Malaria Parasitaemia among Different Haemoglobin Genotypes in Federal Capital Territory, Abuja

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Abstract
Malaria is a common and life-threatening disease in many tropical and subtropical areas and transmitted by the female Anopheles mosquitoes with major impact on global public health. It is endemic in Nigeria with up to 97% of the population at risk. Malaria results to 60% outpatient visits to healthcare facilities, 25% childhood death, and 11% maternal death. Haemoglobin genotype variants have been implicated in reducing malaria parasite replication within the red blood cells and enhance splenic clearance in malaria parasitized erythrocytes. The study of the Malaria parasitaemia among different haemoglobin genotypes in Federal Capital Territory, Abuja, is aimed at determining the effect of haemoglobin genotypes on malaria parasitaemia among residents in FCT. (2mls) of venous blood was collected from 384 randomly selected residents. Determination of malaria parasitaemia was by Microscopy and the cellulose acetate membrane electrophoresis was used for haemoglobin genotype. A structured questionnaire created with Epi-Info version 7 for data collection and analysis. Results of 187 volunteers with malaria parasitaemia indicated that: malaria parasitaemia was more in female (58.8%) and male (41.4%); age groups of 0-10 (42.0%), 11-20 (35.2%) and 21-30 (14.5%) and 91.7% within age range of 0-30 years of the studied group; in Gwagwalada (24.6%), followed by Bwari (21.4%), Kuje (18.7%), Kwali (17.7%), Abaji (15.5%) and was least in AMAC (2.1%) and severe (+++) (> 10,000/µL) in blood genotype AA (47.4%) than in AS (0.0%), AC (0.0%) and least in SS (0.0%). It was concluded that malaria and the haemoglobin S gene were endemic in FCT and variant Haemoglobin C and S could confer protection to malaria parasitaemia.

Keywords: Malaria, Parasitaemia, Haemoglobin Genotypes.

Introduction
Malaria is a life-threatening mosquito-borne blood disease caused by a Plasmodium parasite and transmitted by the female Anopheles mosquitoes to humans. (Akinbeyo, et al., 2009). Malaria remains a topic of public health importance and continues to rank among the foremost killer diseases of our time (Ilozumba and Uzozie, 2009). It is a vector borne disease endemic in Nigeria with up to 97% of the population at risk (Agomuo et al., 2016). Malaria result to 60% outpatient visits to healthcare facilities, 25% childhood death, and 11% maternal death (Lawal et al., 2018 and WHO, 2016). The financial loss due to malaria annually is estimated to be about 132 billion naira in form of treatment cost, prevention, and loss of man hours, among others (Lawal et al., 2018 and WHO, 2016). Malaria disease affects about 500 million people and kills about 2 million, mostly children each year globally (WHO, 2015). In Nigeria about 96 million people are exposed to malaria, and out of these 64 million people get infected and almost 300,000 deaths are being reported annually in the general population, of which over 100,000 deaths are of children (WHO, 2012). Despite the high morbidity and mortality, certain individuals living in malaria endemic regions appear relatively protected compared to those who suffer frequent severe malaria attacks (Burn, 1976; Alouch, 1997; Allison, 2002 and Awah, Nwanedo et al., 2012). Haemoglobin genotype variants have been implicated in reducing parasite replication within the red blood cells and enhance splenic clearance in parasitized erythrocytes (Esan, 2015 and Kreuzberg, 2010). Malaria can be treated...
and controlled with early diagnosis and some countries lack the resources to do this effectively (Peter, 2017).

Methods

Study population

The study population consist of 384 randomly selected FCT residents made of 192 males and 192 females from 0 to >60 years visiting FCT Hospitals.

Sample size

The sample size was determined by:

\[ n = \frac{Z^2pq}{ME^2} \]

Where
\[ n = \text{Sample size for this research} \]
\[ Z = \text{Z-score =1.96 at 95% Confidence interval} \]
\[ p = \text{Estimated proportion (50%, as actual proportion is unknown) of FCT residents with malaria parasitemia among different blood Genotypes.} \]
\[ ME = \text{Marginal error= ±0.05.} \]

Therefore, \[ n = \frac{Z^2pq}{ME^2} = \frac{(1.96x1.96x0.5x0.5)}{0.05x0.05} = 384.16 \approx 384 \]

Ethical approval

Ethical approval was obtained from the FCT Hospitals management board where all the subjects that were used for this study were confirmed to be asymptomatic for malaria and not on any antimalarial medication for at least seven days before sample collection.

Informed consent

Informed consent was obtained from volunteers and from parent’s legal guardian of the under-aged participants.

Inclusion criteria

Included: written/thumb-printed informed consent obtained from the adult volunteers and parent or legal guardian of the under-aged participants, permanent resident in the Federal Capital Territory and all volunteers from 0 - >60 years of age.

Exclusion criteria

Exclusion criteria from the study were those of major congenital defects, any chronic disease, or anaemia defined as measured haemoglobin <6 g/dL and are not living in FCT. All asymptomatic subjects who are on anti-malaria were also excluded.

Sampling Area

Federal Capital Territory (FCT) is north of the confluence of the Niger and Benue Rivers. It has a land area of 8,000sqkm and it falls within latitude 8° 25’ N and 9° 20° North of the Equator and longitude 5° 45’ and 7° 39’ Bordering the FCT are the states of Kaduna to the northeast, Plateau to the east and south, Kogi to the southwest, and Niger to the west and northwest. Abuja have been growing at 20% to 30% per year. Phase 1 of the city is divided into five (5) districts - Central, Garki, Wuse, Maitama, and Asokoro. Phase 2 is divided into five (5) districts - Kado, Durumi, Gudu, Utako and Jabi. Phase 3 districts is divided into four (4) districts - Mabuchi, Katampe, Wuye and Gwarimpa. Total population of Abuja FCT (1,406,239): Abaji (58642), Abuja Municipal-AMAC (776298), Bwari (229274), Gwagwalada (158618), Kuje (97233) and Kwali(86174) (NPC, 2006). More than 70% of the land is rural. Abuja is in tune with nature with abundant hills, highlands and other distinguishing features that make it a delight to behold. The largest indigenous group in Abuja are the Gbabyi (also known as Gwari); the next largest indigineous
group are Koro; smaller indigenous group include; Gade, Egбуra, Gwandara, Bassa and Ganagana (Awah, Nwanedo, et al, 2012).

**Sampling**

Using sterile needle and syringes, two milliliters (2mls) of venous blood was collected from 384 randomly selected FCT residents visiting FCT General Hospitals comprising of 192 males and 192 females by venipuncture (by tying a tourniquet around the upper arm and sterilizing arm with 70% ethanol to sterilize and increase blood pressure in the veins. The 2mls venous blood sample collected was dispensed into ethylene-diamine-tetra-acetic acid (EDTA) anti-coagulated blood containers, properly mixed by standard method and labeled appropriately (Cheesbrough, 1998).

**Malaria diagnosis**

Subjects were tested for malaria parasite by x10 then, x100 microscopic objectives examination of Giemsa stained thick and thin blood films on grease free microscopic slide under oil immersion. The presence of even one parasite per microscopic field indicate positivity for malaria parasite. Level of parasitemia or degree of severity of malaria infection were graded as low+ (1 to 999 /µL), moderate++ (1000 to 9999 /µL) and severe+++ (>10,000 /µL) (Idonije et al., 2011 and Awah, Nwanedo, et al, 2012).

**Determination of haemoglobin genotype**

The haemoglobin genotypes were determined by the cellulose acetate membrane electrophoresis (CAME) as describe in John and Lewis (1986), Awah and Uzoegwu (2006) and Tidi et al. (2013). Briefly: fifty micro-liters of washed cells was added into khán tubes containing 50 micro-liters of 0.1% white saponin and mixed thoroughly (haemolysate). The haemolysate was centrifuged to remove any debris. The supernatant was used for the test. Haemoglobin lysates are spotted in triplicates at 0.5 cm apart of one end of well-dried cellulose acetate strips. Reference HbAS and HbA Chaemolysates was spotted alongside also as control. The spots were allowed to dry and the strips placed in an electrophoretic tank to form a bridge between the anode and cathode of the tank in such a way that haemoglobin bands migrate towards the anode. Both compartments of the tank contained the same working buffer–Tris-EDTA Borate buffer (pH 8.9). A constant voltage of 150 V (2mA) was applied through a Shandon power supply unit to achieve excellent separation within 5–10 minutes. After separation, the haemoglobin bands were visualized directly or otherwise stained for 2 minutes with 2% Ponseau S stain and then de-stained with distilled water. Pink spots of separated haemoglobin spots were observed upon white background of the cellulose acetate membrane. HbA migrated fastest followed by HbS then HbC, etc respectively (Evans, 1971 and Awah, Nwanedo, et al, 2012).

**Statistical analysis of data**

The data from this survey was analysed using Epi-Info Software Version 7. Only the test results with malaria parasitaemia among different genotypes were statistically analysed and presented in frequency tables, bar chart and percentages.

**Results**

The results were as presented in frequency Tables 1 to 5 and Figure 1 below: Malaria parasitaemia was more in female (58.8%) than male (41.4%) (Table1); and more in 0-10 (42.0%), 11-20 (35.2%) and 21-30 (14.5%). Therefore, malaria parasitaemia was 91.7% within age range of 0-30 years of the studied group (Table 2); more (Table 3) in Gwagwalada (24.6%), followed by Bwari (21.4%), Kuje (18.7%), Kwali (17.7%), Abaji (15.5%) and was least in AMAC (2.1%). From figure 1 and Table 4 indicate that malaria parasitaemia was highest in blood genotype AA (69%), AS (24.2%), AC (4.7%) and least in SS (2.1%) and SC (0.0%) and blood genotype identified in FCT include: AA, AC, AS, SC and SS. Table 5 indicated that malaria parasitaemia was severe (Severe (+++) (> 10,000/ µL)) in blood genotype AA (47.4%) than in AS (0.0%), AC (0.0%) and least in SS (0.0%) and SC (0.0%).
Figure 2. Bar chart distribution of malaria parasitaemia by blood genotype

Table 1. Frequency distribution of malaria parasitaemia by sex

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cum. Percent</th>
<th>Exact 95% LCL</th>
<th>Exact 95% UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>77</td>
<td>41.40%</td>
<td>41.40%</td>
<td>34.24%</td>
<td>48.84%</td>
</tr>
<tr>
<td>Female</td>
<td>110</td>
<td>58.80%</td>
<td>100.00%</td>
<td>51.16%</td>
<td>65.76%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>187</td>
<td>100.00%</td>
<td>100.00%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note: N=384 (Total Volunteers Tested); n=187 (Volunteers with Malaria Parasitaemia).**

Table 2. Frequency distribution of malaria parasitaemia by age in years.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cum. Percent</th>
<th>Exact 95% LCL</th>
<th>Exact 95% UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>78</td>
<td>41.97%</td>
<td>41.97%</td>
<td>34.92%</td>
<td>49.27%</td>
</tr>
<tr>
<td>11-20</td>
<td>66</td>
<td>35.23%</td>
<td>77.20%</td>
<td>28.51%</td>
<td>42.42%</td>
</tr>
<tr>
<td>21-30</td>
<td>27</td>
<td>14.51%</td>
<td>91.71%</td>
<td>9.86%</td>
<td>20.28%</td>
</tr>
<tr>
<td>31-40</td>
<td>11</td>
<td>5.70%</td>
<td>97.41%</td>
<td>2.88%</td>
<td>9.97%</td>
</tr>
<tr>
<td>41-50</td>
<td>3</td>
<td>1.55%</td>
<td>98.96%</td>
<td>0.32%</td>
<td>4.48%</td>
</tr>
<tr>
<td>&gt;50</td>
<td>2</td>
<td>1.04%</td>
<td>100.00%</td>
<td>0.13%</td>
<td>3.69%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>187</td>
<td>100.00%</td>
<td>100.00%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note: N=384 (Total Volunteers Tested); n=187 (Volunteers with Malaria Parasitaemia).**

Table 3. Frequency distribution of malaria parasitaemia by location in the fct

<table>
<thead>
<tr>
<th>Location</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cum. Percent</th>
<th>Exact 95% LCL</th>
<th>Exact 95% UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABAJI</td>
<td>29</td>
<td>15.51%</td>
<td>15.51%</td>
<td>10.64%</td>
<td>21.51%</td>
</tr>
<tr>
<td>AMAC</td>
<td>4</td>
<td>2.14%</td>
<td>17.65%</td>
<td>0.59%</td>
<td>5.39%</td>
</tr>
<tr>
<td>BWARI</td>
<td>40</td>
<td>21.39%</td>
<td>39.04%</td>
<td>15.74%</td>
<td>27.97%</td>
</tr>
<tr>
<td>GWAGWALADA</td>
<td>46</td>
<td>24.60%</td>
<td>63.64%</td>
<td>18.61%</td>
<td>31.41%</td>
</tr>
<tr>
<td>KUJE</td>
<td>35</td>
<td>18.72%</td>
<td>82.35%</td>
<td>13.40%</td>
<td>25.06%</td>
</tr>
<tr>
<td>KWALI</td>
<td>33</td>
<td>17.65%</td>
<td>100.00%</td>
<td>12.47%</td>
<td>23.88%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>187</td>
<td>100.00%</td>
<td>100.00%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note: N=384 (Total Volunteers Tested); n=187 (Volunteers with Malaria Parasitaemia).**
**Table 4.** Frequency distribution of malaria parasitaemia by blood genotype.

<table>
<thead>
<tr>
<th>Blood Genotype</th>
<th>Malaria Parasitaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>Low (+) (1-999/µL)</td>
<td>6(3.2%)</td>
</tr>
<tr>
<td>Moderate (++) ((1000-9999/ µL)</td>
<td>35(18.4%)</td>
</tr>
<tr>
<td>Severe (+++) (&gt; 10,000/ µL)</td>
<td>90(47.4%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>131(69%)</td>
</tr>
</tbody>
</table>

**Note:** N=384 (Total Volunteers Tested); n=187 (Volunteers with Malaria Parasiteamia).

**Table 5.** Frequency distribution of malaria parasitaemia severity by blood genotype

<table>
<thead>
<tr>
<th>Haemoglobin Genotypes</th>
<th>Malaria Parasitaemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (+) (1-999/µL)</td>
</tr>
<tr>
<td>AA</td>
<td>3.2</td>
</tr>
<tr>
<td>AC</td>
<td>4.7</td>
</tr>
<tr>
<td>AS</td>
<td>21.6</td>
</tr>
<tr>
<td>SC</td>
<td>0.0</td>
</tr>
<tr>
<td>SS</td>
<td>2.1</td>
</tr>
</tbody>
</table>

**Note:** N=384 (Total Volunteers Tested); n=187 (Volunteers with Malaria Parasiteamia).

**Discussion**

The aim of this research survey include: To determine in study group of FCT residents; (1) Malaria parasitaemia, (2) Haemoglobin genotype distribution, (3) relationship between haemoglobin genotype and malaria parasitaemia and (4) the severity of malaria parasitaemia amongst the various haemoglobin genotypes.

**Malaria parasitaemia in FCT**

In general, the result of this research work portrayed existence of malaria parasitaemia in FCT. From Table 4.3 above, malaria parasitaemia were more in Gwagwalada (24.6%), followed by Bwari (21.4%), Kuje(18.7%), Kwali (17.7%), Abaji(15.5%) and was least in AMAC(2.1%) which agreed with the work of Nasir (2015), who reported that the FCT is one of the endemic area for malaria with a high prevalence malaria parasitemia rate of 82.0% among tested subjects and 26.0% among control subjects and highest malaria density of >10,000. According to WHO (2012) and UNICEF (2000), malaria proliferates in conditions where awareness is low with weak health care systems and humid bushy areas supporting the result of this research work that found only 2.1% parasitaemia in AMAC, an urban area in FCT and higher parasitamia in Gwagwalada (24.6%), followed by Bwari (21.4%), Kuje(18.7%), Kwali (17.7%) and Abaji(15.5%) comprising mostly urban areas within FCT.

Again Ogbu (2015), prevalence of malaria parasitaemia among asymptomatic women at booking visit in a tertiary hospital in FCT North Central Nigeria was 38.8% which disagree with the result of this research work which found that malaria parasitaemia was more in female (58.6%) and male (41.4%) (Table 4.1 above).

From table 4.2 above showing the frequency distribution of malaria parasitaemia by age in years of volunteer subjects used in this study, malaria parasitaemia was more in the youngest 0-10 (42.0%), 11-20 (35.2%) and 21-30(14.5%). Therefore, malaria parasitaemia was 91.7% within young age range of 0-30 years of the studied group and agreed with WHO (2013) that reported that malaria remain a common and life-threatening disease in tropical and subtropical areas affecting mostly the young of over 100 countries.
and territories, and these countries/territories are visited by more than 125 million international travellers every year.

**Haemoglobin genotypes in FCT**

The work of Nwanedo et al (2012), reported that the gene for HbS is distributed widely throughout sub-Saharan Africa and countries with African immigrants, where carrier frequencies range from 5%–40% or more which disagreed with the findings of this research work which indicated AA(69%), AS(24.2%), AC(4.7%) and least in SS(2.1%) and SC(0.1%) as the percentage blood genotype identified in FCT (Bar Chart 4.1 and Table 4.4 above). It could be adduced that the HbS and SS genes are endemic in FCT and again agreed with the work of Allison (2002) who hypothesized that the sickle cell gene arose spontaneously in malarious zones.

**Relationship between haemoglobin genotype and malaria parasitaemia in FCT**

Nasir (2015), reported that the FCT is one of the endemic area for malaria with a high prevalence *malaria parasitemia* rate of 82.0% among tested subjects and highest *malaria* density of >10,000 and this research work in Bar Chart 4.1 and Table 4.4, indicated that malaria parasitaemia was highest in blood genotype AA(69%), AS(24.2%), AC(4.7%) and least in SS(2.1%) and SC(0.0%) both supporting Modiano et al (2001) who emphasized that the high frequency of the sickle-cell haemoglobin (HbS) gene in endemic malarious zones is believed to be due to a heterozygote (HbAS ) advantage against fatal malaria. This implied that HbS gene could shield human from malaria parasitaemia indicating that there exist strong relationship between haemoglobin genotypes and malaria parasitaemia as it has been suggested that HbAS would provide a protective advantage early in life before the acquisition of clinical immunity to malaria though definitive data to support this assumption especially in high malaria transmission areas, are lacking (Modianoet al.,2001 and Awah, Nwanedo, et al, 2012).

**Severity of malaria parasitaemia among various haemoglobin genotypes in FCT**

From table 4.5 above indicated that malaria parasitaemia was severe ((+++> 10,000/ µL)) in blood genotype AA(47.4%) than in AS(0.0%), AC(0.0%) and least in SS(0.0%) and SC(0.0%) which agreed with the work of Michael Aidoo et al (2002), who also indicated that AS and AC genotype is associated with lower incidence of clinical *malaria* relative to AA genotype among the tested subjects and Esan (2015) who reported that haemoglobin genotype variants could reduce parasite replication within the red blood cells and enhance splenic clearance in parasitized erythrocytes. In addition, (Nwanedo, et al (2012) equally reported that HbAS is widely known to confer significant protection from severe and uncomplicated malaria, although underlying mechanisms for this phenomenon was not precisely defined and Kreuzberg (2010), reported that similar protection afforded by haemoglobin C (HbC) was more recently demonstrated; all supporting the result of this research.

**Conclusion**

Malaria had been shown as a complex disease that depends on many host genetic factors including haemoglobin genotypes.

The AC, AS and SC haemoglobin genotypes were associated with lower risk of malaria parasitaemia relative to normal genotype, AA amongst the studied volunteers.

These malaria, the haemoglobin C and haemoglobin S genes were endemic in FCT and that variant Haemoglobin C and S could confer protection to malaria parasitaemia.

Hence, evaluations of anti-malarial interventions in endemic regions like the FCT should consider Haemoglobin genotypes and compliance with national malaria guidelines as a potentially important.
Reference


