Significant Differences in *FcγRIIa, FcγRIIa and FcγRIIIb* Genes Polymorphism and Anti-malarial IgG Subclass Pattern are Associated with Severe Malaria in Saudi Children

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Abstract

Background: The FcyRs genotypes have been reported to play a key role in the defence against malaria parasites through both cellular and humoral immunity. This study aimed to investigate the possible correlation between FcγR (IIa, IIIa, and IIIb) genes polymorphism and the clinical outcome for anti-malarial antibody response of *Plasmodium falciparum* infection among Saudi children.

Material and methods: A 600 volunteers have been enrolled in this study, including 200 malaria-free control (MFC) subjects, 218 patients with uncomplicated malaria (UM) and 182 patients with severe malaria (SM). The FcγR genotypes was analysed using PCR amplification methods, and measurement of immunoglobulins were determine using ELISA.

Results: The data revealed the *FcγRIIa*-R/R131 showed a statistically association with the increased susceptibility to SM when compared to UM patients. Furthermore, higher levels of IgG1, IgG2, and IgG4 were associated with the *FcγRIIa*-H/H131 genotypes among UM patients. Although the *FcγRIIa*-F/V176 genotype was not associated with UM, it showed a significant association with severe malaria. Interestingly, the *FcγRIIa*-V/V176 genotype was This study aimed to associated with protection against SM. Moreover, severe malaria patients carrying the *FcγRIIa*-F/F genotype showed higher levels of AMA-1-specific IgG2 and IgG4 antibodies. The *FcγRIIIb* NA1/NA1 and *FcγRIIIb* NA2/NA2 genotypes did not show significant differences between UM and the MFC. However, the genotype *FcγRIIIb*-NA2/NA2 was statistically associated with severe malaria.

Conclusions: The data presented in this study strongly suggest the possible impact of FcyR (IIa, RIIIa and RIIIb) gene variants and anti-malaria IgG subclasses play a role in susceptibility to malaria infection and disease outcomes in Saudi children.

Background

Malaria is a parasitic infectious disease caused by five species of Plasmodium (*P.*) that are transmitted to humans *via* mosquito bites. Of these species, *P. falciparum,* which is most prevalent in Africa, and *P. vivax* pose the greatest threat to health [1]. In Saudi Arabia, *P. falciparum* represents about 99 % of the total cases of malaria, while only 1% of the patients are infected by *P. vivax* [1]. Globally, malaria affects approximately 228 million people and causes 416,000 deaths in 2018; children less than 5 years old are more vulnerable group affected by malaria [2]. In Saudi Arabia, the number of recorded malaria patients was steadily below 100 between 2010 and 2015, but rose to 272 cases in 2016, mostly due to increased migration of people from war zones along the border with Yemen, as well as difficulties in providing adequate medical services in these regions [1]. However, the health service in this country remains vigilant and offers free diagnosis and treatment for all patients [1].

There are three sub-families of surface receptors for the Fc region of IgG, designated as FcγRI, II, and III [3]. Most immune cells express Fc receptors which are crucial for determining the specificity of IgG antibodies [4]. FcγR induces monocyte activation features such as phagocytosis, degranulation,

superoxide generation, antibody-dependent cell inhibition, cytokine production, and antibody regulation, which are essential for host defence and immune regulation [5, 6]. The effectiveness of IgG-induced FcγR activity demonstrates inter-individual heterogeneity due to the genetic polymorphisms of the three subclasses of FcγR; *FcγRIIa* (CD32a), *FcγRIIa* (CD16a), and *FcγRIIIb* (CD16b) [6].

The present study aimed to investigate the possible relationship between the expression of *FcγRIIa* (CD32a), *FcγRIIa* (CD16a), and *FcγRIIIb* (CD16b) gene variants and the antibodies against the malarial Apical Membrane Antigen 1 (AMA-1) in association with the susceptibility to malaria infection among Saudi children.

Material And Methods

Study area

This study was conducted at Bani Malik General Hospital in Jazan Region (BMGHJ), located in the Southern part of the Kingdom of Saudi Arabia (KSA), during three transmission seasons from October 2015 to March 2018. The highlights of this study setting have already been described in related previous studies [4, 7-10].

Study design and patients

A prospective case-control study was conducted in children attending the outpatient clinic of BMGHJ, with a confirmed clinically diagnosed *P. falciparum* infection. Patients with positive thick blood film for *P. falciparum* asexual parasites were recruited based on the microscopic diagnosis.

Participants with no features of severe malaria were defined as having uncomplicated *P. falciparum* infection. Children were diagnosed with severe malaria on the basis of one or more of the following: severe malarial anaemia, cerebral malaria, hypoglycaemia, jaundice, acidosis, acute kidney injury (renal impairment), significant bleeding, pulmonary oedema, and shock as described in detailed by the WHO [11]. These clinical manifestations occurred in the absence of any identified alternative cause other than *P. falciparum* asexual parasitemia. Children with cerebral malaria had a Blantyre Coma Score (BCS) < 3 at or 4 hours post-admission. Children with severe malarial anaemia had a blood haemoglobin concentration 5 g/dl or a haematocrit value <15% together with a parasite count > 10000/μL. All other children recruited in the study had a haemoglobin concentration above this level. Control children were selected from Child and Woman Health Clinics (CWHC), the clinics provide children's health services, including providing/following-up their routine vaccinations, as well as providing seasonal vaccines for children, frequency-matching the patients for age, sex, and ethnicity. Control group were recruited who appeared healthy by clinical physical examination and did not have serious illness or any malaria according to information provided by guardians or parents.

The study excluded children with any co-infectious diseases and none of the participants was positive for HIV. All the children were recruited during three malaria transmission seasons, from October 2015 to

March 2018.

Sample collection

After the diagnosis of malaria and before the start of the pharmacological course of treatment, 100 µL of blood was spotted and dried on filter paper (Qualitative filter paper, Grade 1, circles, diam. 42.5 mm from Whatman®, Sigma-Aldrich®). This collected sample was used for investigating Fcγ receptors genes polymorphism, parasite detection using PCR, and measurement of immunoglobulins as described earlier in our report [12, 13].

Serum elution from filter-paper samples

To elute dried samples from filter-paper, a hole puncher of ϕ 6 mm was used for punching out filter-paper discs and placed in Eppendorf tubes with 100 µL of phosphate-buffered saline (PBS). Subsequently, the discs were transferred to 10 ml tubes. Then, 500 µL of (PBS) with 0.05% Tween and 0.5% bovine serum albumin (BSA) were added to the tubes and incubated under shaking for 2 hours at room temperature. After incubation, the samples were vigorously shaken with a vortex for 30 seconds, and the supernatants containing the eluted sera were aliquoted in cryotubes (1.5 ml) and stored at −20 °C till analysis. Each extracted sample contained an approximately 1:100 diluted serum [12].

DNA extraction

DNA was extracted from 50 µL dried drop of blood sample on the filter paper using the QIAamp DNA Mini Kit (Qiagen®, Hamburg, Germany). The extracted DNA was re-suspended in a 150 µL of Tris-borate-EDTA (TBE) buffer as previously described [14].

Parasite genotype

Detection of *P. falciparum* was based on targeting the *AMA-1_3D7* gene using polymerase chain reaction (PCR) from 5 µl of the extracted DNA samples as previously detailed [15-17].

Enzyme-linked immunosorbent assays (ELISA)

IgG subclasses antibodies were measured against the recombinant AMA-1 anti-malarial antigen. The total levels of IgG and its subclasses were measured using enzyme-linked immunosorbent assays (ELISA) as previously described in detail [18, 19] and as recently indicated [20].

Genotyping of FcγRs polymorphisms

The *FcγRIIa-131Arg/His* (rs1801274, assay ID: C__9077561_20) and *FcγRIIa-176F/V* (rs396991, assay ID: C__25815666_10) polymorphisms were genotyped using the high-throughput TaqMan® 5' allelic discrimination assay-by-design method, as per the instructions of the manufacturer (Applied Biosystems, Foster City, CA, USA). The *FcγRIIIb-NA1/NA2* genotyping for the rs448740 (N65S) and rs147574249

(N82D) was carried out in accordance with the formerly described RFLP (Restriction Fragment Length Polymorphism) method [21, 22].

Statistical analysis

Statistical analysis was done by SPSS statistical software version 23 for Windows (IBM© SPSS© statistics). In this study, the antibodies (total IgG and IgG subclasses) levels were analysed using one-way analysis of variance tests and the *P* values were determined. The values were deduced from the log–log correlative coefficient for each of the respective antibody standard curve.

The boxes shown in the results illustrate the total observations corresponding to the 25% and 75% quartile, and the horizontal line represents the median. The whiskers illustrate the 10% and 90% quartile, excluding outliers. With respect to the risk of malaria infection during the pregnancy period, all values of *P* <0.05, 95% confidence intervals (CI) for odds ratio (OR) that did not cross 1.00 were considered as statistically significant. In the analysis, *FcγRIIa- Arg/ His 131* polymorphism was used as a reference, due to its utmost prevalence in humans [23]. Using the same software, a 2 x 2 chi-square test was used to compare the overall allele frequency. The *Hardy–Weinberg equilibrium (HWE)* for genotypic deviations were assessed using a chi-squared statistical test The logistic regression analysis was performed to test for the associations between the *FcγRs* genotypes related to higher levels of anti-malarial IgG subclass among severe malaria compared to uncomplicated malaria patients. Associations were quantified using odds ratios [OR] with 95% confidence intervals [CI] that did not cross 1.00 with P value <0.05, defined as statistically significant. As shown below, each IgG subclass was ranked in malaria-free controls in two categories based on the levels of antimalarial antibodies.

Antibodies	Normal Levels µg/mL	Higher Levels µg/mL
IgG1	≤3.7	≥3.71
IgG2	≤1.1	≥1.2
IgG3	≤2.14	≥2.15
IgG4	≤0.41	≥0.42

Results

Classification of the study participants

In this study, demographic data on malaria, parasite density, and disease complication variables were analysed for 600 children of matched sex and age. The 600 subjects were categorized into three different groups. Group I: The malaria-free control [MFC, n = 200 (33.3%) subjects]; included subjects without symptoms of the disease and showed negative results for blood film examination and PCR of the malaria parasites. Group II: Uncomplicated malaria [UM, n = 218 (36.3%) patients]. Group III: severe malaria [SM, n = 182 (30.3%) patients]. Group III included patients with severe malarial anaemia [n = 56 (30.8%)],

cerebral malaria [n = 61 (33.5%)] and children suffering from other complications such as hypoglycaemia, jaundice, pulmonary oedema and acute respiratory distress [n = 65 (35.7%)]. The mean number of parasites in severe malaria patients was significantly higher compared to uncomplicated malaria, P<0.001 (Table 1). The body temperature was significantly different between the study population, P < 0.001 (Table 1).

Comparison between the distribution of the *FcγRIIa* genotype and its allelic frequencies among the different study groups

The genotype frequencies for *FcγRIIa*, *FcγRIIa*, and *FcγRIIIb* did not deviate from the expectations of the HWE in each genotype group (Table 2). The frequencies for the homozygotes carrying the *FcγRIIa-R/R131* in UM were lower than the ones in MFC. The logistic regression analysis revealed that there was no statistically significant difference between UM and MFC amongst both homozygotes *R/R131* [18.0% in UM versus 15.1% in MFC; OR=1.39, 95% CI (0.89 to 2.19) and *P* value = 0.15] and H/H131 [36.7% in UM versus 30% MFC; OR = 0.92, 95% CI (0.62 to 1.36) and *P* value = 0.66] using the heterozygotes as the reference group (Table 3). The *FcγRIIa-R/R131* genotype was statistically associated with SM compared to UM [34.6% in SM versus 15.1% in UM; OR = 2.132, 95% CI (1.287 to 3.533) and *P* value = 0.003]. In contrast, the *FcγRIIa-H/H131* genotype was negatively associated with SM compared to UM [13.7% in SM versus 36.7% in UM; OR = 0.349, 95% CI (0.206 to 0.592) and *P* value < 0.001] (Table 2). The frequencies for the heterozygotes carrying the *FcγRIIa-H/R131* genotype were almost the same among the three groups of MFC, UM, and SM (52.0%, 48.2%, and 51.6%, respectively) (Table 2).

Comparison between the *FcγRIIa* genotypes distributions and their allelic distributions among the different study groups.

As shown in Table 2, the genotype frequencies for *FcγRIIa*, *FcγRIIa* and *FcγRIIIb* did not deviate from expectations of the *HWE* in each genotype group (Table 2). The frequencies for the homozygotes carrying the *FcγRIIa-R/R131* in UM (group II) were lower than the ones in MFC (group I). The logistic regression analysis revealed that there was no statistically significant difference between UM and MFC amongst both homozygotes R/R131 [18.0% in UM versus 15.1% in MFC; OR=1.39, 95% CI (0.89- 2.19) and *P* value= 0.15] and H/H131 [For 36.7% in UM versus 30% MFC; OR= 0.92, 95% CI (0.62- 1.36) and *P* value=0.66] using the Heterozygotes as the reference group (Table 3). The *FcγRIIa-R/R131* was statistically associated with SM patients compared to UM [34.6% in SM versus 15.1% in UM; OR= 2.132, 95% CI (1.287- 3.533) and *P* value= 0.003]. In contrast, the patients carrying *FcγRIIa-H/H131* genotype were statistically negatively associated with SM compared to UM [13.7% in SM versus 36.7% in UM; OR= 0.349, 95% CI (0.206- 0.592) and *P* value <0.001] (Table 2). The frequencies for the heterozygotes carrying the *FcγRIIa-H/R131* genotype were almost the same among the three groups of MFC, UM and SM (52.0%, 48.2% and 51.6%, respectively) (Table 2).

Comparison between the distribution of *FcγRIIa* genotype and its allelic frequencies among the different study groups

The genotype frequencies showed no statistically significant difference among the homozygotes *FcγRIIa-F/F* with UM compared to MFC (Table 2). The logistic regression analysis confirmed the absence of significant differences between UM and MFC among homozygotes F/F [39% in UM versus 45.5% in MFC; OR = 1.95, 95% CI (0.65 to 2.38) and *P* value = 0.79]. Similarly, *FcγRIIa*-V/V genotype showed no statistically significant association with UM compared to MFC [12.8% in UM versus 14.5% in MFC; OR = 1.72, 95% CI (1.04 to 2.82) and *P* value = 0.13] using the heterozygotes as a reference group (Tables 2 and 3). On the other hand, *FcγRIIa-F/F* genotype was statistically associated with SM compared to UM [72.5% in SM versus 39% in UM; OR = 11.51, 95% CI (6.71 to 19.77) and *P* value<0.001] (Tables 2 and 3). In contrast, the *FcγRIIa-V/V* genotype was statistically negatively associated with SM compared to UM [3.8% in SM versus 12.8%in UM; OR = 0.20, 95% CI (0.09 to 0.47) and *P* value <0.001] (Tables 2 and 3). The frequencies analyses also showed differences in the distributions of the heterozygotes *FcγRIIa*-F/V genotype among the three groups (40% in MFC, 48.2% in UM, and 23.6% in SM) (Table 2).

The genotype analyses showed similar frequencies between UM and MFC for homozygotes carrying the *FcγRIIIb-NA1/NA1* of (Table 2). This was confirmed by logistic regression analysis which revealed the lack of statistically significant difference between these two groups in the homozygotes NA1/NA1 [15.1% in UM 17.0% in MFC; OR = 0.79, 95% CI (0.48-1.30) and *P* value = 0.354] (Tables 2 and 3). Furthermore, NA2/NA2 genotype was not statistically significantly different among UM patients compared to MFC [36.7% in UM versus 37.0% in MFC; OR = 1.24, 95% CI (0.85-1.79) and *P* value = 0.263] using the heterozygotes as a reference group (Tables 2 and 3). Similarly, there was no statistical differences between patients with SM and UM for *FcγRIIIb-NA1/NA1* genotype [9.9% in SM versus 15.1% UM; OR = 0.82, 95% CI (0.43 to 1.57) and *P* value = 0.545] (Tables 2 and 3). However, patients carrying the *FcγRIIIb-NA2/NA2* genotype were significantly associated with SM compared to UM [51.6% in SM versus 36.7% in UM; OR= 1.76, 95% CI (1.15- 2.70) and *P* value= 0.009] (Table 2 and 3). The frequencies analyses also showed differences in the distributions of the heterozygotes *FcγRIIa-NA1/NA2* genotype among the three groups (46% in MFC, 48.2% in UM, and 38.5% in SM) (Table 2).

Specific IgG subclass reactivity in the different study groups

The antibody responses for the *P. falciparum* blood-stage antigen AMA-1 was analysed in the different study groups. Our results showed statistically significant differences among the anti-malarial IgG subclasses antibody levels in the different study groups; the overall *P* value < 0.001 (Table 4). In general, the median value of IgG1 and IgG3 subclasses were expressed at higher levels than IgG2 and IgG4 antibodies in the UM group when compared to both SM and MFC subjects (Table 4). To investigate the potential association between the antimalarial IgG subclass responses and protection against infections, we first, used a logistic regression model to compare the levels of IgG subclasses between the UM infection and MFC (Table 5). The results showed that a higher level of IgG3 against the AMA-1 antigen was associated with UM patients compared to MFC subjects [OR = 2.6; 95% CI (2 to 3.3) and *P* value < 0.001]. In addition, the levels of AMA-1-specific IgG4 were significantly lowered in UM patients compared MFC [OR = 0.7; 95% CI (0.5 to 1.9) and *P* value < 0.001] (Table 5). There was no observed association for the AMA-1-specific IgG1 and IgG2 responses in UM compared to MFC (Table 5). The same logistic

regression model confirmed that the apparent anti-malarial IgG1 to IgG4 antibodies were significantly associated with SM when compared to UM patients (Table 5). Collectively, the levels of AMA-1-specific IgG1, IgG2, IgG3 and IgG4 were significantly higher in SM compared to UM patients [for IgG1: OR = 3.4; 95% CI (2.1 to 4.7) and *P* value < 0.001, for IgG2: OR = 2.7; 95% CI (1.9 to 3.5) and P value < 0.001, for IgG3: OR = 1.8; 95% CI (1.1 to 4.6) and *P* value < 0.001) and for IgG4: OR = 1.5; 95% CI (1.2 to 1.8) and *P* value < 0.001] (Table 5).

The results indicated that patients carrying the *FcγRIIa*-H/H131 genotype are significantly associated with higher expression levels of the antimalarial IgG1, IgG2 and IgG4 antibodies, but not IgG3 in UM patients [for IgG1: OR = 0.3; 95% CI (0.2 to 0.6) and *P* value <0.001, for IgG2: OR = 0.5; 95% CI (0.3 to 0.8) and *P* value= 0.006 and for IgG4: OR = 0.5; 95% CI (0.3 to 0.8) and *P* value= 0.006] (Table 6). Comparatively, patients harboring the *FcγRIIa-R/R131* genotype show significantly increased levels of antimalarial IgG2 antibodies and associated with SM compared to UM [OR = 3.7; 95% CI (2.0 to 6.7) and *P* value < 0.001] (Table 6). However, patients carrying the genotype *FcγRIIa-R/R131* are statistically negatively associated with higher levels of AMA-1-specific IgG3 [OR = 0.4; 95% CI (0.2 to 0.6) and P value < 0.001] (Table 6). Independently, the model of the multivariate logistic regression analysis of individuals carrying the *FcγRIIa-F/F* genotype is significantly associated with higher levels of AMA-1-specific IgG2 and IgG4 antibodies in SM compared to UM patients [for IgG2: OR = 3.9; 95% CI (2.4 to 6.4) and *P* value < 0.001 and for IgG4: OR= 3.2; 95% CI (2.1 to 5.3) and *P* value < 0.001] (Table 6). These results together clearly show that the *FcγRIIa-F/F* genotype is negatively associated with higher expression levels of AMA-1-specific IgG3 among SM compared to UM patients [OR = 0.2; 95% CI (0.1 to 0.4) and *P* value < 0.001] (Table 6). Similarly, our data show that the *FcγRIIa-V/V* genotype is negatively associated with higher levels of AMA-1-specific IgG4 in SM compared to UM subjects [OR = 0.4; 95% CI (0.2 to 0.6) and *P* value < 0.001] (Table 6).

Furthermore, our analyses show that the *FcγRIIIb-NA2/NA2* genotype is significantly associated with a higher level of AMA-1-specific IgG4 among SM compared to UM group [OR = 1.7; 95% CI (1.1 to 2.7) and *P* value= 0.011] (Table 6). Our results indicate that the *FcγRIIIb* genotypes are not associated with the independent action of the three IgG subclasses (IgG1, IgG2, and IgG3) of antibodies, maybe due to the absence of interaction in the logistic regression model.

Discussion

This study aimed to evaluate the possible relationship between the variants of *FcγRIIa* (CD32a), *FcγRIIa* (CD16a), *FcγRIIIb* (CD16b) gene polymorphism and *P. falciparum* AMA-1-specific IgG subclass and its importance in the susceptibility to complicated malaria infections among children in Saudi Arabia. To our best knowledge, this study is the country's first report investigating this association among children.

The data of this investigation suggested that there was no significant impact of the *FcγRIIa*-R/H131 genotypes polymorphism on the susceptibility to UM infection compared to MFC. This finding is in parallel with the previously published report from Eastern Sudan by Giha and co-workers which

suggested the lack of statistically significant association between *FcγRIIa-R/H131* and HbAS genotypes polymorphism on immunity and susceptibility to UM infection [24]. This may be due to the similarities in malaria epidemiology, malaria transmission, and patient's semi-immunity to malaria infection [25, 26]. In contrast, the study of Shi et al. has demonstrated a protective effect against UM for *FcγRIIa-R/R131* compared with the heterozygote *FcγRIIa-R/H131* genotype carriers in infants below one year of age [27]. Therefore, there is no general agreement regarding the role of *FcγRIIa-R/H131* in UM infection. In the present study, the logistic regression model suggests that the genotypes *FcγRIIa-R/R131* are statistically significantly associated with increased susceptibility to SM infection (2.1-fold) when compared to UM patients. On the other hand, our data indicate that the *FcγRIIa-H/H131* is negatively associated with SM (3.3-fold decrease) compared to UM patients. Similarly, in a former study, we have reported that the *FcγRIIa-R/R131* genotypes are associated with SM, while the *FcγRIIa-H/H131* genotypes show a significant association with mild malaria among Sudanese patients residing in East Sudan [28]. Previously published case-control investigation have demonstrated that *FcγRIIa-R/R131* homozygosity is associated with protection against high parasite density [27], and the genotypes of *FcγRIIa-H/H131* are correlated with high risk of either severe malaria or placental malaria [29-31]. In addition, several hospital-based case-control reports have confirmed the association between *FcγRIIa-H/H131* and protection against bacterial infections, while *FcγRIIa-R/R131* is associated with increased susceptibility to similar bacterial infections [32-34]. Interestingly, we found here that the levels of IgG1, IgG2, and IgG4 are associated with *FcγRIIa-H/H131* in the UM patients. Similar results have been suggested by Nasr et al. among the Fulani ethnic group that are less susceptible to severe malaria infection [19]. In contrast, previous data on pregnant women with asymptomatic malarial infection (ASM) revealed that the high levels of AMA-1-specific anti-malarial IgG1, IgG2, and IgG4 antibodies are statistically associated with R/R131 carriers rather than the genotype *FcγRIIa-H/H131* [26]. This contradiction may be due to the different levels of malaria endemicity, variations in the individual's genetic background, and the variations in the study designs. The results of this study suggest that the relative reduction in malaria infection in the UM group cannot be explained solely by the magnitude and quality of humoral response to malaria. Additional studies are needed to clarify whether the *FcγRIIa-R/H131* polymorphism is a causative factor in the variance predisposition to malaria that is demonstrated among the different groups.

This study also revealed that the *FcγRIIa-F/V176* genotypes are not associated with UM patients compared to MFC. On the other hand, the *FcγRIIa-F/F176* genotype is statistically associated with SM compared to UM patients. However, patients carrying the *FcγRIIa-V/V176* genotypes are statistically associated with protection against SM compared to UM. The latter finding is in line with a recent Kenyan study which shows that the polymorphisms in the *FcγRIIa-V/V* are associated with protection against severe malarial anaemia and modulations in circulating IFNγ levels [22]. In contrast, a previous investigation on Thai patients did not show an association between *FcγRIIa-F/V176* genotypes and the severity of the disease [31]. Again, these discrepancies may be attributed to the difference in ethnicity and study design.

The current study suggests that individuals carrying the *FcγRIIa-F/F* genotype are significantly expressing higher levels of AMA-1-specific IgG2 and IgG4 antibodies in the SM group compared to patients with UM.

In agreement with this finding, Koene et al. have shown that the *FcγRIIa-F/F* is significantly less bound to IgG1, IgG3, and IgG4 compared to the *FcγRIIa-V/V* genotypes [35].

Our results suggest that there are no statistically significant differences between UM and MFC for the *FcγRIIIbNA1/NA1* and *FcγRIIIbNA2/NA2* genotypes. In contrast, the patients carrying the *FcγRIIIb*-NA2/NA2 genotype are significantly associated with SM compared to patients with UM. Recent work on children living in Western Kenya suggests that the *FcγRIIIbNA1/NA2* gene polymorphisms are not significantly associated with susceptibility to severe malarial anaemia [22]. In addition, the study performed by Adu and co-workers demonstrates that the *FcγRIIIb* NA2/NA2 in Ghanaian children is associated with clinical malaria [5]. A master's degree thesis published in 2010 has demonstrated an association between the *FcγRIIIb-NA2/NA2* and susceptibility to severe and uncomplicated malaria among Ghanaian children [36]. These contradicting results may be attributed to the different malaria transmission settings and malaria epidemic. Moreover, different ethnicity associated with variations in the genetic background may significantly contribute to the *FcγR* gene polymorphism and susceptibility/protection to severe malaria [19].

Conclusion

This study reveals the lack of significant influence of *FcγRIIa-R/H131* genotypes polymorphism on the susceptibility to UM infection. Whereas, the *FcγRIIa*-H/H131 genotype is negatively associated with SM. In addition, the higher levels of antimalarial IgG1, IgG2, and IgG4 antibodies are associated with *FcγRIIa-H/H131* in patients with UM. Furthermore, our data show that the *FcγRIIa-F/V176* genotypes are not associated with UM. In contrast, the *FcγRIIa-F/F176* genotype is correlated with SM. However, patients carrying the *FcγRIIa*-V/V176 genotype were more protected against the severity of the disease. Patients with severe malaria and carrying the *FcγRIIa*-F/F genotype also show higher levels of AMA-1-specific IgG2 and IgG4 antibodies. Furthermore, our data suggest that there are no significant statistical differences between UM and MFC for the *FcγRIIIb* NA1/NA1 and *FcγRIIIb* NA2/NA2 genotypes. Nevertheless, the *FcγRIIIb*-NA2/NA2 genotype is significantly associated with SM infection. Further studies are underway in our laboratory to elucidate if the *FcγRIIa*, *FcγRIIa*, and *FcγRIIIb* genotypes polymorphism contribute to the differential susceptibility to malaria among the different study groups.

Strength And Limitations

To our knowledge, this is the first study in the Kingdom of Saudi Arabia highlighted the relation between *FcγR* genotypes polymorphism, IgG subclass and malaria infection among Saudi children. This will hopefully lead to further research in the area. However, its small sample size and being performed in one region in Saudi Arabia limit the study. Findings need to be confirmed in a large sample size from various regions representing the whole endemic area.

List Of Abbreviations

AMA-1: Apical Membrane Antigen 1

AMPSJ: Aledabi Malaria Prevention Station in Jazan

ASRED: Allele Specific Restriction Enzyme Digestion

BMGHJ: Bani Malik General Hospital in Jazan

FcγR: Fc-gamma receptors

PCR: Polymerase Chain Reaction

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Reviewed Board (IRB) of King Abdulaziz Medical City, Health Affairs, Ministry of National Guard, Riyadh, Saudi Arabia. Prior to participation, informed consent was also obtained from children and their parents\guardians.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contribution

AN, AA and SAM draft the idea of the research and the research proposal, researched data, designed experiments, and AN, AA, HO, HAE, ME, AA, ATH, AA, AA and SAM wrote the first draft of the manuscript. AN, AA, HAE, SAM, and AA performed the ELISA and participated in the gene polymorphism analysis. AN and ME conducted the data analysis and contributed to the writing of the statistical components in the study. AN, OH and ATH contributed to patient recruitment, diagnosis management and reviewed the research project protocol.

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Tables

Table 1: Description of study participants

Characteristics	Malaria-Free controls (MFC) n=200	Uncomplicated malaria (UM) n=218	Severe malaria (SM) n=182	P value Between Groups
Gender				
Girls n=321	104(52%)	115 (52.8%)	102 (56%)	0.704
Boys n=279	96 (48%)	103 (47.2%)	80 (44%)	
Age (mean± SD)	4.0± 1.4	4.1± 1.4	4.1± 1.1	0.89
Parasite density (mean± SD)	NA	6438.4± 990	15911.4± 403	<0.001
Temperature (mean± SD)	36.9± 0.28	38.9± 0.72	41.1± 0.7	<0.001
Malaria Complication				
Severe anaemia n (%)	NA	NA	56 (30.8%)	<0.001
Cerebral malaria n (%)	NA	NA	61 (33.5%)	
Other complication* n (%)	NA	NA	65 (35.7%)	

* Other complication (hypoglycaemia, jaundice, pulmonary oedema and acute respiratory distress)

Table 2: Distribution of FcγRIIa, FcγRIIIa and FcγRIIIb genotypes and alleles frequency in the different study groups

Genotypes	Malaria-Free controls (n=200)	Uncomplicated malaria (n=218)	Severe malaria (n=182)
FcγRIIa			
R/R (%)	36 (18.0%)	33 (15.1%)	63 (34.6%)
H/R (%)	104 (52.0%)	105 (48.2%)	94 (51.6%)
H/H (%)	60 (30.0%)	80 (36.7%)	25 (13.7%)
Allele frequency			
R allele	0.44	0.40	0.61
H allele	0.56	0.60	0.39
*HWE**	0.44	0.87	0.28
FcγRIIIa			
F/F (%)	91 (45.5%)	85 (39.0%)	132 (72.5%)
F/V (%)	80 (40.0%)	105 (48.2%)	43 (23.6%)
V/V (%)	29 (14.5%)	28 (12.8%)	7 (3.8%)
Allele frequency			
F allele	0.65	0.63	0.84
V allele	0.35	0.37	0.16
*HWE**	0.10	0.62	0.15
FcγRIIIb			
NA1/NA1 (%)	34 (17.0%)	33 (15.1%)	18 (9.9%)
NA1/NA2 (%)	92 (46.0%)	105 (48.2%)	70 (38.5%)
NA2/NA2 (%)	74 (37.0%)	80 (36.7%)	94 (51.6%)
Allele frequency			
NA1 allele	0.6	0.39	0.29
NA2 allele	0.4	0.61	0.71
*HWE**	0.56	0.88	0.36

(*if < 0.05 - not consistent with *Hardy–Weinberg equilibrium "HWE")*

Table 3: Association between individual FcγRIIa, FcγRIIIa and FcγRIIIb genotypes and severity of malaria

	UM versus MFC		SM versus UM	
	Adjusted[†] OR (95% CI)	P value	Adjusted[*] OR (95% CI)	P value
FcγRIIa				
R/R	1.39 (0.89- 2.19)	0.15	2.132 (1.287- 3.533)	0.003
H/R	1.00		1.00	
H/H	0.92 (0.62- 1.36)	0.026	0.349 (0.206- 0.592)	<0.001
FcγRIIIa				
F/F	1.95 (0.65- 2.38)	0.79	11.51 (6.71- 19.77)	<0.001
F/V	1.00		1.00	
V/V	1.72 (1.04- 2.82)	0.13	0.20 (0.09- 0.47)	<0.001
FcγRIIIb				
NA1/NA1	0.79 (0.48- 1.30)	0.354	0.82 (0.43- 1.57)	0.545
NA1/NA2	1.00		1.00	
NA2/NA2	1.24 (0.85- 1.79)	0.263	1.76 (1.15- 2.70)	0.009

[†]Odds Ratio (OR) adjusted with sex and age.

[*]Odds Ratio (OR) adjusted with sex, age and Parasite density

Table 4: Comparison of Anti-AMA1 IgG subclasses (µg/mL) among different study groups

Group	IgG1	IgG2	IgG3	IgG4
	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)
Malaria-Free controls(n=200)	3.91 (0.2-35.6)	0.89 (0.1- 15.1)	1.1 (0.02-15.4)	0.4 (0.1- 1.7)
Uncomplicated malaria(n=218)	4.23 (0.3- 47)	0.73 (0.1- 43.6)	3.6 (1- 29.6)	0.3 (0.1- 3.9)
Severe malaria(n182)	2.97 (0.3-25.7)	3.35 (0.3- 45.3)	2 (1- 8.5)	1.2 (0.2- 14.2)
P value*	<0.001	<0.001	<0.001	<0.001

AMA-1: Apical membrane antigen 1

*P value was derived from *Kruskal Wills* between the study groups

Table 5: *logistic regression* analysis of malaria specific (anti-AMA1) IgG subclasses levels among the different study groups

Dependent Variable	Model of independent variables		OR (95% CI)	P value
IgG1 AMA1	**Uncomplicated malaria**	**MFC†**	0.9 (0.3 to 1.3)	0.670
		SM¥	3.4 (2.1 to 4.7)	**<0.001**
IgG2 AMA1	**Uncomplicated malaria**	**MFC**	1.4 (0.7 to 2.0)	0.912
		SM	2.7 (1.9 to 3.5)	**<0.001**
IgG3 AMA1	**Uncomplicated malaria**	**MFC**	2.6 (2 to 3.3)	**<0.001**
		SM	1.8 (1.1 to 4.6)	**<0.001**
IgG4 AMA1	**Uncomplicated malaria**	**MFC**	0.7 (0.5 to 1.9)	**<0.001**
		SM	1.5 (1.2 to 1.8)	**<0.001**

† OR represent odds ratios while CI represents confidence intervals. In model (A) uncomplicated versus malaria-free control "MFC": malaria-free controls were assigned 0 uncomplicated malaria were assigned 1 in the logistic regression analysis. OR above 1 represented value higher levels antimalarial IgG subclass associated to uncomplicated malaria while less than 1 value represented malaria-free controls.

¥In model (B) severe malaria versus uncomplicated malaria: uncomplicated malaria was assigned 0 severe malaria were assigned 1 in the logistic regression analysis. OR above 1 represented value higher

levels antimalarial IgG subclass associated to severe malaria while less than 1 value represented uncomplicated malaria.

Table 6: *Logistic regression* analysis of individual carrying FcγRIIa, FcγRIIIa, FcγRIIIb genotypes in relation of the levels specific IgG subclasses associated with severe malaria compared to uncomplicated

FcγRs genotypes	Antimalrial IgG-AMA-1 subclasses	Genotypes	Adjusted OR⁺ (95% CI)	P value
FcgRIIa	IgG1	R/R131	2.3 (1.4- 3.4)	0.485
		H/R131	1	
		H/H131	0.3 (0.2- 0.6)	<0.001
	IgG2	R/R131	3.7 (2.0- 6.7)	<0.001
		H/R131	1	
		H/H131	0.5 (0.3- 0.8)	0.006
	IgG3	R/R131	0.4 (0.2- 0.6)	<0.001
		H/R131	1	
		H/H131	1.4 (0.8- 2.3)	0.28
	IgG4	R/R131	3.2 (1.8- 5.7)	<0.001
		H/R131	1	
		H/H131	0.5 (0.3- 0.8)	0.006
FcγRIIIa	IgG1	F/F	0.8 (0.5- 1.2)	0.272
		F/V	1	
		V/V	1.3 (0.8- 2.2)	0.326
	IgG2	F/F	3.9 (2.4- 6.4)	<0.001
		F/V	1	
		V/V	0.4 (0.3- 0.7)	0.002
	IgG3	F/F	0.2 (0.1- 0.4)	<0.001
		F/V	1	
		V/V	1.8 (0.9- 3.7)	0.096
	IgG4	F/F	3.2 (2.1- 5.3)	<0.001
		F/V	1	
		V/V	0.4 (2.0- 0.6)	<0.001
FcγRIIIb	IgG1	NA1/NA1	1.3 (0.7- 2.4)	0.47
		NA1/NA2	1	
		NA2/NA2	0.9 (0.6- 1.4)	0.71
	IgG2	NA1/NA1	1.0 (0.6- 1.9)	0.94
		NA1/NA2	1	
		NA2/NA2	1.4 (0.9- 2.1)	0.14
	IgG3	NA1/NA1	0.9 (0.5- 1.7)	0.72
		NA1/NA2	1	
		NA2/NA2	0.9 (0.6- 1.4)	0.71
	IgG4	NA1/NA1	1.2 (0.6- 2.2)	0.61
		NA1/NA2	1	
		NA2/NA2	1.7 (1.1- 2.7)	0.011

† OR represent odds ratios while CI represents confidence intervals. OR adjusted with parasite density. Higher levels of antimalarial IgG subclasses among uncomplicated malaria patients were assigned 0 while higher levels antimalarial IgG subclasses among severe malaria patients were assigned 1 in the logistic regression analysis. OR above 1 represented value associated to higher levels antimalarial IgG subclasses among severe malaria patients while less than 1 value represented higher levels antimalarial IgG subclasses among uncomplicated malaria patients.