Malaria Infection and its Relation to ABO Blood Grouping in Khartoum, Singa and Al Genaid, Sudan

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Abstract:
The study was conducted in a period from March to July (2013) in 3 areas in Sudan (Khartoum, Al Genaid and Singa). The study was conducted on 302 blood sample, 83 samples were found positive for malaria infection. This constituted an overall infection rate of 27.5 %. The prevalence rates reported in Khartoum, Al-Genaid, and Singa were 14.4 %, 31.5 %, and 42.4 % respectively. The results showed that the highest prevalence rate (31.2 %) was reported among males in the areas, while females reported 24.7 %. The highest

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prevalence rate (41.7 %) was reported among the less than 10 years old and the lowest rate (18.1 %) was reported among the 10 - 20 years old. The investigation revealed that the highest prevalence rate (54.6 %) was reported among the O + ve blood group and the lowest prevalence rate (14.3 %) was reported among the AB - ve blood group. High parasitaemia (++++) was strictly confined to the A +ve, B + ve and B-ve with rates of 0.3 %, 0.7 %, and 0.3 % respectively. Low parasitaemia was more evident with the O +ve blood group (8.3 %) while moderate parasitaemia (++) was mostly observed with the O +ve blood group (7 %).

Key words: ABO Blood Grouping, Khartoum, Al Genaid, Singa.

INTRODUCTION:

ABO blood group system is genetically controlled and proportions of various ABO groups differ significantly in different populations and ethnic groups. Thus, any national or international study reporting association of ABO groups with a disease must use population frequency of ABO groups as the base for comparison. Cserti and Dzik (2007) (1) reviewed 22 publications in the year 2007 that investigated relationship between P. falciparum malaria and ABO groups. They did not find a study with adequate sample size and also commented that most of the studies are influenced by the absence or inappropriate control groups. In a recent study in Brazilian Amazon region, only 142 control and 98 cases of malaria have been investigated (2). A, B, H antigen synthesis involves addition of sugars to paragloboside; N-acetyl galactose amine is specific for ‘A’, D galactose for ‘B’ and L fructose for ‘H’1. ‘O’ group erythrocytes are less prone to form rosettes with P. falciparum parasite-infected RBC because of the reduced cyto-adhesion and rosette formation with parasites. Hence, ‘O’ group individuals have reduced risk of severe malaria. However, ‘O’ group shows significant association with placental P.
falciparum malaria infection. Akanbi et al. (2010) (3) have observed that ‘A’ group has more parasite density than ‘B’ and ‘O’. ‘O’ group red cells have minimum density. Fry et al. (2008) (4) have tested three African populations for ABO alleles by molecular methods. They observed that haplotypes in ‘O’ and non-‘O’ individuals are different and might lead to malaria susceptibility of the population. The main objectives of this study were to determine the relationship between malaria infection and ABO blood grouping, to study the establishment of malaria infection in different blood groups, to study relationship between parasitaemia and different blood groups in patients with malaria, to study malaria infection in study subjects according to age groups and to study malaria infection in study subjects according to gender.

MATERIALS AND METHODS:

Study design:  
It is a cross-sectional hospital based study.

Study area: 
The study was carried out in Khartoum (capital of Sudan), Singa (central Sudan) and Al Genaid (northeastern part of Sudan).

Study population: 
For purpose of this study, patients suspected of having malaria were selected from different hospitals and health care centers in different endemic areas mentioned above in a period from March to July 2013.
Ethical consideration:
Approval of the ethical committee of the faculty was taken. Informed consent was obtained from all participants in the study.

Collection of blood samples:
5 ml of capillary or venous blood were collected.

Collection of capillary blood:
With cotton wool dipped in 70% alcohol, the tip of the third finger was cleaned, and with sterile lancet, finger was pricked firmly and rapidly. The first drop of blood was wiped.

Collection of venous blood:
For venous blood, tourniquet was tied around the right or left elbow and then cotton wool in 70% alcohol was used to clean the arm on a visible vein. The needle was then introduced to the vein and blood collected and placed in a blood container with EDTA anticoagulant.

PREPARATION OF BLOOD SMEARS:

Preparation of thick blood smears:
Three drops of collected blood were placed in clean and dry slide (about 2 cm from edge of slide) and then stirred by a corner of another clean and dry slide until appropriate thick smear obtained, the smear was left to dry.

Preparation of thin blood smears:
Drop of blood was placed on the middle of clean and dry slide and by edge of another slide (called spreader) placed just in front of the drop of blood and the spreader turned until it touched the drop of blood, then blood allowed to run along the
edge of spreader, and then spreader was pushed forward to the end of the slide with suitable speed. The smear was left to dry.

**Staining of blood film:**
All thick and thin blood films were stained using Giemsa stain. Only thin films were fixed with methanol for 1-2 minutes. The slides were covered with 10% Giemsa solution for 10 minutes. All slides were washed using clean water and allowed to air dry.

**Examination of blood films:**
The slides were examined using light microscope (Olympus x100 oil immersion lenses). The number of parasites were counted and reported by using the following grading:
- 1 - 10 parasites per 100 thick film fields  
  +.
- 11 - 100 parasites per 100 thick film fields  
  ++.
- 1 - 10 parasites per thick film field  
  +++.
- 11 - 100 parasites per thick film field  
  ++++.

**Procedure of ABO blood groups:**
With a grease pencil two circle are drawn on clean and dry slide, and labeled one (A) and another (B), and on another slide circle was drawn and labeled (D), then a drop of blood was placed on each circle, then to the circle (A) drop of anti-serum A was added, drop of anti-serum B also was added to circle (B), and drop of anti-serum D was added to circle (D) on the another slide. Then each suspension was mixed with different wood stick.

**Interpretation of ABO blood groups:**
The interpretation of ABO blood was group as follows:
- Agglutination on (A) circle and no agglutination on (B) circle mean the ABO blood group is A.
- Agglutination on (B) circle and no agglutination on (A) circle mean the ABO blood group is B.
- Agglutination on Both (A) circle and (B) circle mean the ABO blood group is AB.
- No agglutination on both (A) circle and (B) circle mean the ABO blood group is O.
- Agglutination on (D) circle means the Rhuses factor (Rh-factor) is positive (+ve).
- No agglutination on (D) circle means the Rhuses factor (Rh-factor) is negative (-ve).

**Data analysis:**
Data were analyzed using Statistical Package for the Social Sciences program (SPSS program). The Chi-square test was used for difference in proportion. 0.05 % was taken as cut off limit for 95% statistical significance. Frequencies and percentages tests were used and then the data were presented in tables.

**RESULTS:**
Out of the 302 blood samples examined in the 3 areas investigated, 83 samples were found positive for malaria infection. This constituted an overall infection rate of 27.5% in table (1). The prevalence rates reported in Khartoum, Al Genaid, and Singa were 14.4%, 31.5%, and 42.4 % respectively in table (2). The difference in rates was found to be statistically highly significant at p=0.000. The results showed that the highest prevalence rate (31.2%) was reported among males in the areas, while females reported 24.7% prevalence rate in table (3). The difference in rates was found to be statistically insignificant at p=0.293. The highest prevalence rate (41.7%) was reported among the less than 10 years old and the lowest rate (18.1%) was reported among the 10-20 years old in table.
The difference in rates between all age groups was found to be statistically significant at p=0.006. The investigation revealed that the highest prevalence rate (54.6%) was reported among the O +ve blood group and the lowest prevalence rate (14.3%) was reported among the Ab -ve blood group in table (5). The difference in rates was found to be highly significant at p=0.000. High parasitaemia (++++) was strictly confined to the A +ve, B +ve and B -ve blood groups with rates of 0.3%, 0.7%, and 0.3% respectively. Low parasitaemia was more evident with the O +ve blood group (8.3%) while moderate parasitaemia (+) was mostly observed with the O +ve blood group (7%) in table (6). The difference in rates between parasitaemia and blood groups was statistically highly significant at p=0.000.

Table (1): The overall prevalence rate of malaria in all areas investigated

<table>
<thead>
<tr>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>302</td>
<td>83</td>
<td>27.5%</td>
</tr>
</tbody>
</table>

Table (2): The prevalence rates of malaria in each area investigated

<table>
<thead>
<tr>
<th>Area</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khartoum</td>
<td>118</td>
<td>17</td>
<td>14.4%</td>
</tr>
<tr>
<td>Al Genaid</td>
<td>111</td>
<td>35</td>
<td>31.5%</td>
</tr>
<tr>
<td>Singa</td>
<td>73</td>
<td>31</td>
<td>42.4%</td>
</tr>
</tbody>
</table>

p=0.000

Table (3): The prevalence rates of malaria infection in all areas investigated according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>128</td>
<td>40</td>
<td>31.2</td>
</tr>
<tr>
<td>Female</td>
<td>174</td>
<td>43</td>
<td>24.7</td>
</tr>
</tbody>
</table>

p=0.293

Table (4): The prevalence rates of malaria in all areas investigated according to age groups

<table>
<thead>
<tr>
<th>Age groups (Year)</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 10</td>
<td>79</td>
<td>33</td>
<td>41.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. Examined</th>
<th>No. Positive</th>
<th>Prevalence%</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 10-20</td>
<td>116</td>
<td>21</td>
<td>18.1</td>
</tr>
<tr>
<td>From 21-30</td>
<td>63</td>
<td>17</td>
<td>26.9</td>
</tr>
<tr>
<td>Above 30</td>
<td>44</td>
<td>12</td>
<td>27.2</td>
</tr>
</tbody>
</table>

p= 0.006

Table (5): The prevalence rates of malaria infection according to blood groups

<table>
<thead>
<tr>
<th>Blood group</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+ve</td>
<td>62</td>
<td>14</td>
<td>22.6</td>
</tr>
<tr>
<td>A−ve</td>
<td>9</td>
<td>4</td>
<td>44.4</td>
</tr>
<tr>
<td>B+ve</td>
<td>64</td>
<td>12</td>
<td>19.4</td>
</tr>
<tr>
<td>B−ve</td>
<td>3</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td>O+ve</td>
<td>141</td>
<td>47</td>
<td>54.6</td>
</tr>
<tr>
<td>O−ve</td>
<td>13</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>AB+ve</td>
<td>7</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>AB−ve</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

p=0.000

Table (6): The different levels of parasitaemia for different blood groups

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+ve</td>
<td>5 (1.7%)</td>
<td>4 (1.3%)</td>
<td>4 (1.3%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>A−ve</td>
<td>1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>3 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>B+ve</td>
<td>4 (1.3%)</td>
<td>1 (0.3%)</td>
<td>5 (1.7%)</td>
<td>2 (0.7%)</td>
</tr>
<tr>
<td>B−ve</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>O+ve</td>
<td>25 (8.3%)</td>
<td>21 (7%)</td>
<td>1 (0.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>O−ve</td>
<td>2 (0.7%)</td>
<td>2 (0.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>AB+ve</td>
<td>0 (0%)</td>
<td>1 (0.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>AB−ve</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

p=0.000

DISCUSSION:

From the results, it was obvious that the overall prevalence rate in the 3 areas investigated was relatively high (27.5%). This rate was found to be equal to the rate reported by Abdallah (2010) (5) in El-Duem (27.5%); however, it was lower than the rate reported by Ibrahim (2008) (6) in Soba hospital.
From the investigation, the highest rate (41.7%) was reported with the age group less than 10 years old. This rate was higher than the rate reported by Abdallah (2010) (5) (26.7%) in the same group, however, it was lower than the rate reported by Ibrahim (2008) (6) for the same age group (60%). As far as gender is concerned, our results showed that males reported the highest rate (31.2%), while females reported a 24.7% rate. These rates were closer to the rates reported by Abdallah (2010) (5) in El-Duem for males and females (26.1% and 28.6% respectively), however, it was lower than the rates reported by Ibrahim (2008) (6) (53.3% and 26% respectively). In our opinion, gender does not play a great role in the establishment of malaria infection. From table 5, it was clear that the highest rate of malaria infection was reported with the O +ve blood group (54.6%), while the rates in other blood groups ranged from 14.3% (AB +ve) and 44.4% (A-ve). This higher rate of infection was in agreement with the finding of Akhingbe et al. (2011) (7) who reported that subjects with group O had a higher prevalence of malaria parasitaemia. Compared to the study performed by Tekeste and Petros (2010) (8) in Ethiopia, our rate with O group (54.6%) was higher than their rate (22.9%) in the same group, however, their rates in other blood groups were almost closer to ours (35.7%, 21.4% and 20%) for A, B and AB respectively. From table 6, it is obvious that despite the high prevalence of malaria infection in subjects with blood group O, they were less likely to exhibit high parasitaemia compared to the A and B blood group. This finding was in line with the finding of Igbeneghu et al. (2012) (9) who reported that O individuals appeared to be the most protected against high parasite density followed by B individuals while A and AB individuals were more likely to experience high parasite density.
CONCLUSION:

This study concluded that Malaria infection in the areas investigated is still in the increase. Gender does not have any role in the establishment of the disease. As usual, age group of less than 10 years old is the more susceptible to infection than other age groups. Individuals with O blood group showed high susceptibility to malaria infection, however, they were less likely to exhibit high parasitaemia. High parasitaemia was most likely to occur in subjects with blood groups A and B.

REFERENCES:


