin variants,\(^9,10\) glucose-6-phosphate dehydrogenase,\(^11\) pyruvate kinase,\(^12\) and haptoglobin.\(^13\)

Cytokines orchestrate the initial immune response against invading malarial parasites. Previous studies have reported an increased concentration of pro-inflammatory cytokines like IL-6 and anti-inflammatory cytokines like IL-10 in *P. falciparum*-infected patients.\(^14\) IL-18 is a pro-inflammatory cytokine encoded by the *IL-18* gene on chromosome 11q23.1 and belongs to the IL-1 superfamily. IL-18 is expressed by a wide range of cells, including macrophages, Kupffer cells, keratinocytes, osteoblasts, astrocytes, and dendritic cells (DCs).\(^15\) IL-18 is synthesized as 24 kD pro-IL-18, a biologically inactive precursor peptide, which is subsequently cleaved by caspase-1 into a biologically active IL-18 cytokine.\(^15\) The most salient biological property of IL-18 that distinguishes it from IL-1 is its ability to induce secretion of high levels of interferon (IFN)-γ in the presence of IL-12.\(^16\) Also, a substantial pool of intracellular IL-18 is present in the cytoplasm and is regulated mainly by caspase-1-mediated pro-IL-18 processing.\(^15\) IL-18 has a wide array of biological functions, many of which are accomplished synergistically with other cytokines, such as IFN-γ, as an early immune response to pathogenic invasions.\(^16\) Several investigations into biological role of IL-18 have shown that IL-18 is a potent inducer of (i) tumor necrosis factor alpha (TNFα), Fas-ligand, and IL-2 from T and NK cells; (ii) IL-1β, IL-8, and macrophage inflammatory protein (MIP)-1α from peripheral blood mononuclear cells; and (iii) cyclooxygenase (COX2), and inducible nitric oxide synthase.\(^17,19\)

The initial immune response to *P. falciparum* infection is mediated by IFN-γ that is induced by the combination of interleukin 12 (IL-12) and 18 (IL-18). This cytokine secretion is crucial for the control of parasitemia and resolution of malarial infection through TNFα induction and enhanced release of reactive nitrogen and oxygen radicals.\(^20\) Torre et al (2001) demonstrated that IL-18 may have a pro-inflammatory role in patients with uncomplicated *P. falciparum* infection.\(^21\) In another study, it was found that IL-18 and IL-12 levels were higher in children with mild malaria than in children with a severe form of the disease.\(^22\) Also, high quantities of both IL-18 and IFN-γ molecules was found to be associated with severe forms of malarial infections.\(^20\) A study in western Kenya investigated the relationship between IL-18 and clinical malaria phenotypes in children, and reported association between higher secretion of IL-18 with severe malarial anemia (SMA).\(^23\) Similarly, a study in adults demonstrated a close association between increased IL-18 levels and severe *falciparum* malaria.\(^24\) Taken together, these findings indicate that IL-18 is an important factor in the pathogenesis of *P. falciparum* induced malaria.

Several polymorphisms within the *IL-18* gene have been reported to play an important role in the etiology of several diseases such as atherosclerosis,\(^25\) rheumatoid arthritis (RA), systemic lupus erythematosus (SLE),\(^26,27\) allergic asthma, and allergic dermatitis,\(^28\) mostly by altering the circulating levels of IL-18. However, very few studies have investigated *IL-18* gene polymorphisms in relation to its role in malaria pathogenesis.

Previously, we have shown that genetic variations in the *IL-22* gene could be correlated with susceptibility to malaria infection.\(^29\) In this study, we investigated whether the single nucleotide polymorphisms (SNPs) spanning the *IL-18* gene were associated with the susceptibility to *P. falciparum* infection and related parasitemia. We also analyzed the influence of patient age and genetic polymorphisms in relation to extent of *P. falciparum* parasitemia.

**Materials and Methods**

**Ethical Considerations**

Ethical approval to conduct this study was granted by Ethical Review Board (ERB) of King Fahad Central Hospital (KFCH), Jazan (Registry no. 041). The study followed ethical guidelines set by the Helsinki
Declaration of 1975. Prior to study subject enrolment, informed consent was obtained from each subject; legal guardians provided consent on behalf of children. All patient-related research data including human biological samples were de-identified and secured to preserve the privacy of study participants.

Study Design and Population

This case–control study was conducted and a total of 450 subjects were enrolled. Blood samples were collected from 250 patients diagnosed with *P. falciparum* malaria infection admitted at Malaria Center, Jazan region, situated in the Southwest part of the Saudi Arabia. In addition, 200 healthy controls were recruited as a control cohort. We used WHO manual (“plus system” scale) to identify the parasite density in a thick blood smear from infected individuals. The *P. falciparum* infected malaria subjects were classified into four categories based on the parasite density; 1) *Group I*: presence of 1–10 malarial parasites per 100 thick blood smear fields; 2) *Group II*: presence of 11–100 malarial parasites per 100 thick blood smear fields; 3) *Group III*: presence of 1–10 malarial parasites per single thick blood smear field; and 4) *Group IV*: presence of over 10 malarial parasites per single thick blood smear field. Patients were excluded from the study if they were co-infected with Dengue virus (DENV), Hepatitis C virus (HCV), Hepatitis B virus (HBV) or Human immunodeficiency virus (HIV).

Selection and Genotyping of IL-18 SN Ps

The **IL-18** gene is made up of 20.9 kb and consists of a 5ʹ-untranslated region (UTR) promoter, 6 exons and a 3ʹUTR region. The SNPs were genotyped using the polymerase chain reaction (PCR)-based direct DNA sequencing and SNP-specific TaqMan assays. Ten tag SNPs were identified based on the following criteria: minor allele frequency (MAF) more than 5% and linkage disequilibrium (LD) threshold of $r^2 \geq 0.8$. The 10 identified tag SNPs were: rs11214101, rs544354, rs360729, rs5744260, rs549908, rs360714, rs7106524, rs5744231, rs360718, and rs1293344. In addition, six SNPs, rs187238, rs1946518 and rs1946519 from the promoter region and rs360727, rs5744292 and rs360728 from and 3ʹUTR of the **IL-18** gene were selected. The details of the SNPs are summarized in Figure 1 and Supplementary Table 1.

**DNA Extraction and Polymerase Chain Reaction (PCR)**

Using the manufacturer’s guide, DNA was extracted via the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany). The PCR was carried out using GoTaq® Master Mixes (Promega Corporation, Madison, WI, USA). Primers were designed through the Primer3 Input version 0.4.0 available at [http://bioinfo.ut.ee/primer3-0.4.0/](http://bioinfo.ut.ee/primer3-0.4.0/) and they are shown in Supplementary Table 2. All primers were M13-tailed to improve sequencing of the PCR products. The resultant PCR products were run on

**Figure 1** Schematic drawing illustrating the positions of IL-18 SNPs investigated in this study.
2% agarose gel electrophoresis to ensure PCR products’ quality. The amplified PCR products underwent direct sequencing by a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) as per the recommended protocol. Afterward, each of the PCR products was subjected to both reverse and forward sequencing. The final sequences were evaluated and modified using the SeqMan Pro 15 Lasergene (DNASTAR, Inc., Madison, WI, USA).

**Transfection and Luciferase Reporter Assay**

Several DNA fragments of the *IL-18* promoter and 3’ UTR region were cloned and transfected in HuH7 (obtained from the Japanese Collection of Research Bioresources Cell Bank, catalogue number: JCRB0403) cells and the variations in the luciferase gene activity were monitored. The chromosomal positions of the fragments and the primers used for amplification are shown in Supplementary Tables 3 and 4 for the promoter and 3’ UTR regions, respectively. All of the fragments were cloned in pGL3 Basic Vector (accession number: U47297) (Promega, Madison, WI, USA). HotStar DNA Polymerase (Qiagen, Hilden, Germany) was used for PCR amplification using the guidelines of the manufacturer. For colony selection on ampicillin plates, *Escherichia coli* DH5α competent cells (catalogue number: 18265017) (ThermoFisher Scientific, Sunnyvale, CA, USA) were utilized. Replicates were selected and DNA was extracted through QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Extracted DNA fragments were then transfected into HuH7 cells, which were cultivated and evaluated for luciferase activity after 48 hours.

**Statistical Analyses**

We used HaploView software version 4.0 (Broad Institute of MIT and Harvard, Cambridge, MA, USA) to analyze SNPs and measure linkage disequilibrium (LD), haplotype frequencies and MAFs. To assess the genotypic association for selected genetic variants, we used the De Finetti program available at [http://ihg.gsdf.org/cgi-bin/hw/hwa1.pl](http://ihg.gsdf.org/cgi-bin/hw/hwa1.pl). In addition, we conducted analyses to identify for the allelic, recessive and dominant modes of inheritance for each genetic variant. The study findings were presented as odds ratios (OR) with their 95% confidence intervals (CI). The Hardy–Weinberg equilibrium (HWE) was performed to test for deviation; *P* value <0.05 was considered as a statistically significant deviation from HWE. The *P* value <0.05, obtained in a two-tailed test, was set as a threshold for statistically significant association.

**Results**

**Genotype and Allele Frequency Analysis Between *P. falciparum*-Infected Patients and Healthy Control Group**

Genotypic distributions of SNPs in the healthy control group and the *P. falciparum*-infected patients are shown in Table 1. We found that SNPs rs5744292 (OR = 70.446; 95% CI= 4.318–1149.323; p<0.0001) and rs544354 (OR = 1.498; 95% CI= 1.088–2.063; *p* = 0.013) were of significantly higher occurrence in *P. falciparum*-infected patients when compared to healthy control group. The rs5744292-G allele (8%) and rs544354-A allele (26%) showed increased frequency among infected patients in comparison with the control group. We also observed that the individuals carrying either the homozygous genotype rs5744292-GG or the heterozygous AG were associated with increased risk of malarial infection with an OR of 13.828 and 48.857, respectively, when compared to homozygous AA carriers. Under a dominant model of inheritance, the rs5744292-G allele was found to be associated with an increased risk of infection (OR = 61.763). Similarly, we found that individuals carrying the homozygous AA genotype with SNP rs544354 had an increased risk of parasitemia (OR = 5.253) when compared to homozygous GG carrying individuals. In addition, the rs544354-A allele was significantly associated with risk of *P. falciparum* infection in both dominant (OR = 1.435) and recessive (OR = 0.209) models. The homozygous SNP rs360714 GG genotype was found to be associated with an increased risk of malaria infection (OR = 1.47) compared to the homozygous AA genotype, whereas the heterozygous rs7106524 AG genotype was associated with an increased risk of the infection (OR = 1.652) compared to the homozygous GG genotype.

**Genotype and Allele Frequency Analysis Based on Parasite Density**

Patients were divided into 4 groups based on increasing parasite density: Group 1 (lowest density; 14 patients), Group 2 (24 patients), Group 3 (52 patients), and Group 4 (highest density; 160 patients). The 16 selected polymorphisms were further investigated to determine their association with varying levels of parasitemia in the *P.
| SNPs     | Genotype/ Allele         | Controls n=200 | Patients n=250 | OR (95% C.I.)       | $\chi^2$ | P-value* |
|----------|--------------------------|----------------|----------------|---------------------|----------|------------|
| rs1946518 | GG                       | 55 (27.5%)     | 78 (31.2%)     | Ref                 |          |            |
|          | GT                       | 100 (50%)      | 120 (48%)      | 0.846 (0.547–1.308) | 0.57     | 0.452      |
|          | TT                       | 45 (22.5%)     | 52 (20.8%)     | 0.815 (0.481–1.381) | 0.58     | 0.446      |
|          | T                        | 210 (52.5%)    | 276(55.2%)     | 0.897 (0.689–1.168) | 0.65     | 0.419      |
|          | TT+GT vs GG              | 190 (47.5%)    | 224(44.8%)     | 0.836 (0.56–1.26)  | 0.73     | 0.393      |
|          | TT vs GT+GG              |                | 1.105 (0.70–1.73) | 0.19    |            |
| rs1946519 | CC                       | 55 (27.5%)     | 82 (32.8%)     | Ref                 |          |            |
|          | AC                       | 103 (51.5%)    | 119 (47.6%)    | 0.775 (0.503–1.193) | 1.34     | 0.246      |
|          | AA                       | 42 (21%)       | 49 (19.6%)     | 0.783 (0.458–1.337) | 0.81     | 0.368      |
|          | C                        | 213 (53.25%)   | 283 (56.6%)    | 0.873 (0.671–1.138) | 1.01     | 0.315      |
|          | A                        | 187 (46.75%)   | 217 (43.4%)    | 0.777 (0.517–1.168) | 1.47     | 0.225      |
|          | AA+AC vs CC              |                | 1.090(0.687–1.730) | 0.13 |            |
|          | AA vs AC+CC              |                | 0.713          |            |            |
| rs187238  | CC                       | 122 (61%)      | 148 (59.2%)    | Ref                 | 1.043    | 0.838      |
|          | CG                       | 64 (32%)       | 81 (32.4%)     | 1.236 (0.603–2.534) | 0.34     | 0.561      |
|          | GG                       | 14 (7%)        | 21 (8.4%)      | 1.092 (0.802–1.488) | 0.31     | 0.576      |
|          | C                        | 308 (77%)      | 377 (75.4%)    | 1.078 (0.737–1.576) | 0.15     | 0.698      |
|          | G                        | 92 (23%)       | 123 (24.6%)    | 0.821 (0.406–1.658) | 0.3      | 0.582      |
|          | GG+CG vs CC              |                | 1.052          |            |            |
|          | GG vs CG+CC              |                | 0.713          |            |            |
| rs360728  | CC                       | 151 (75.5%)    | 197 (78.8%)    | Ref                 | 0.912    | 0.708      |
|          | CG                       | 37 (18.5%)     | 44 (17.6%)     | 0.575 (0.236–1.400) | 0.14     | 0.708      |
|          | GG                       | 12 (6%)        | 9 (3.6%)       | 0.787 (0.538–1.151) | 1.52     | 0.216      |
|          | C                        | 339 (84.75%)   | 438 (87.6%)    | 0.829 (0.533–1.291) | 1.709    | 0.406      |
|          | G                        | 61 (15.25%)    | 62 (12.4%)     | 1.709 (0.705–4.142) | 1.44     | 0.230      |
|          | GG+CG vs CC              |                | 1.092          |            |            |
|          | GG vs CG+CC              |                | 0.831          |            |            |

(Continued)
Table 1 (Continued).

| SNPs    | Genotype/ Allele | Controls n=200 | Patients n=250 | OR (95% C.I.) | $\chi^2$ | P-value* |
|---------|------------------|----------------|----------------|---------------|---------|----------|
| rs5744292 | AA               | 200 (100%)     | 217 (86.8%)    | Ref           | 48.857 | <0.0001  |
|         | AG               | 0 (0%)         | 26 (10.4%)     | 13.828        | 6.35   | 0.012    |
|         | GG               | 0 (0%)         | 7 (2.8%)       | 70.446        | 33.49  | <0.0001  |
|         | A                | 400 (100%)     | 460 (92%)      | 61.763        | 28.49  | <0.0001  |
|         | G                | 0 (0%)         | 40 (8%)        | 0.081         | 5.69   | 0.017    |
|         | GG+AG vs AA      | 61.763         | 0.081          |               |        |          |
|         | GG vs AG+AA      | 0.081          |               |               |        |          |
| rs360727 | GG               | 149 (74.5%)    | 196 (78.4%)    | Ref           | 0.834  | 0.453    |
|         | AG               | 41 (20.5%)     | 45 (18%)       | 0.684         | 0.65   | 0.419    |
|         | AA               | 10 (5%)        | 9 (3.6%)       | 0.801         | 1.31   | 0.252    |
|         | G                | 339 (84.75%)   | 437 (87.4%)    | 0.805         | 0.94   | 0.331    |
|         | A                | 61 (15.25%)    | 63 (12.6%)     | 1.409         | 0.54   | 0.463    |
|         | AA+AG vs GG      | 0.805          |               |               |        |          |
|         | AA vs AG+AA      | 1.409          |               |               |        |          |
| rs549908 | TT               | 120 (60%)      | 152 (60.8%)    | Ref           | 0.892  | 0.579    |
|         | GT               | 69 (34.5%)     | 78 (31.2%)     | 1.435         | 0.85   | 0.357    |
|         | GG               | 11 (5.5%)      | 20 (8%)        | 1.049         | 0.09   | 0.764    |
|         | T                | 309 (77.25%)   | 382 (76.4%)    | 0.967         | 0.03   | 0.863    |
|         | G                | 91 (22.75%)    | 118 (23.6%)    | 0.669         | 1.08   | 0.298    |
|         | GG+GT vs TT      | 0.967          |               |               |        |          |
|         | GG vs GT+TT      | 0.669          |               |               |        |          |
| rs11214101 | TT          | 200 (100%)     | 249 (99.6%)    | Ref           | 2.411  | 0.370    |
|          | CT               | 0 (0%)         | 1 (0.4%)       | 0.804         | 0.80   | 1.000    |
|          | CC               | 0 (0%)         | 0 (0%)         | 2.405         | 0.80   | 1.234    |
|          | T                | 400 (100%)     | 499 (99.8%)    | 2.411         | 0.80   | 0.370    |
|          | C                | 0 (0%)         | 1 (0.2%)       | 1.249         | Nan    | 1.000    |
|          | CC+CT vs TT      | 2.411          |               |               |        |          |
|          | CC vs CT+TT      | 1.249          |               |               |        |          |
| rs544354 | GG | 127 (63.5%) | 137 (54.8%) | Ref (0.860–1.880) | 1.45 | 0.229 |
| AG | 70 (35%) | 96 (38.4%) | 5.253 (1.504–18.351) | 8.21 | 0.004 |
| AA | 3 (1.5%) | 17 (6.8%) | 1.498 (1.088–2.063) | 6.17 | 0.013 |
| G | 324 (81%) | 370 (74%) | 1.435 (0.981–2.100) | 3.47 | 0.006 |
| A | 76 (19%) | 130 (26%) | 0.209 (0.060–0.723) | 7.35 | 0.007 |
| AA+AG vs GG | | | | | |
| AA vs AG+AA | | | | | |

| rs360729 | AA | 126 (63%) | 152 (60.8%) | Ref (0.705–1.606) | 0.09 | 0.768 |
| AT | 60 (30%) | 77 (30.8%) | 1.243 (0.607–2.545) | 0.36 | 0.550 |
| TT | 14 (7%) | 21 (8.4%) | 1.107 (0.809–1.515) | 0.41 | 0.524 |
| T | 88 (22%) | 119 (238%) | 1.098 (0.748–1.610) | 0.23 | 0.633 |
| TT+AT vs AA | | | | | |
| TT vs AT+AA | | | | | |

| rs5744260 | GG | 193 (96.5%) | 239 (95.6%) | Ref (0.346–2.412) | 0.03 | 0.856 |
| AG | 7 (3.5%) | 11 (4.4%) | 0.582 (0.011–29.518) | N/N | 1.000 |
| AA | 0 (0%) | 0 (%) | 0.916 (0.351–2.389) | 0.03 | 0.857 |
| G | 393 (98.25%) | 489 (97.8%) | 0.914 (0.346–2.412) | 0.03 | 0.856 |
| A | 7 (1.75%) | 11 (2.2%) | 1.710 (0.034–86.361) | N/N | 1.000 |
| AA+AG vs GG | | | | | |
| AA vs AG+AA | | | | | |

| rs360714 | AA | 142 (71%) | 193 (77.5%) | Ref (0.504–1.199) | 1.30 | 0.254 |
| AG | 53 (26.5%) | 56 (22.4%) | 0.147 (0.017–1.273) | 4.03 | 0.045 |
| GG | 5 (2.5%) | 1 (0.4%) | 0.702 (0.478–1.030) | 3.29 | 0.069 |
| G | 337 (84.25%) | 442 (88.4%) | 0.723 (0.473–1.106) | 2.25 | 0.134 |
| A | 63 (15.75%) | 58 (11.6%) | 6.385 (0.740–55.097) | 3.72 | 0.054 |
| GG+AG vs AA | | | | | |
| GG vs AG+AA | | | | | |

| rs7106524 | GG | 92 (46%) | 97 (38.8%) | Ref (1.082–2.522) | 5.44 | 0.019 |
| AG | 62 (31%) | 108 (43.2%) | 0.928 (0.563–1.530) | 0.09 | 0.769 |
| AA | 46 (23%) | 45 (18%) | 1.047 (0.800–1.371) | 0.11 | 0.737 |
| G | 246 (61.5%) | 302 (60.4%) | 1.344 (0.922–1.959) | 2.36 | 0.124 |
| A | 154 (38.5%) | 198 (39.6%) | 1.361 (0.858–2.158) | 1.72 | 0.189 |
| AA+AG vs GG | | | | | |
| AA vs AG+AA | | | | | |

(Continued)
| SNPs      | Genotype/Allele | Controls n=200 | Patients n=250 | OR (95% C.I.) | $\chi^2$ | P-value* |
|----------|----------------|----------------|----------------|----------------|---------|----------|
| rs5744231| CC             | 194 (97%)      | 241 (96.4%)    | Ref            | 0.19   | 0.661    |
|          | CT             | 5 (2.5%)       | 8 (3.2%)       | 1.288 (0.415–4.000) | 0.2     | 0.878    |
|          | TT             | 1 (0.5%)       | 1 (0.4%)       | 0.805 (0.050–12.953) | 0.02    | 0.874    |
|          | T              | 393 (98.25%)   | 490 (98%)      | 1.146 (0.432–3.038) | 0.07    | 0.874    |
|          | TT+CT vs.CC    | 1 (0.5%)       | 1 (0.4%)       | 1.207 (0.422–3.451) | 0.12    | 0.724    |
|          | TT vs.CT+CC    | 0.805 (0.050–12.953) | 0.784    |
| rs360718 | AA             | 121 (60.5%)    | 146 (58.4%)    | Ref            | 0.01   | 0.924    |
|          | AC             | 65 (32.5%)     | 80 (32%)       | 1.020 (0.679–1.531) | 0.07    | 0.325    |
|          | CC             | 14 (7%)        | 24 (9.6%)      | 1.421 (0.704–2.866) | 0.66    | 0.416    |
|          | A              | 307 (76.75%)   | 372 (74.4%)    | 1.136 (0.836–1.544) | 0.02    | 0.652    |
|          | C              | 93 (23.25%)    | 128 (25.6%)    | 1.091 (0.747–1.594) | 0.097   | 0.324    |
|          | CC+AC vs.AA    | 121 (60.5%)    | 145 (58%)      | Ref            | 0.01   | 0.924    |
|          | CC vs.AC+AA    | 0.709 (0.357–1.409) | 0.324    |
| rs1293344| AA             | 121 (60.5%)    | 145 (58%)      | Ref            | 0.25   | 0.618    |
|          | AG             | 64 (32%)       | 85 (34%)       | 1.108 (0.740–1.661) | 0.09    | 0.769    |
|          | GG             | 15 (7.5%)      | 20 (8%)        | 1.13 (0.546–2.267) | 0.27    | 0.602    |
|          | A              | 306 (76.5%)    | 375 (75%)      | 1.085 (0.798–1.475) | 0.27    | 0.602    |
|          | G              | 94 (32.5%)     | 125 (25%)      | 1.109 (0.759–1.620) | 0.29    | 0.592    |
|          | GG+AG vs.AA    | 0.932 (0.464–1.872) | 0.844    |
|          | GG vs.AG+AA    |                   |               |                |        |          |

*Bold indicates significance.*
Table 2 Genotype Distribution and Allele Frequency of IL-18 Gene SNPs When Group 1+2 of Parasite Density Was Compared with Group 3+4

| SNPs     | Genotype/Allele Distribution | Group (1+2) n=38 | Group (3+4) n=212 | OR (95% C.I.) | $\chi^2$ | P-value* |
|----------|-------------------------------|------------------|-------------------|---------------|----------|----------|
| rs360727 |                                |                  |                   |               |          |          |
|          | GG                            | 27 (71%)         | 169 (79.7%)       | Ref           | 0.559    | 0.248–1.259 | 2.01     | 0.156 |
|          | AG                            | 10 (26.3%)       | 35 (16.5%)        | 0.559        | 0.248–1.259 | 2.01     | 0.156 |
|          | AA                            | 1 (2.7%)         | 8 (3.8%)          | 1.278        | 0.154–10.629 | 0.83     | 0.362 |
|          | G                             | 64 (84.2%)       | 373 (87.98%)      | 0.729        | 0.369–1.443 | 0.83     | 0.362 |
|          | A                             | 12 (15.8%)       | 51 (12.02%)       | 0.625        | 0.287–1.358 | 0.12     | 0.232 |
|          | AA+AG vs. GG                  |                  |                   |              | 0.689    | 0.084–5.567 | 1.43     | 0.232 |
|          | AA vs. AG+AA                  |                  |                   |              | 0.275    | 0.036–2.14 | 1.75     | 0.185 |
|          |                                |                  |                   |              |          |          |
| rs549908 |                                |                  |                   |               |          |          |
|          | TT                            | 22 (57.9%)       | 130 (61.32%)      | Ref           | 0.711    | 0.345–1.463 | 0.86     | 0.325 |
|          | GT                            | 15 (39.5%)       | 63 (29.71%)       | 3.171        | 1.049–10.254 | 1.37     | 0.241 |
|          | GG                            | 1 (2.6%)         | 19 (8.97%)        | 1.085        | 0.605–1.946 | 0.08     | 0.783 |
|          | T                             | 59 (77.6%)       | 323 (76.18%)      | 0.867        | 0.430–1.748 | 0.16     | 0.690 |
|          | G                             | 17 (22.4%)       | 101 (23.82%)      | 0.275        | 0.036–2.14 | 1.75     | 0.185 |
|          | GG+GT vs. TT                  |                  |                   |              |          |          |
|          | GG vs. GT+TT                  |                  |                   |              |          |          |
|          |                                |                  |                   |              |          |          |
| rs11214101 |                               |                  |                   |               |          |          |
|          | TT                            | 38 (100%)        | 211 (99.5%)       | Ref           | 0.546    | 0.022–13.654 | 0.18     | 0.671 |
|          | CT                            | 0(0%)            | 1(0.5%)           | 0.182        | 0.004–9.313 | 0.18     | 1.014 |
|          | CC                            | 0(0%)            | 0(0%)             | 0.542        | 0.022–13.426 | 0.18     | 0.671 |
|          | T                             | 76(100%)         | 423(99.76%)       | 0.546        | 0.022–13.654 | 0.18     | 1.014 |
|          | C                             | 0(0%)            | 1(0.24%)          | 5.519        | 0.108–282.36 | 0.18     | 0.671 |
|          | CC+CT vs. TT                  |                  |                   |              |          |          |
|          | CC vs. CT+TT                  |                  |                   |              |          |          |
|          |                                |                  |                   |              |          |          |
| rs544354 |                                |                  |                   |               |          |          |
|          | GG                            | 20(52.6%)        | 117(55.2%)        | Ref           | 0.923    | 0.446–1.910 | 0.05     | 0.829 |
|          | AG                            | 15(39.5%)        | 81 (38.2%)        | 0.798        | 0.210–3.029 | 0.11     | 0.739 |
|          | AA                            | 3(7.9%)          | 14(6.6%)          | 0.906        | 0.524–1.568 | 0.12     | 0.724 |
|          | G                             | 55(72.4%)        | 315(74.3%)        | 0.902        | 0.452–1.802 | 0.09     | 0.770 |
|          | A                             | 21(27.6%)        | 109(25.7%)        | 1.212        | 0.331–4.438 | 0.08     | 0.770 |
|          | AA+AG vs. GG                  |                  |                   |              |          |          |
|          | AA vs. AG+AA                  |                  |                   |              |          |          |
|          |                                |                  |                   |              |          |          |
(Continued)
Table 2 (Continued).

| SNPs   | Genotype/Allele Distribution | Group (1+2) n=38 | Group (3+4) n=212 | OR (95% C.I.) | \( \chi^2 \) | P-value* |
|--------|-------------------------------|------------------|-------------------|----------------|-----------|----------|
| rs360729 | AA                            | 21 (55.3%)       | 131 (61.8%)       | Ref            | 0.611 (0.298–1.253) | 1.83     | 0.176   |
|        | AT                            | 16 (42%)         | 61 (28.8%)        | 3.206 (0.408–25.16) | 1.36     | 0.243   |
|        | TT                            | 1 (2.7%)         | 20 (9.4%)         | 1.008 (0.567–1.789) | 0        | 0.979   |
|        | A                             | 58 (76.3%)       | 323 (76.2%)       | 0.764 (0.380–1.533) | 0.58     | 0.447   |
|        | T                             | 18 (23.7%)       | 101 (23.8%)       | 0.259 (0.034–1.993) | 1.94     | 0.163   |
|        | TT+AT vs AA                   | 0.611 (0.298–1.253) | 1.83     | 0.176   |
|        | TT vs AT+AA                   | 3.206 (0.408–25.16) | 1.36     | 0.243   |
|        | AA                            | 58 (76.3%)       | 323 (76.2%)       | 0.764 (0.380–1.533) | 0.58     | 0.447   |
|        | A                             | 16 (42%)         | 61 (28.8%)        | 3.206 (0.408–25.16) | 1.36     | 0.243   |
|        | T                             | 1 (2.7%)         | 20 (9.4%)         | 1.008 (0.567–1.789) | 0        | 0.979   |
|        | TT+AT vs AA                   | 0.611 (0.298–1.253) | 1.83     | 0.176   |
|        | TT vs AT+AA                   | 3.206 (0.408–25.16) | 1.36     | 0.243   |
| rs574260 | GG                            | 35 (92.1%)       | 204 (96.2%)       | Ref            | 0.458 (0.116–1.809) | 1.3      | 0.254   |
|        | AG                            | 3 (7.9%)         | 8 (3.8%)          | 0.174 (0.003–8.891) | Nan      | 1       |
|        | AA                            | 0 (0%)           | 0 (0%)           | 0.468 (0.121–1.805) | 1.27     | 0.225   |
|        | A                             | 73 (96.05%)      | 416 (98.1%)       | 0.458 (0.116–1.809) | 1.3      | 0.254   |
|        | AA+AG vs GG                   | 0.458 (0.116–1.809) | 1.3      | 0.254   |
|        | AA vs AG+AA                   | 5.519 (0.108–282.3) | Nan      | 1       |
| rs1946518 | GG                            | 9 (23.7%)        | 69 (32.5%)        | Ref            | 0.652 (0.280–1.517) | 0.99     | 0.318   |
|        | GT                            | 20 (52.6%)       | 100 (47.2%)       | 0.623 (0.229–1.693) | 0.87     | 0.350   |
|        | TT                            | 9 (23.7%)        | 43 (20.3%)        | 0.782 (0.479–1.274) | 0.98     | 0.322   |
|        | G                             | 38 (50%)         | 238 (56.1%)       | 0.643 (0.289–1.4333) | 1.18     | 0.277   |
|        | T                             | 38 (50%)         | 186 (43.9%)       | 0.643 (0.289–1.4333) | 1.18     | 0.277   |
|        | TT+GT vs GG                   | 0.652 (0.280–1.517) | 0.99     | 0.318   |
|        | TT vs GT+GG                   | 0.623 (0.229–1.693) | 0.87     | 0.350   |
| rs1946519 | CC                            | 12 (31.6%)       | 70 (33.02%)       | Ref            | 1.104 (0.492–2.475) | 0.06     | 0.810   |
|        | AC                            | 16 (42.1%)       | 103 (48.58%)      | 0.669 (0.265–1.688) | 0.73     | 0.392   |
|        | AA                            | 10 (26.3%)       | 39 (18.4%)        | 0.828 (0.507–1.350) | 0.57     | 0.448   |
|        | A                             | 40 (52.6%)       | 243 (57.3%)       | 0.936 (0.446–1.965) | 0.03     | 0.861   |
|        | AA+AC vs CC                   | 0.936 (0.446–1.965) | 0.03     | 0.861   |
|        | AA vs AC+CC                   | 1.584 (0.711–3.530) | 1.28     | 0.257   |
| SNP           | Allele 1 | Allele 2 | Allele 3 | Allele 4 | Allele 5 | Allele 6 | Allele 7 | p Value 1 | p Value 2 | p Value 3 | p Value 4 | p Value 5 | p Value 6 | p Value 7 |
|--------------|---------|---------|---------|---------|---------|---------|---------|----------|----------|----------|----------|----------|----------|----------|
| rs187238     | CC      | CG      | GG      | C       | G       | Ref     |          | 0.452    | 1.01     | 0.44     | 0.568    | 2.6      | 0.107    | 0.163    |
|              |         |         |         |         |         |         |          | (0.222–0.921) | (0.350–21.901) | (0.479–1.438) | (0.284–1.137) | (0.034–1.993) |          |
|              |         |         |         |         |         |         |          | 4.93     | 1.315    | 0.505    | 2.6      | 0.107    | 0.163    |          |
| rs360728     | CC      | CG      | GG      | C       | G       | Ref     |          | 0.671    | 0.89     | 0.345    | 3.326    | 1.54     | 0.214    | 0.872    |
|              |         |         |         |         |         |         |          | (0.292–1.542) | (0.188–58.703) | (0.501–2.258) | (0.372–1.913) | (0.016–4.880) |          |
|              |         |         |         |         |         |         |          | 0.89     | 0.345    | 0.195    | 0.17     | 0.684    | 0.195    |          |
| rs5744292    | AA      | AG      | GG      | A       | G       | Ref     |          | 5.139    | 3.07     | 0.079    | 3.116    | 1.43     | 0.231    | 0.019    |
|              |         |         |         |         |         |         |          | (0.675–39.122) | (0.174–55.751) | (1.028–56.156) | (0.871–49.662) | (0.020–6.360) |          |
|              |         |         |         |         |         |         |          | 3.07     | 0.079    | 0.019    | 1.43     | 0.231    | 0.019    |          |
| rs360714     | AA      | AG      | GG      | A       | G       | Ref     |          | 1.595    | 0.98     | 0.321    | 0.065    | 0.024    | 0.750    | 0.017    |
|              |         |         |         |         |         |         |          | (0.629–4.041) | (0.003–1.623) | 1.136 (0.516–2.503) | (0.567–3.293) | (0.68–425.21) |
|              |         |         |         |         |         |         |          | 0.98     | 0.321    | 0.024    | 0.059    | 0.484    | 0.484    | 0.017    |
| rs7106524    | GG      | AG      | AA      | G       | A       | Ref     |          | 1.225    | 0.27     | 0.603    | 1.072    | 0.02     | 0.887    | 0.780    |
|              |         |         |         |         |         |         |          | (0.570–2.631) | (0.407–2.824) | (0.650–1.774) | (0.583–2.371) | (0.424–2.523) |          |
|              |         |         |         |         |         |         |          | 0.27     | 0.603    | 0.887    | 0.08     | 0.649    | 0.941    |          |

(Continued)
### Table 2 (Continued).

| SNPs   | Genotype/Allele Distribution | Group (1+2) n=38 | Group (3+4) n=212 | OR (95% C.I.) | $\chi^2$ | P-value* |
|--------|------------------------------|------------------|-------------------|---------------|---------|----------|
| **rs5744231** |                              |                  |                   |               |         |          |
| CC     |                              | 38 (100%)        | 203 (95.8%)       | Ref           | 1.49    | 0.222    |
| CT     |                              | 0 (0%)           | 8 (3.7%)          | 3.216 (0.182–56.889) | 0.19    | 0.665    |
| TT     |                              | 0 (0%)           | 1 (0.5%)          | 3.876 (0.225–66.836) | 1.83    | 0.372    |
| T      |                              | 76 (100%)        | 414 (97.6%)       |               |         |          |
|        | TT+CT vs. CC                 |                  | 0 (0%)            |               |         |          |
|        | TT vs. CT+CC                 |                  | 10 (2.4%)         |               |         |          |
| **rs360718** |                              |                  |                   |               |         |          |
| AA     |                              | 20 (52.63%)      | 126 (59.43%)      | Ref           | 0.58    | 0.444    |
| AC     |                              | 14 (36.84%)      | 66 (31.132%)      | 0.748 (0.355–1.576) | 0.15    | 0.698    |
| CC     |                              | 4 (10.53%)       | 20 (9.434%)       | 0.794 (0.246–2.564) | 0.53    | 0.467    |
| A      |                              | 54 (71.1%)       | 318 (75%)         |               |         |          |
| C      |                              | 22 (28.9%)       | 106 (25%)         |               |         |          |
|        | CC+AC vs. AA                 |                  | 0.758 (0.379–1.517) | 0.61    | 0.433    |
|        | CC vs. AC+AA                 |                  | 1.129 (0.363–3.509) | 0.04    | 0.833    |
| **rs1293344** |                              |                  |                   |               |         |          |
| AA     |                              | 18(47.37%)       | 127(59.91%)       | Ref           | 3.92    | **0.047**|
| AG     |                              | 19(50%)          | 66(31.13%)        | 0.492 (0.242–1.001) | 0.95    | 0.330    |
| GG     |                              | 1(2.63%)         | 19(9.96%)         | 2.693 (0.340–21.353) | 0.33    | 0.565    |
| A      |                              | 55(72.37%)       | 320(75.47%)       | 0.851 (0.491–1.474) |         |          |
| G      |                              | 21(27.63%)       | 104(24.53%)       | 0.602 (0.301–1.205) | 2.08    | 0.149    |
|        | GG+AG vs. AA                 |                  | 0.275 (0.036–2.114) | 1.75    | 0.185    |
|        | GG vs. AG+AA                 |                  |                   |               |         |          |

**Note:** Bold indicates significance.
falciparum-infected individuals. Allelic and genotypic frequency comparisons were done between the patients in group 1+2 with the patients in group 3+4 (Table 2). We found that SNP rs5744292 (OR = 7.597; 95% CI = 1.028–56.156; p = 0.01967) was associated with increased parasite density in *P. falciparum*-infected patients. Furthermore, the rs5744292-G allele was significantly associated with higher parasitemia levels under the dominant model with an OR of 6.578. We also found heterozygous genotype CG for SNP rs187238 (OR = 0.452) and AG for SNP rs1293344 (OR = 0.492), to be associated with variations in malarial parasitemia, when compared with homozygous genotypes CC and AA, respectively. Under a recessive model of inheritance, rs360714-G allele showed a significant association with increased levels of parasitic density (OR = 17).

We also compared allele frequency for these SNPs in group 1+2+3 against group 4. Under a recessive mode of inheritance, we found that rs549908 G allele (OR = 0.290) and rs360729 T allele (OR = 0.272) were both significantly associated with increased level of parasitemia. No other association was observed between these two groups (Table 3).

**Haplotype Association Analysis**

Haplotype analysis was done between the healthy control group and *P. falciparum*-infected patients and the result is summarized in Table 4. Out of the three haplotypes identified, the occurrence frequency of two haplotypes, GT and AT were significant between these two groups. GT (rs544354 and rs549908) was found to be more frequent within the healthy controls (f = 0.586), compared to *P. falciparum*-infected patients (f = 0.516), ($\chi^2$ = 4.352; p = 0.037). However, AT (rs544354 and rs49908) was more prevalent among the *P. falciparum*-infected patients (f = 0.248), compared to the healthy controls (f = 0.187), ($\chi^2$ = 4.834; p = 0.028).

Similar haplotype analysis was done between *P. falciparum*-infected patients in group 2+3+4 and group 1 for parasitic density. We observed that the haplotype CAT (rs360728, rs360729 and rs549908) had significantly higher frequency (f=0.821) within the group 1 patients, compared to combined group 2+3+4 patients (f = 0.637), ($\chi^2$ = 3.926; p = 0.048) (Table 5).

**Genotype and Allele Frequency Analysis Based on Age**

We also examined the relation of the 16 selected SNPs in the IL-18 gene with the age of the *P. falciparum*-infected patients. For this analysis, the 250 patients with parasitemia were divided into different age groups. The genotypic distribution of patients in 1–9 years age group compared to the patient group ≥10 years of age is represented in Table 6. We observed rs544354 (OR = 0.407; 95% CI = 0.204–0.812; p = 0.008) and rs360714 (OR = 0.256; 95% CI = 0.119–0.554; p=0.001) to be significantly associated with age in *P. falciparum*-infected patients. Heterozygous rs544354-AG genotype was present in significantly higher frequency (f = 0.77) in age group 1–9 years, compared to patients in ≥10 years age group (f = 0.35). It was observed that both under dominant (OR = 0.146; 95% CI = 0.041–0.519; p <0.001) and recessive (OR = 0.794; 95% CI = 0.099–6.355; p <0.001) models of inheritance, rs544354-A allele occurrence was associated with the age of the infected patients. Similarly, for SNP rs360714, heterozygous-AG genotype showed a higher incidence (f = 0.61) in *P. falciparum*-infected patients in the 1–9 years age group compared to patients above 10 years of age (f = 0.19). In the dominant model, rs360714-G allele was also significantly associated with patient age (OR = 0.157; 95% CI = 0.058–0.428; p <0.001).

Further analysis was performed between the patient group 1–9 years age group compared to the patient group ≥41 years of age (Table 7). The results showed that rs5744292 A allele (OR = 9.456; 95% CI = 0.546–163.840; p = 0.037), rs544354 AG genotype (OR = 0.080; 95% CI = 0.020–0.330; p=0.0001) and rs360714 AG genotype (OR = 0.163; 95% CI = 0.051–0.517; p=0.001) are significantly associated with age in *P. falciparum*-infected patients. Heterozygous rs544354-AG genotype was present in significantly higher frequency (f = 0.77) in age group 1–9 years, compared to patients in ≥41 years age group (f = 0.22). Also, heterozygous rs360714-AG was present in a significantly higher frequency (f = 0.61) in age group 1–9 years, compared to patients in ≥41 years age group (f = 0.20)

**Haplotype Analysis Between Different Age Groups**

We performed a haplotype analysis between the different age groups within the *P. falciparum*-infected patients. Haplotype analysis between the different age groups within the *P. falciparum* infected patients identified two haplotypes GG (rs544354 and rs360727) with a significantly higher frequency among ≥10 years age group (f = 0.674) compared to 1–9 years age group (f = 0.491), ($\chi^2$ =
Table 3 Genotype Distribution and Allele Frequency of *IL-18* Gene SNPs When Group 1+2+3 of Parasite Density Was Compared with Group 4

| SNPs   | Genotype/Allele | Group (1+2+3) n=90 | Group (4) n=160 | OR (95% C.I.) | $\chi^2$ | P-value* |
|--------|-----------------|---------------------|-----------------|---------------|----------|----------|
| rs1946518 |                |                     |                 |               |          |          |
|        | GG              | 26 (28.9%)          | 52 (32.5%)      | Ref           |          |          |
|        | GT              | 46 (51.1%)          | 74 (46.25%)     | 0.804 (0.443–1.462) | 0.51     | 0.474    |
|        | TT              | 18 (20%)            | 34 (21.25%)     | 0.944 (0.450–1.980) | 0.02     | 0.879    |
|        | G               | 98 (54.4%)          | 178 (55.6%)     | 0.953 (0.661–1.376) | 0.06     | 0.798    |
|        | T               | 82 (45.6%)          | 142 (44.4%)     |               |          |          |
|        | TT+GT vs. GG    | 0.844 (0.480–1.482) |                 | 0.35          |          | 0.554    |
|        | TT vs. GT+GG    |                     |                 |               |          |          |
| rs1946519 |                |                     |                 |               |          |          |
|        | CC              | 28 (31.1%)          | 54 (33.75%)     | Ref           |          |          |
|        | AC              | 45 (25%)            | 74 (46.25%)     | 0.853 (0.474–1.535) | 0.28     | 0.595    |
|        | AA              | 17 (18.9%)          | 32 (20%)        | 0.976 (0.464–2.055) | 0        | 0.949    |
|        | C               | 101 (56.1%)         | 182 (56.9%)     | 0.969 (0.671–1.401) | 0.03     | 0.868    |
|        | A               | 79 (43.9%)          | 138 (43.1%)     |               |          |          |
|        | AA+AC vs. CC    | 0.886 (0.510–1.542) |                 | 0.18          |          | 0.669    |
|        | AA vs. AC+CC    | 0.932 (0.484–1.793) |                 | 0.05          |          | 0.831    |
| rs187238 |                |                     |                 |               |          |          |
|        | CC              | 50 (55.6%)          | 98 (61.25%)     | Ref           |          |          |
|        | CG              | 36 (40%)            | 45 (28.1%)      | 0.638 (0.366–1.111) | 2.54     | 0.111    |
|        | GG              | 4 (44.4%)           | 14 (8.75%)      | 1.786 (0.559–5.709) | 0.98     | 0.322    |
|        | C               | 136 (75.6%)         | 241 (76.7%)     | 0.936 (0.610–1.438) | 0.09     | 0.763    |
|        | G               | 44 (24.4%)          | 73 (23.25%)     | 0.753 (0.445–1.274) | 1.12     | 0.289    |
|        | GG+CG vs. CC    | 0.753 (0.445–1.274) |                 | 1.12          |          | 0.289    |
|        | GG vs. CG+CC    |                     |                 |               |          |          |
| rs360728 |                |                     |                 |               |          |          |
|        | CC              | 69 (76.7%)          | 128 (80%)       | Ref           |          |          |
|        | CG              | 19 (21.1%)          | 25 (15.6%)      | 0.709 (0.365–1.378) | 1.03     | 0.309    |
|        | GG              | 2 (22.2%)           | 7 (4.4%)        | 1.887 (0.381–9.331) | 0.62     | 0.429    |
|        | C               | 157 (87.2%)         | 281 (87.81%)    | 0.947 (0.546–1.644) | 0.04     | 0.847    |
|        | G               | 23 (12.8%)          | 39 (12.19%)     |               |          |          |
|        | GG+CG vs. CC    | 0.821 (0.440–1.532) |                 | 0.38          |          | 0.535    |
|        | GG vs. CG+GG    | 0.497 (0.101–2.444) |                 | 0.77          |          | 0.380    |

*P-value calculated for group 1+2+3 vs. group 4.*
| SNP     | AA       | AG       | GG       | Ref     | 137 (85.6%) | 17 (10.6%) | 6 (3.8%) | 29 (9.1%) | 1.103 (0.470–2.590) | 0.05 | 0.821 |
|---------|----------|----------|----------|---------|-------------|------------|---------|-----------|---------------------|------|--------|
| rs5744292 | 80 (88.9%) | 9 (10%)  | 1 (0.1%) | 11 (6.1%) | 1.103 (0.470–2.590) | 0.05 | 0.821 |
| rs360727 | 68 (75.6%) | 19 (21.1%) | 3 (3.3%) | 25 (13.9%) | 1.103 (0.470–2.590) | 0.05 | 0.821 |
| rs549908 | 54 (75.6%) | 33 (46.7%) | 3 (3.3%) | 141 (78.3%) | 1.103 (0.470–2.590) | 0.05 | 0.821 |
| rs121410 | 89 (98.9%) | 1 (0.1%)  | 1 (0.1%) | 17 (9.9%)  | 1.103 (0.470–2.590) | 0.05 | 0.821 |
| rs543454 | 46 (61.5%) | 40 (44.4%) | 4 (4.4%) | 132 (73.3%) | 1.103 (0.470–2.590) | 0.05 | 0.821 |
| SNPs     | Genotype/Allele | Group (1+2+3) n=90 | Group (4) n=160 | OR (95% C.I.) | \( \chi^2 \) | P-value* |
|----------|-----------------|--------------------|-----------------|---------------|-----------|----------|
| rs360729 | AA              | 53 (58.9%)         | 99 (61.9%)      | Ref           | 1.87      | 0.171    |
|          | AT              | 34 (37.8%)         | 43 (26.9%)      | 0.677 (0.387–1.185) | 3.57      | 0.058    |
|          | TT              | 3 (3.3%)           | 18 (11.2%)      | 1.147 (0.744–1.770) | 0.39      | 0.534    |
|          | A               | 140 (77.8%)        | 241 (75.3%)     | 0.883 (0.521–1.496) | 0.22      | 0.642    |
|          | T               | 40 (22.2%)         | 79 (24.7%)      | 0.272 (0.078–0.950) | 4.69      | 0.030    |
|          | TT+AT.vs.AA     |                    |                 | 0.883 (0.521–1.496) | 0.22      | 0.642    |
|          | TT.vs.AT+AA     |                    |                 | 0.272 (0.078–0.950) | 4.69      | 0.030    |
| rs5744260 | GG              | 85 (94.4%)         | 154 (96.25%)    | Ref           | 0.45      | 0.504    |
|          | AG              | 5 (5.6%)           | 6 (3.75%)       | 0.662 (0.196–2.235) | 0.45      | 0.504    |
|          | AA              | 0 (0%)             | 0 (0%)          | 0.553 (0.011–28.14) | Nan       | I        |
|          | G               | 175 (97.2%)        | 314 (98.1%)     | 0.669 (0.201–2.223) | 0.44      | 0.535    |
|          | A               | 5 (2.8%)           | 6 (1.9%)        | 0.662 (0.196–2.235) | 0.45      | 0.505    |
|          | AA+AG.vs.GG     |                    |                 | 1.773 (0.04–90.14) | Nan       | I        |
|          | AA.vs.AG+GG     |                    |                 |               |           |          |
| rs360714 | AA              | 70 (77.8%)         | 123 (76.9%)     | Ref           | 0.108     | 0.747    |
|          | AG              | 19 (21.1%)         | 37 (23.1%)      | 1.108 (0.593–2.073) | 1.74      | 0.186    |
|          | GG              | 1 (1.1%)           | 0 (0%)          | 0.190 (0.008–4.734) | 0        | 0.972    |
|          | A               | 159 (88.3%)        | 283 (88.4%)     | 0.990 (0.560–1.750) | 0.03      | 0.870    |
|          | G               | 21 (11.7%)         | 37 (11.6%)      | 1.053 (0.567–1.953) | 0.03      | 0.870    |
|          | GG+AG.vs.AA     |                    |                 |               |           |          |
|          | GG.vs.AG+AA     |                    |                 |               |           |          |
| rs7106524 | GG              | 37 (41.1%)         | 60 (37.5%)      | Ref           | 1.136     | 0.660    |
|          | AG              | 38 (42.2%)         | 70 (43.75%)     | 1.233 (0.587–2.593) | 0.31      | 0.579    |
|          | AA              | 15 (16.7%)         | 30 (13.75%)     | 1.127 (0.775–1.640) | 0.39      | 0.532    |
|          | G               | 112 (62.2%)        | 190 (59.4%)     | 1.164 (0.686–1.973) | 0.32      | 0.573    |
|          | A               | 68 (37.8%)         | 130 (40.6%)     | 0.857 (0.438–1.714) | 0.17      | 0.680    |
|          | AA+AG.vs.GG     |                    |                 |               |           |          |
|          | AA.vs.AG+AA     |                    |                 |               |           |          |
| rs5744231 |   |   |   |   |   |   |   |   |   |
|-----------|---|---|---|---|---|---|---|---|---|
|           | CC | CT | TT | C | T | TT+CT vs. CC | TT vs. CT+CC | Ref | 0.942 (0.220–4.035) |
| rs360718  |   |   |   |   |   |   |   |   |   |
|           | AA | AC | CC | A | C | CC+AC vs. AA | CC vs. AC+AA | Ref | 0.781 (0.445–1.372) |
| rs129344  |   |   |   |   |   |   |   |   |   |
|           | AA | AG | GG | A | G | GG+AG vs. AA | GG vs. AG+AA | Ref | 0.716 (0.413–1.242) |
| rs5744260 |   |   |   |   |   |   |   |   |   |
|           | GG | AG | AA | G | A | AA+AG vs. GG | AA vs. AG+GG | Ref | 0.662 (0.196–2.235) |

**Note:** *Bold indicates significance.*
Table 4 Haplotype Analysis of IL-18 Gene Polymorphisms Among Plasmodium Infected Patients versus Healthy Control Subjects

| Haplotype Block | Freq. | Plasmodium Infected Patients, Healthy Control Ratio Counts | Plasmodium Infected Patients, Healthy Control Frequencies | Chi Square | P value * |
|-----------------|-------|----------------------------------------------------------|----------------------------------------------------------|------------|-----------|
| rs544354        | rs549908 | 0.547 | 0.516 | 0.586 | 4.352 | 0.037 |
| G T             |       | 258.1: 241.9, 234.3: 165.7 | 0.607 | 0.048 |
| G G             | 0.224 | 0.224, 0.224 | 0.000 | 0.990 |
| A T             | 0.221 | 0.248, 0.187 | 4.834 | 0.028 |

Note: *Bold indicates significance.

Table 5 Haplotype Analysis of IL-18 Gene Polymorphisms in Group 1 versus Group 2+3+4 of Plasmodium Infected Patients

| Haplotype Block | Freq. | Group (2+3+4), Group (1) Ratio Counts | Group (2+3+4), Group (1) Frequencies | Chi Square | P value * |
|-----------------|-------|--------------------------------------|--------------------------------------|------------|-----------|
| rs360728        | rs360729 | rs549908 | 0.647 | 294.2: 167.8, 23.0: 27.0 | 0.637, 0.821 | 3.926 | 0.048 |
| C A T           |       | 89.1: 372.9, 4.0: 24.0 | 0.193, 0.142 | 0.509 |
| C T G           | 0.19  | 46.6: 415.4, 1.0: 27.0 | 0.101, 0.036 | 0.258 |
| G A T           | 0.097 | 12.8: 449.2, 0.0: 28.0 | 0.028, 0.000 | 0.373 |
| G T G           | 0.026 | 10.2: 451.8, 0.0: 28.0 | 0.002, 0.000 | 0.436 |
| C T T           | 0.021 | 7.6: 454.4, 0.0: 28.0 | 0.016, 0.000 | 0.505 |
| C A G           | 0.015 |                                            |                                       |            |

Note: *Bold indicates significance.

4.93; P = 0.026), and haplotypes AA (rs544354 and rs360727) with a significantly higher frequency among 1–9 years age group (f = 0.130) compared to in patients above 10 years of age (f = 0.045), (χ² = 4.984; P = 0.026) (Table 8).

Haplotype analysis was also done for the IL-18 SNPs between the patients within the age groups 1–9 years and ≥41 years. Two significant haplotypes were identified for these age groups (Table 9). This included haplotype AAG (rs360729, rs360714 and rs7106524), present with increased frequency within the age group ≥41 years (f = 0.375) compared to patients within age group 1–9 years (f = 0.168), (χ² = 4.917; p = 0.027), while haplotype AGG (rs360729, rs360714 and rs7106524) was present with increased frequency within the group 1–9 years (f = 0.278), compared to patients above 41 years of age (f = 0.103), (χ² = 5.687; p = 0.017).

Effect of IL-18 SNPs on Expression Levels in Luciferase Reporter Assay

Luciferase reporter assay was performed to investigate the effect of the SNPs in the promoter region of IL-18 on its expression levels. Several DNA fragments of the IL-18 promoter were cloned and transfected in HuH7 cells and the variations in the luciferase gene activity were monitored. Figure 2 shows the schematic representation of the influence of IL-18 promoter segments, with the corresponding SNPs, on its transcriptional activity. Three different promoter fragments represented as; fragment 1 [from −795 to +28], fragment 2 [from −795 to −523] and fragment 3 [from −182 to +28] were analyzed. We found that fragment 1 exhibited high luciferase activity compared to fragments 2 and 3 (Figure 2A). Fragment 1 includes the two SNPs, rs1946518 and rs187238 that were studied here. As such, we generated three additional fragments that contain different haplotype combinations. Haplotype TG (rs1946518 and rs187238) showed the highest luciferase activity (Figure 2B).

The luciferase reporter assay was also conducted for different DNA segments of the 3'UTR region of the IL-18 gene. Three different fragments of the 3'UTR IL-18 region analyzed were represented as fragment 1 [from +20472 to +20984], fragment 2 [from +20500 to +20984] and fragment 3 [from +20472 to +20733]. Fragment 3 containing 2 SNPs, rs360728 and rs5744292, showed the highest level of luciferase activity (Figure 3A). As it showed the highest luciferase activity, fragment 3 was selected for haplotype expression analysis. Three different allelic combinations for SNPs rs360728 and rs5744292 were tested for their effect on luciferase expression. Haplotype CG (rs360728 and rs5744292) was found to have the highest luciferase activity (Figure 3B).
| SNPs         | Genotype/Allele | Group (1–9 Years) n=18 | Group (10 Years and Above) n=232 | OR (95% C.I.) | χ²   | P-value* |
|-------------|-----------------|------------------------|-----------------------------------|---------------|------|----------|
| rs1946518   | GG              | 3 (16.7%)              | 75 (32.3%)                        | Ref           | 0.360 (0.098–1.320) | 2.56 | 0.109    |
|             | GT              | 12 (66.6%)             | 108 (46.6%)                       | 0.653 (0.127–3.369) | 0.26 | 0.608    |
|             | TT              | 3 (16.7%)              | 49 (21.1%)                        | 0.798 (0.405–1.574) | 0.42 | 0.514    |
|             | G               | 18 (50%)               | 258 (55.6%)                       | 0.419 (0.118–1.490) | 1.91 | 0.167    |
|             | T               | 18 (50%)               | 206 (44.4%)                       | 0.747 (0.208–2.684) | 0.20 | 0.653    |
|             | TT+GT vs. GG    |                        |                                   | 0.419 (0.118–1.490) | 1.91 | 0.167    |
|             | TT vs. GT+GG    |                        |                                   | 0.747 (0.208–2.684) | 0.20 | 0.653    |
| rs1946519   | CC              | 3 (16.7%)              | 79 (34.05%)                       | Ref           | 0.339 (0.092–1.240) | 2.9 | 0.088    |
|             | AC              | 12 (66.7%)             | 107 (46.12%)                      | 0.582 (0.113–3.005) | 0.43 | 0.513    |
|             | AA              | 3 (16.6%)              | 46 (19.83%)                       | 0.751 (0.381–1.480) | 0.69 | 0.406    |
|             | C               | 18 (50%)               | 265 (57.1%)                       | 0.387 (0.109–1.378) | 2.29 | 0.130    |
|             | A               | 18 (50%)               | 199 (42.9%)                       | 0.809 (0.225–2.911) | 0.11 | 0.744    |
|             | AA+AC vs. CC    |                        |                                   | 0.387 (0.109–1.378) | 2.29 | 0.130    |
|             | AA vs. AC+CC    |                        |                                   | 0.809 (0.225–2.911) | 0.11 | 0.744    |
| rs187238    | CC              | 14(77.8%)              | 134 (57.75%)                      | Ref           | 2.011 (0.639–6.326) | 1.48 | 0.224    |
|             | CG              | 4(22.2%)               | 77 (33.2%)                        | 4.636 (0.268–80.6) | 2.17 | 0.141    |
|             | GG              | 0(0%)                  | 21 (9.05%)                        | 2.759 (0.956–7.966) | 3.81 | 0.051    |
|             | C               | 32(88.9%)              | 345 (74.4%)                       | 2.560 (0.818–8.014) | 2.77 | 0.095    |
|             | G               | 4(11.1%)               | 119 (25.6%)                       | 0.266 (0.015–4.568) | 1.78 | 0.182    |
|             | GG+CG vs. CC    |                        |                                   | 2.560 (0.818–8.014) | 2.77 | 0.095    |
|             | GG vs. CG+CC    |                        |                                   | 0.266 (0.015–4.568) | 1.78 | 0.182    |
| rs360728    | CC              | 12(66.7%)              | 185 (79.74%)                      | Ref           | 0.506 (0.16–1.5)    | 1.52 | 0.216    |
|             | CG              | 5(27.8%)               | 39 (16.81%)                       | 0.519 (0.06–4.49) | 0.37 | 0.544    |
|             | GG              | 1(5.5%)                | 8 (3.45%)                         | 0.557 (0.23–1.33) | 1.77 | 0.189    |
|             | C               | 29(80.6%)              | 409 (88.1%)                       | 0.508 (0.18–1.4) | 1.71 | 0.191    |
|             | G               | 7(19.4%)               | 55 (11.9%)                        | 1.647 (0.19–13.9) | 0.21 | 0.643    |
|             | GG+CG vs. CC    |                        |                                   | 0.508 (0.18–1.4) | 1.71 | 0.191    |
|             | GG vs. CG+GG    |                        |                                   | 1.647 (0.19–13.9) | 0.21 | 0.643    |
| SNPs     | Genotype/Allele | Group (1–9 Years) n=18 | Group (10 Years and Above) n=232 | OR (95% C.I.) | $\chi^2$ | P-value* |
|----------|-----------------|------------------------|-----------------------------------|---------------|---------|----------|
| rs5744292 | AA              | 18 (100%)              | 199 (85.8%)                       | Ref           |         |          |
|          | AG              | 0 (0%)                 | 26 (11.2%)                        | 4.915 (0.28–83.96) | 2.33    | 0.126    |
|          | GG              | 0 (0%)                 | 7 (3.0%)                          | 1.391 (0.07–25.33) | 0.63    | 0.426    |
|          | A               | 36 (100%)              | 424 (91.4%)                       | 6.965 (0.42–115.6) | 3.37    | 0.101    |
|          | G               | 0 (0%)                 | 40 (8.6%)                         | 6.213 (0.36–105.5) | 2.95    | 0.085    |
|          | GG+AG vs AA     |                       |                                   | 0.813 (0.04–14.8) | 0.56    | 0.454    |
|          | GG vs AG+AA     |                       |                                   |               |         |          |
| rs360727  | GG              | 12 (66.7%)             | 184 (79.31%)                      | Ref           |         |          |
|          | AG              | 5 (27.8%)              | 40 (17.24%)                       | 0.522 (0.174–1.564) | 1.39    | 0.238    |
|          | AA              | 1 (5.5%)               | 8 (3.45%)                         | 0.522 (0.060–4.521) | 0.36    | 0.548    |
|          | G               | 29 (80.6%)             | 408 (87.93%)                      | 0.569 (0.238–1.359) | 1.65    | 0.195    |
|          | A               | 7 (19.4%)              | 56 (12.07%)                       | 0.522 (0.186–1.462) | 1.58    | 0.209    |
|          | AA+AG vs GG     |                       |                                   | 1.647 (0.194–13.951) | 0.21    | 0.643    |
|          | AA vs AG+AA     |                       |                                   |               |         |          |
| rs549908  | TT              | 13 (72.2%)             | 139 (59.9%)                       | Ref           |         |          |
|          | GT              | 5 (27.8%)              | 73 (31.5%)                        | 1.365 (0.469–3.979) | 0.33    | 0.566    |
|          | GG              | 0 (0%)                 | 20 (8.6%)                         | 3.968 (0.227–69.317) | 1.85    | 0.173    |
|          | T               | 31 (86.1%)             | 351 (75.6%)                       | 1.996 (0.758–5.255) | 2.03    | 0.154    |
|          | G               | 5 (13.9%)              | 113 (24.4%)                       | 1.740 (0.600–5.043) | 1.06    | 0.302    |
|          | GG+GT vs TT     |                       |                                   | 0.280 (0.016–4.821) | 1.69    | 0.194    |
|          | GG vs GT+TT     |                       |                                   |               |         |          |
| rs11214101| TT              | 18 (100%)              | 231 (99.6%)                       | Ref           |         |          |
|          | CT              | 0 (0%)                 | 1 (0.4%)                          | 0.240 (0.009–6.094) | 0.08    | 0.780    |
|          | CC              | 0 (0%)                 | 0 (0%)                            | 0.080 (0.002–4.144) | Nan    | 1.000    |
|          | T               | 36 (100%)              | 463 (99.8%)                       | 0.236 (0.009–5.903) | 0.08    | 1.002    |
|          | C               | 0 (0%)                 | 1 (0.2%)                          | 0.240 (0.009–6.064) | 0.08    | 0.780    |
|          | CC+CT vs TT     |                       |                                   | 12.568 (0.242–651.669) | Nan    | 1        |
|          | CC vs CT+TT     |                       |                                   |               |         |          |
| SNP      | Ref Allele | Minor Allele | Genotype Distribution | Odds Ratio (95% CI) | p-Value | OR (95% CI) | p-Value |
|----------|------------|--------------|-----------------------|--------------------|---------|-------------|---------|
| rs544354 | GG         | A            | 3 (16.66%)            | 0.131 (0.037–0.470) | 0.036   | 1.282       | 0.001   |
|          | AG         | G            | 14 (77.78%)           | 0.358 (0.035–3.652) | 0.008   | 0.82        | 0.366   |
|          | AA         |               | 1 (5.56%)             | 0.407 (0.204–0.812) | 0.008   | 6.86        | 0.008   |
|          | AA+AG      |               | 0.146 (0.041–0.519)   | 11.39              | 0.001   | 0.05        |         |
|          | AA         |               | 0.794 (0.099–6.355)   |                   | <0.001  |             |         |
|          | AG+AA      |               | 0.794 (0.099–6.355)   |                   |         |             |         |
| rs360729 | AA         | T            | 13 (72.2%)            | 1.707 (0.537–5.422) | 0.039   | 0.84        | 0.359   |
|          | AT         | T            | 4 (22.2%)             | 1.871 (0.23–15.08)  | 0.029   | 1.09        | 0.296   |
|          | TT         | T            | 1 (5.6%)              | 1.610 (0.63–3.966)  | 0.056   |             |         |
|          | A          | T            | 30 (83.3%)            | 1.740 (0.60–5.043)  | 0.039   | 1.06        | 0.302   |
|          | G          | T            | 1 (25.6%)             | 0.624 (0.079–4.933) | 0.039   | 0.2         | 0.651   |
| rs360714 | AA         | A            | 7 (38.89%)            | 0.725 (0.057–0.419) | 0.001   | 1.09        | 0.302   |
|          | AG         | G            | 11 (61.1%)            | 0.154 (0.005–3.215) | 0.001   | 1.04        | 0.302   |
|          | GG         |               | 0 (0%)                | 0.256 (0.119–0.554) | 0.001   | 1.39        | 0.296   |
|          | A          |               | 25 (69.44%)           | 0.157 (0.058–0.428) | 0.001   | 1.06        | 0.302   |
|          | G          |               | 11 (30.56%)           | 0.171 (0.164–106.0) | 0.001   | 0.2         | 0.651   |
| rs7106524| GG         | A            | 6 (33.3%)             | 0.725 (0.248–2.118) | 0.035   | 0.35        | 0.555   |
|          | AG         | G            | 9 (50%)               | 0.923 (0.220–3.870) | 0.014   | 0.04        | 0.846   |
|          | AA         |               | 3 (16.7%)             | 0.912 (0.458–1.815) | 0.070   | 1.35        | 0.001   |
|          | G          |               | 21 (58.3%)            | 0.775 (0.281–2.137) | 0.238   | 0.24        | 0.621   |
|          | A          |               | 15 (41.7%)            | 0.905 (0.251–3.267) | 0.023   | 0.02        | 0.878   |
| rs5744231| CC         | T            | 18 (100%)             | 1.407 (0.078–25.35) | 0.035   | 0.64        | 0.422   |
|          | CT         | T            | 0 (0%)                | 0.248 (0.010–6.312) | 0.070   | 0.08        | 0.776   |
|          | TT         | T            | 0 (0%)                | 1.686 (0.097–29.36) | 0.79    | 1.0         | 1.000   |
|          | C          |               | 36 (100%)             | 1.573 (0.09–28.103) | 0.269   | 0.72        | 0.394   |
|          | T          |               | 0 (0%)                | 4.171 (0.16–106.0)  | 0.035   | 0.08        | 0.780   |

(Continued)
Discussion

IL-18, an important immune response mediator that has been reported to be involved in the course of malarial infection. IL-18 is known as a potent proinflammatory cytokine, and its physiological levels have been reported to increase at different stages of \textit{P. falciparum} infection. Information about the influence of genetic variations within the IL-18 gene on parasitemia caused by \textit{P. falciparum} is scarce. In this study, we examined the plausible role and influence of polymorphisms within the IL-18 gene on parasitemia levels in \textit{P. falciparum}-infected patients. We found SNP rs5744292 to be significantly associated with an increased risk of \textit{P. falciparum} infection when compared to healthy controls. It was observed that the rs5744292-G allele occurred more frequently in infected individuals than in the control group. rs5744292 has been implicated in several diseases such as cardiovascular diseases and coronary atherosclerosis. It has also been reported that a decrease in IL-18 concentration is associated with the G allele of rs5744292. We also identified another SNP, rs544354-A allele, to be significantly associated with an increased risk of parasitemia in infected patients. Further, individuals with homozygous AA genotype were found to have an elevated risk of parasitemia caused by \textit{P. falciparum}. There is very little information available about the clinical impact of the SNP rs544354 which is located in the 3’UTR of the IL-18 gene. Another polymorphism found to be significantly associated with parasitemia in the infected patients was the rs360718-GG homozygote, present in the intron region of the IL-18 gene. However, this result needs to be interpreted with caution as the number of individuals in study population carrying GG genotype at the rs360714 SNP were very few.

SNP rs7106524, also located in the intron region of the IL-18 gene, has been investigated for its role in several clinical conditions such as those involving liver transplant. A previous study by He et al (2010) found an association between polymorphism rs7106524 with IL-18 levels, where the minor allele genotype (AA) was found to be associated with the highest concentration of serum IL-18. We found rs7106524 heterozygous GA genotype to be associated with an increased risk of \textit{P. falciparum} parasitemia in comparison to the other two homozygous genotypes. Possibly, moderate levels of IL-18 linked to rs7106524-GA genotype may have a functional consequence in increasing the risk of malarial

Table 6 (Continued).

| Genotype/Allele | Group (1–9 Years) n=18 | Group (10 Years and Above) n=232 | OR (95% C.I.) | \( \chi^2 \) | P-value* |
|----------------|------------------------|----------------------------------|--------------|--------|---------|
| AA | 13 (72.2%) | 123 (63.3%) | Ref | | |
| AC | 5 (27.8%) | 74 (34.0%) | | | |
| CC | 0 (0%) | 10 (5%) | | | |
| AC+CC vs. AA | 12 (66.7%) | 138 (60.0%) | 0.83 (0.32–2.14) | 0.73 | 0.39 |
| CC vs. AC+AA | 13 (66.7%) | 127 (54.4%) | 1.00 (0.50–2.00) | 0.99 | 0.32 |
| rs1293344 | | | | | |
| AA | 12 (66.7%) | 185 (79.5%) | Ref | | |
| AG | 6 (33.3%) | 45 (19.5%) | | | |
| GG | 0 (0%) | 20 (8.6%) | | | |
| AG+GG vs. AA | 18 (100%) | 205 (87.1%) | 2.08 (0.90–4.44) | 2.4 | 0.12 |
| GG vs. AG+AA | 0 (0%) | 10 (4.3%) | 0.01 (0.00–0.23) | 23.8 | <0.001 |

Note: *Bold indicates significance.
Table 7 Genotype Distribution and Allele Frequency of IL-18 Gene SNPs When Age Group 1–9 Years Was Compared with Group 41 Years and Above

| SNPs     | Genotype/ Allele Distribution | Group (1–9 Years) n=18 | Group(41 Years and Above) n=54 | OR (95% C.I.)          | χ²  | P-value* |
|----------|-------------------------------|------------------------|--------------------------------|------------------------|-----|---------|
| rs1946518 |                               |                        |                                |                        |     |         |
|          | GG                            | 3 (16.7%)              | 20 (37.03%)                    | Ref                    |     |         |
|          | GT                            | 12 (66.6%)             | 21 (38.9%)                     | 0.263 (0.064–1.070)    | 3.76| 0.052   |
|          | TT                            | 3 (16.7%)              | 13 (24.07%)                    | 0.650 (0.113–3.726)    | 0.24| 0.627   |
|          | G                             | 18 (50%)               | 61 (56.5%)                     | 0.770 (0.362–1.641)    | 0.46| 0.498   |
|          | T                             | 18(50%)                | 47 (43.5%)                     | 0.340 (0.088–1.321)    | 2.58| 0.108   |
|          | TT+GT vs. GG                  |                        |                                | 0.631 (0.157–2.527)    | 0.43| 0.512   |
|          | TT vs. GT+GG                  |                        |                                |                        |     |         |
| rs1946519 |                               |                        |                                |                        |     |         |
|          | CC                            | 3 (16.7%)              | 18 (33.33%)                    | Ref                    |     |         |
|          | AC                            | 12 (66.6%)             | 22 (40.74%)                    | 0.306 (0.075–1.252)    | 2.89| 0.089   |
|          | AA                            | 3 (16.7%)              | 14 (25.92%)                    | 0.778 (0.136–4.458)    | 0.08| 0.777   |
|          | C                             | 18 (50%)               | 58 (53.7%)                     | 0.862 (0.405–1.834)    | 0.15| 0.699   |
|          | A                             | 18(50%)                | 50 (46.3%)                     | 0.400 (0.102–1.563)    | 1.82| 0.177   |
|          | AA+AC vs. CC                  |                        |                                | 0.571 (0.144–2.274)    | 0.64| 0.423   |
|          | AA vs. AC+CC                  |                        |                                |                        |     |         |
| rs187238  |                               |                        |                                |                        |     |         |
|          | CC                            | 14 (77.8%)             | 37 (68.51%)                    | Ref                    |     |         |
|          | CG                            | 4 (22.2%)              | 13 (24.07%)                    | 1.230 (0.342–4.415)    | 0.1 | 0.750   |
|          | GG                            | 0 (0%)                 | 4 (7.4%)                       | 3.480 (0.176–68.785)   | 1.47| 0.224   |
|          | C                             | 32 (88.9%)             | 87 (80.6%)                     | 1.931 (0.615–6.059)    | 1.31| 0.252   |
|          | G                             | 4 (11.1%)              | 21 (19.4%)                     | 1.608 (0.460–5.617)    | 0.56| 0.454   |
|          | GG+CG vs. CC                  |                        |                                | 0.303 (0.016–5.911)    | 1.41| 0.234   |
|          | GG vs. CG+CC                  |                        |                                |                        |     |         |
| rs360728  |                               |                        |                                |                        |     |         |
|          | CC                            | 12 (66.7%)             | 42 (77.8%)                     | Ref                    |     |         |
|          | CG                            | 5 (27.7%)              | 10 (18.5%)                     | 0.571 (0.164–1.996)    | 0.78| 0.376   |
|          | GG                            | 1 (5.6%)               | 2 (3.7%)                       | 0.571 (0.048–6.856)    | 0.2 | 0.655   |
|          | C                             | 29 (80.5%)             | 94 (87.04%)                    | 0.617 (0.227–1.674)    | 0.91| 0.339   |
|          | G                             | 7 (19.4%)              | 14 (12.96%)                    | 0.571(0.177–1.844)     | 0.89| 0.345   |
|          | GG+CG vs. CC                  |                        |                                | 1.529 (0.130–17.939)   | 0.12| 0.733   |
|          | GG vs. CG+GG                  |                        |                                |                        |     |         |

(Continued)
| SNPs  | Genotype/ Allele Distribution | Group (1–9 Years) n=18 | Group(41 Years and Above) n=54 | OR (95% C.I.) | \( \chi^2 \) | P-value* |
|-------|-------------------------------|------------------------|-------------------------------|---------------|------|--------|
| rs5744292 | AA                            | 18 (100%)              | 44 (81.5%)                    |              |      |        |
|  | AG                            | 0 (0%)                  | 8 (14.8%)                     |              |      |        |
|  | GG                            | 0 (0%)                  | 2 (3.7%)                      |              |      |        |
|  | A                             | 36 (100%)               | 96 (88.9%)                    |              |      |        |
|  | G                             | 0 (0%)                  | 12 (11.1%)                    |              |      |        |
|  | GG+AG vs AA                   | 0 (0%)                  | 8.730 (0.486–156.833)         | 3.87          | 0.049|        |
|  | GG vs AG+AA                   | 0.568 (0.026–12.377)    | 0.69                          | 0.407        |      |        |
| rs360727 | GG                            | 12 (66.7%)              | 44 (81.5%)                    |              |      |        |
|  | AG                            | 5 (27.7%)               | 8 (14.8%)                     |              |      |        |
|  | AA                            | 1 (5.6%)                | 2 (3.7%)                      |              |      |        |
|  | G                             | 29 (80.5%)              | 96 (88.9%)                    |              |      |        |
|  | A                             | 7 (19.4%)               | 12 (11.1%)                    |              |      |        |
|  | GG+AG vs GG                   | 0.455 (0.137–1.504)     | 1.71                          | 0.190        |      |        |
|  | GG vs GT+TT                   | 1.529 (0.130–17.939)    | 0.12                          | 0.733        |      |        |
| rs549908 | TT                            | 13 (72.2%)              | 39 (72.2%)                    |              |      |        |
|  | GT                            | 5 (27.8%)               | 10 (18.5%)                    |              |      |        |
|  | GG                            | 0 (0%)                  | 5 (9.3%)                      |              |      |        |
|  | T                             | 31 (86.1%)              | 88 (81.5%)                    |              |      |        |
|  | G                             | 5 (13.9%)               | 20 (18.5%)                    |              |      |        |
|  | GG+GT vs TT                   | 1.00 (0.304–3.290)      | 0                             | 1.000        |      |        |
|  | GG vs GT+TT                   | 0.243 (0.013–4.619)     | 1.79                          | 0.180        |      |        |
| rs11214101 | TT                            | 18 (100%)               | 54 (100%)                     |              |      |        |
|  | CT                            | 0 (0%)                  | 0 (0%)                        |              |      |        |
|  | CC                            | 0 (0%)                  | 0 (0%)                        |              |      |        |
|  | T                             | 36 (100%)               | 108 (100%)                    |              |      |        |
|  | C                             | 0 (0%)                  | 0 (0%)                        |              |      |        |
|  | CC+CT vs TT                   | 0.339 (0.007–17.722)    | 0.339                         | 0.790        | 1.000|        |
|  | CC vs CT+TT                   | 2.946 (0.056–153.803)   | 2.946                         | 1.000        |      |        |
| SNP       | Genotype | Ref | Test Stat | P Value |
|-----------|----------|-----|-----------|---------|
| rs544354  | GG       | 3 (16.7%) | 32 (59.3%) | Ref     |
|           | AG       | 14 (77.8%) | 12 (22.2%) | 0.080 (0.020–0.330) |
|           | AA       | 1 (5.6%) | 10 (18.5%) | 0.938 (0.087–10.048) |
|           | G        | 20 (55.6%) | 76 (70.4%) | 0.526 (0.242–1.144) |
|           | A        | 16 (44.4%) | 32 (29.6%) | 0.138 (0.036–0.532) |
|           | AA+AG vs. GG | 0.020–0.330 | 2.67 | 0.102 |
|           | AA vs. AG+AA | 0.087–10.048 | 0.957 |
|           | G        | 20 (55.6%) | 76 (70.4%) | 2.67 |
|           | A        | 16 (44.4%) | 32 (29.6%) | 0.102 |
| rs360729  | AA       | 13 (72.2%) | 39 (72.2%) | Ref     |
|           | AT       | 4 (22.2%) | 10 (18.5%) | 0.833 (0.223–3.115) |
|           | TT       | 1 (5.6%) | 5 (9.3%) | 1.667 (0.178–15.607) |
|           | T        | 30 (83.3%) | 88 (81.5%) | 1.136 (0.417–3.095) |
|           | T+AT vs. AA | 0.223–3.115 | 0.07 | 0.786 |
|           | T vs. AT+AA | 0.178–15.607 | 0.2 | 0.651 |
| rs5744260 | GG       | 16 (88.9%) | 52 (96.3%) | Ref     |
|           | AG       | 2 (11.1%) | 2 (3.7%) | 0.308 (0.040–2.363) |
|           | AA       | 0 (0%) | 0 (0%) | 0.314 (0.006–16.466) |
|           | G        | 34 (94.4%) | 106 (98.1%) | 0.321 (0.044–2.365) |
|           | A        | 2 (5.6%) | 2 (1.9%) | 0.308 (0.040–2.363) |
|           | AA+AG vs. GG | 0.040–2.363 | 1.41 | 0.234 |
|           | AA vs. AG+AA | 0.006–16.466 | 1.41 | 0.260 |
|           | G        | 21 (58.3%) | 69 (63.9%) | 2.946 (0.056–153.803) |
|           | A        | 15 (41.7%) | 39 (36.1%) | 0.576 (0.063–5.291) |
| rs360714  | AA       | 7 (38.9%) | 43 (79.6%) | Ref     |
|           | AG       | 11 (61.1%) | 11 (20.4%) | 0.163 (0.051–0.517) |
|           | GG       | 0 (0%) | 0 (0%) | 0.172 (0.003–9.374) |
|           | G        | 25 (69.4%) | 97 (89.8%) | 0.258 (0.100–0.663) |
|           | A        | 11 (30.6%) | 11 (10.2%) | 0.163 (0.051–0.517) |
|           | GG+AG vs. AA | 0.051–0.517 | 10.56 | 0.001 |
|           | GG vs. AG+AA | 0.003–9.374 | 10.56 | 0.001 |
| rs7106524 | GG       | 6 (33.3%) | 23 (42.6%) | Ref     |
|           | AG       | 9 (50%) | 23 (42.6%) | 0.667 (0.204–2.178) |
|           | AA       | 3 (16.7%) | 8 (14.8%) | 0.696 (0.140–3.454) |
|           | G        | 21 (58.3%) | 69 (63.9%) | 0.791 (0.366–1.709) |
|           | A        | 15 (41.7%) | 39 (36.1%) | 0.674 (0.220–2.063) |
|           | AA+AG vs. GG | 0.204–2.178 | 0.45 | 0.500 |
|           | AA vs. AG+AA | 0.140–3.454 | 0.2 | 0.565 |
|           | G        | 21 (58.3%) | 69 (63.9%) | 0.36 | 0.550 |
|           | A        | 15 (41.7%) | 39 (36.1%) | 0.48 | 0.487 |
|           | GG+AG vs. AA | 0.220–2.063 | 0.04 | 0.850 |
|           | GG vs. AG+AA | 0.270–4.899 | 0.04 | 0.850 |

(Continued)
| SNPs       | Genotype/Allele Distribution | Group (1-9 Years) n=18 | Group (41 Years and Above) n=54 | OR (95% C.I.) | χ² | P-value* |
|------------|------------------------------|------------------------|---------------------------------|---------------|----|---------|
| rs5744231  |                              |                        |                                 |               |    |         |
| CC         | 18 (100%)                    | 53 (98.1%)             | Ref                             | 1.037 (0.040-26.594) | 0.34 | 0.560   |
| CT         | 0 (0%)                       | 1 (1.9%)               | 1.019 (0.041-25.559)           | 0.34          | 0.560 |         |
| TT         | 0 (0%)                       | 0 (0%)                 | 1.037 (0.040-26.594)           | 0.34          | 0.560 |         |
| rs360718   |                              |                        |                                 |               |    |         |
| AA         | 13 (72.2%)                   | 36 (66.7%)             | Ref                             | 4.068 (0.210-78.641) | 1.75 | 0.186   |
| AC         | 5 (27.8%)                    | 1 (0.8%)               | 1.678 (0.087-4.799)            | 0.95          | 0.330 |         |
| CC         | 0 (0%)                       | 23 (41.5%)             | 1.300 (0.401-4.15)             | 0.19          | 0.611 |         |
| rs1293344  |                              |                        |                                 |               |    |         |
| AA         | 12 (66.7%)                   | 37 (68.3%)             | Ref                             | 0.959 (0.280-3.151) | 0.01 | 0.918   |
| AG         | 6 (33.3%)                    | 12 (20.8%)             | 3.667 (0.189-71.113)           | 1.57          | 0.209 |         |
| GG         | 0 (0%)                       | 30 (53.6%)             | 3.279 (0.473-3.455)            | 0.24          | 0.626 |         |
| rs3881016  |                              |                        |                                 |               |    |         |

Note: *Bold indicates significance.

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Table 7 (Continued).
Table 8 Haplotype Analysis for IL-18 Gene Polymorphisms Between Group 1 (1–9 Years) Was Compared to 10 Years and Above

| Haplotype | Freq. | Case, Control Ratio Counts | Case, Control Frequencies | Chi Square | P-value * |
|-----------|-------|----------------------------|---------------------------|------------|-----------|
| rs544354  | rs360727 | 0.66 | 292.5: 141.5, 17.7: 18.3 | 0.674, 0.491 | 4.93 | 0.026 |
| rs360729  | rs360714 | 0.21 | 87.5: 346.5, 11.3: 24.7 | 0.202, 0.314 | 2.53 | 0.112 |
| rs7106524 | rs360714 | 0.08 | 34.5: 399.5, 2.3: 33.7 | 0.080, 0.064 | 1.11 | 0.740 |
| rs360729  | rs360714 | 0.05 | 19.5: 414.5, 4.7: 41.3 | 0.045, 0.130 | 4.984 | 0.026 |

Note: *Bold indicates significance.

Table 9 Haplotype Analysis for IL-18 Gene Polymorphisms Between Groups (1–9 Years) Was Compared to 41 Years and Above

| Haplotype | Freq. | Case, Control Ratio Counts | Case, Control Frequencies | Chi Square | P-value * |
|-----------|-------|----------------------------|---------------------------|------------|-----------|
| rs360729  | rs360714 | 0.36 | 27.8: 50.2, 13.9: 22.1 | 0.356, 0.387 | 0.105 | 0.746 |
| G         | A     | 0.31 | 29.2: 48.8, 6.1: 29.9 | 0.375, 0.168 | 4.917 | 0.027 |
| G         | G     | 0.15 | 8.0: 70.0, 10.0: 26.0 | 0.103, 0.278 | 5.687 | 0.017 |
| G         | A     | 0.14 | 11.8: 66.2, 4.9: 31.1 | 0.151, 0.137 | 0.036 | 0.850 |
| G         | A     | 0.01 | 1.2: 76.8, 0.1: 35.9 | 0.016, 0.002 | 0.442 | 0.506 |

Note: *Bold indicates significance.
parasitemia and such a conclusion needs to be investigated further.

Haplotype analysis also revealed 2 haplotypes GT and AT (rs544354 and rs549908) that show significant differences between the controls and the infected patients. Haplotype GT was found more frequently in the controls whereas haplotype AT was found more frequently in the infected patient group. Haplotype AT includes rs544354 risk allele A whereas haplotype GT contains rs544354 allele G at the corresponding position. This haplotype analysis confirms the association of polymorphism rs544354 with an increased risk of *P. falciparum* infection. We also analyzed the influence of these SNPs in the *IL-18* gene on the extent of parasitemia among the *P. falciparum*-infected patients and found the rs5744292-G allele to be significantly associated with increased parasite density in patients. Polymorphisms within the cytokine genes have been reported to be associated with various degrees of parasitemia. Sortica et al (2013) identified variations in IL-6 and IL-12B to be associated with the levels of parasitemia in *P. vivax*-infected Brazilian population. Additionally, rs5744292 G-allele has been reported to be associated with downregulation of IL-18 levels. The cellular/molecular mechanism through which rs5744292-G

**Figure 2** Luciferase activity assay for the fragments containing *IL-18* promoter. (A) Nested deletions of this region were generated, cloned into pGL3 luciferase vector and transfected in HuH7 cell line for 24 h. (B) As fragment 1 showed the highest luciferase activity, it was further tested to evaluate the effect of the SNPs included in this fragment on the expression activity. Cells were harvested and tested for luciferase activity after normalization using renilla luciferase enzyme. Each experiment was performed in triplicates. Values represent mean ±SD.

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allele contributes to an increase in parasitemia in *P. falciparum*-infected patients is unclear and needs further investigation. Three more SNPs, rs187238, rs360714 and rs1293344, were also found to be significantly associated with varying parasitemia levels in our study group. We observed that rs187238-CG, rs360714-GG and rs1293344-AG genotypes were found to be associated with protection from a higher degree of parasitemia. SNP rs187238, located in the promoter region of *IL-18* gene, has been previously associated with hepatitis C and type 1 diabetes. Anyona et al (2011) observed that rs187238-C allele correlated with decreased IL-18 levels. The authors of this study suggested that this G to C substitution destroys a histone 4 transcription factor-1 (H4TF-1) nuclear factor-binding site, resulting in lower promoter activity with subsequent downregulation of IL-18 levels. Based on these results, it can be inferred that the protective effect of rs187238 heterozygote variants CG
may be due to its regulation of IL-18 levels thereby controlling parasitemia.

Balavarca et al (2014) found that polymorphism rs1293344, located in the 5' flanking region of the IL-18 gene, is associated with improved overall survival of colorectal cancer.\(^{42}\) Also, no significant association was found between polymorphism at rs1293344 and the changes in the serum concentration of IL-18.\(^{43}\) In this study, we found rs1293344-AG genotype to be correlated with improved disease outcomes by protecting against increased parasitemia in P. falciparum-infected patients.

Our analysis also identified several haplotypes with differential frequencies among infected patients with varying degrees of parasitemia. A comparison of patients with the lowest degree of parasitemia with those that had a higher degree of parasitemia revealed one significant haplotype. The haplotype CAT exhibited a higher frequency in group 1 parasitemia patients than the combined group 2+3 +4 parasitemia patients. The mechanism through which this haplotype could affect the course of infection and/or expression of IL-18 requires further investigation.

Age is regarded as one of the crucial factors affecting the susceptibility and severity of the malarial infection.\(^{44}\) It has been observed to correlate with protective immunity in malaria-endemic areas.\(^ {45}\) When compared to adolescents and adults, young children have been reported to be more susceptible to malarial infection, usually with severe complications that may be fatal if untreated.\(^ {46,45,46}\) Due to the development of acquired immunity, adults are protected from severe malaria.\(^ {46,47}\) In this study, we investigated the influence of the selected SNPs in the IL-18 gene within the different age groups of the P. falciparum-infected patients. On comparing genotypic distribution among patients in the 1–9 years group with patients in age group ≥10 years, 2 SNPs- rs544354 and rs360714 were found to show a significant association with the latter group. We found that increased frequency of rs544354-A allele was predictive of parasitemia in the P. falciparum-infected patients included in the 1–9 years age group. Additionally, the A allele had a similar association in both dominant and recessive modes of inheritance as well as heterozygotic AG genotype. Similarly, a higher prevalence of the rs360714-G allele was associated with an increased risk of parasitemia in the patients of age group 1–9 years. Our results are consistent with the observation in the Kenyan population, where parasitemia was found to be strongly dependent on the age factor, with children under 5 years exhibiting the highest malarial parasitemia levels.\(^ {8}\) Further, another study has reported overall higher levels of parasitemia in children ≤9 years.\(^ {48}\) IL-18 levels have also been reported to exhibit an age-related pattern in malarial patients.\(^ {49,50}\) The functional mechanism(s) of these IL-18 SNPs must be studied to elucidate the role of these polymorphisms in determining age-related susceptibility to parasitemia.

We also performed a haplotype analysis between different age groups within the P. falciparum-infected patients. Single haplotype CA was found to be differentially distributed between the younger and older age groups. CA occurred more frequently within the 1–5 year age groups than in the older parasitemia patients. SNP rs187238 is positioned in the promoter region of the IL-18 gene and its C allele has been reported to correlate with decreased concentration of IL-18.\(^ {26}\) The haplotype analysis among the parasitemia patients within the age groups 1–9 years and ≥ 41 years revealed the presence of two haplotypes AAG and AGG of SNPs rs360729, 360714 and rs7106524, respectively, which showed differential distribution between these groups. AAG occurred more frequently in older patients whereas haplotype AGG was found to occur at a higher frequency among the 1–9 years age group. Although rs360714 is located in the intron region of IL-18 gene, the two haplotypes, AAG and AGG differ only with regards to rs360714 A or G allele. This suggest that rs360714 does have some functional impact in different age groups, a conclusion that needs further testing to confirm its biological/physiological importance.

The functional activity of the SNPs in the promoter and 3'UTR of the IL-18 gene was analyzed by luciferase reporter assay. Two SNPs, rs1946518 and rs187238, of the promoter region within the IL-18 gene were found to modulate the transcriptional activity in the vector system. Our results are in agreement with the previous studies that have reported an association of promoter polymorphism with IL-18 expression. A study investigating patients with sarcoidosis analyzed the promoter activity by luciferase assay and found polymorphisms rs1946519 and rs1946518 to be significantly associated with serum levels of IL-18.\(^ {51}\) Liang et al (2005) also reported the association of rs187238, among several other SNPs, with the transcriptional activity in the reporter assay system.\(^ {52}\) Another study examining the functional activity of IL-18 promoter region found polymorphisms rs1946518 and rs187238 to be associated with the transcriptional activity.\(^ {53}\) Similarly, the SNPs in the potential regulatory 3'UTR region have been shown to
affect the expression levels of IL-18. Previous studies have reported that the 3’UTR region of the IL-18 gene contains regulatory elements that may be responsible for mRNA stability and translation rate.\(^{41,54}\) Polymorphism rs5744292 has been previously found to influence the IL-18 expression in various clinical conditions such as coronary artery disease patients.\(^{25,32}\) Although not much is known about the polymorphism rs360728 in relation to IL-18 expression, we found rs360728-C/rs5744292-G haplotype to be associated with enhanced luciferase activity. This was in contrast to the previous report that linked rs5744292-G containing haplotypes with decreased transcriptional activity,\(^{54}\) as well as our observation that rs5744292-G allele is linked to upregulation of IL-18 expression. These variations may be attributed to the fact that in-vitro studies using isolated fragments of the regulating regions may not be able to correctly reflect the functional activity in-vivo. Different elements including both cis- and trans-acting can influence the folding of the regulatory regions and subsequently their transcriptional activity. These results need to be confirmed in-vivo to clarify the role of these SNPs in modulating IL-18 expression.

In conclusion, this study has identified several individual polymorphisms and haplotypes within the pro-inflammatory IL-18 gene and revealed their association with the risk and extent of parasitemia in P. falciparum-infected patients. This is the first time such a detailed analysis of IL-18 gene polymorphism concerning malaria has been reported in Saudi Arabia, which in turn provides important insights into the role of population genetics in malaria.

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Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure
The authors have nothing to declare with respect to the research, authorship, and/or publication of this article.

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