MALARIA AMONG PREGNANT WOMEN AND CHILDREN AND THE PROTECTIVE ROLES OF ABO BLOOD GROUP AND HB-GENOTYPE

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ABSTRACT

The prevalence of malaria among pregnant women and children attending Agbonchia Health Centre Eleme, Rivers State, Nigeria was investigated using thick and thin blood films stained with Giemsa. The prevalence of malaria among the pregnant women was 40.6%, the prevalence among children was 48.8%; whereas the overall prevalence was 40.8%. The prevalence of malaria by age groups showed that pregnant women between ages 21-35 yrs had prevalence of 10.4%-12.4%. Statistical analysis at p<0.05 did not show significant difference in prevalence of malaria by age groups among pregnant women; but there was significant difference among children from 0-4 yrs and the other age groups. Prevalence of malaria by ABO blood group, blood group O had high prevalence of malaria and less clinical episode than the non O blood group. The prevalence of malaria by Hb-genotype, HbAA had prevalence of 33.2% and HbAS 7.8% among pregnant women, whereas children HbAA was 39.6% and HbAS 9.2%. Severe malaria by Hb-genotype, pregnant women HbAA 7.2% and HbAS 1.2%, whereas children HbAA 8.0% and HbAS 1.6%. Statistical analysis at p<0.05 did not show significant difference in prevalence malaria between HbAA and HbAS among pregnant women and children. HbAS pregnant women and children had les clinical episode. Blood group O and HbAS protects against severe P. falciparum malaria in pregnancy and children.

Key words: Malaria, pregnancy, children, ABO-blood group, Hb-genotype.

INTRODUCTION

Malaria is a life threatening parasitic infection in pregnancy and among children. It is a mosquito born infectious disease affecting both humans and animals caused by the genus plasmodium (Fairhurst and Wallems, 2010). Presently, malaria is endemic in the equatorial regions of America, Asia and in the Sub-Saharan Africa were about 85 – 90% of malaria fatality occur (Layne, 2006). Malaria is the most prevalent disease with high morbidity, mortality with high economic and social impact (WHO, 2001).

Severe malaria had been reported among other blood groups as compared to O blood group (Migot-Nabias et al., 2000). Low parasitemia and uncomplicated malaria cases of P. falciparum among blood O group subjects (Pathirana et al., 2005). Significant advantage had been reported of P. falciparum with ABO-blood group O (Zerihum et al., 2011; Row et al., 2007). Malaria is associated with miscarriages, stillbirth, preterm birth, low birth weight of babies and 3.8% of all infants deaths (FMOH, 2002, Feresus et al., 2004; WHO, 2006).

MATERIALS AND METHODS

Study Area

The study was conducted among pregnant women and children attending clinic in Agbonchia Health Centre, Eleme in Eleme Local Government area of Rivers State, Nigeria. Eleme Local Government covers an area of about 138km², with a population of 200,884. It is located between latitudes 4°45E and 4°50N, longitudes 7°05E and 7°10SW. Agbonchia is the largest community in Eleme, located in the fresh water zone with characteristic rain forest vegetation of the Niger Delta Area. The people are predominantly farmers.

Study Subjects

A total of 250 pregnant women attending antenatal care and 250 children from June – August, 2014 were selected randomly without prior knowledge of their clinical or family
Determination of Blood Genotype of subjects

The pregnant women and children were of varying age ranging from 20 – 40 yrs and 0-13 yrs respectively.

Sample Collection

Venous blood was collected by phlebotomy with the use of tourniquet applied on the upper arm of the pregnant women to enable location of vein, but for the children, blood was collected by capillary. Ethanol (70%) was used to clean the site of collection, allowed to dry and blood collected with hypodermic syringe directly into EDTA bottle. This was mixed properly to avoid coagulation.

Preparation of Blood Smears

The preparation of thick and thin blood smears were done according to (Monica Chesbrough, 2002).

Preparation of thin smear

The thin blood smears were allowed to air dry for 10 minutes and fixed with methanol by dipping the thin smear carefully in methanol for 5 seconds (to avoid methanol touching the thick smear). This was allowed to dry and Giemsa stain was applied for 8 minutes. This was rinsed with distilled water and allowed to dry.

Thick smear

The thick smears were air dried for about 30 minutes (not fixed in methanol) but dipped in water to de-haemoglobinize, allowed to dry and stained by the same procedure as the thin smears. When the smears were dry, immersion oil was applied and they were viewed with x100 (oil immersion applied) under the microscope (Chessbrough, 2002).

Microscopic Examination

The back of each air dried blood film slides were carefully cleaned with cotton wool and examined using x10 and x40 firstly, for morphology staining of cells and detection of malaria schizonts, trophozoites and gametocytes and x100 objective for Plasmodium species

Parasite per ml = \( \frac{\text{Parasite count} \times 500}{\text{Set range of WBC (500)}} \)

Haemolysate of each blood samples were prepared by washing the EDTA blood three times with normal saline. Distilled water was added to the test tube containing the blood, this was allowed for 5 minutes to lyse completely before use. The haemoglobin genotype separation was carried out using electrophoretic tank method as described by (Chesbrough, 2002).

Determination of blood group

The blood groups of subjects were determined by rapid tile grouping method. Antisera A, B, AB and antisera D were reacted with the EDTA anticoagulated blood for the determination of blood group. They were spun in a centrifuge and observed for agglutination both macroscopically and microscopically.

Statistical analysis: Data obtained were analyzed by Chi-square.

Ethical consent: Ethical consent was sought from Agbonchia Health Centre Authority, the pregnant women and the parents of the children used as subjects.

RESULTS

The prevalence of malaria among 250 pregnant women was 102(40.6%) while the prevalence among 250 children was 122(48.8%) respectively. The overall prevalence of the sampled population of pregnant women and children (500) was 224 (40.8). The result of the prevalence of malaria by age groups among the pregnant women and children examined for malaria parasitemia, pregnant women, age 21 – 25 yrs, 71 subjects 31(12.4%) was positive, 26 – 30 yrs, 83 subjects, 30 (12.0%) had malaria; 31 – 35 yrs, were 64, 26(10.4%) had malaria parasites and 36 – 40 yrs were 32, 15(6.0%) were infected. For the children age 0-4 yrs were 200, 102(43.6%) had malaria, age 5 – 8 years were 36, 11(4.4%) were infected, age 9 – 12 yrs were 11 children, 2(0.8%) had malaria parasites and ages 13 above 3, 1(0.4%) was infected as shown in Table 2. The percentage occurrences of ABO-blood group among pregnant women was A, 54(20.1%), B 30(12.8%), AB, 7(2.8%) and O, 162(64.8%) while among children A, 83(33.2%), B, 44(17.6%), AB, 12(4.8%) and O, 111(44.4%) respectively.

Table 1. Prevalence of malaria among pregnant women and children

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Numbers examined</th>
<th>Numbers positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>250</td>
<td>102 (40.6)</td>
</tr>
<tr>
<td>Children</td>
<td>250</td>
<td>122 (48.8)</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>204 (40.8)</td>
</tr>
</tbody>
</table>

Numbers in parenthesis = percentages.

Table 2. Prevalence of malaria among pregnant women and children by age group

<table>
<thead>
<tr>
<th>Age group (yrs)</th>
<th>Pregnant women</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number sampled</td>
<td>Number positive</td>
<td>Number positive</td>
</tr>
<tr>
<td>21 – 25</td>
<td>71</td>
<td>31 (12.4)</td>
</tr>
<tr>
<td>26 – 30</td>
<td>83</td>
<td>30(12.0)</td>
</tr>
<tr>
<td>31 – 35</td>
<td>64</td>
<td>26(10.4)</td>
</tr>
<tr>
<td>36 – 40</td>
<td>32</td>
<td>15(6.0)</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>102 (40.8)</td>
</tr>
</tbody>
</table>

Determination of Blood Genotype of subjects

Erythrocytes of each blood sample were prepared by washing the EDTA blood three times with normal saline. Distilled water was added to the test tube containing the blood, this was allowed for 5 minutes to lyse completely before use. The haemoglobin genotype separation was carried out using electrophoretic tank method as described by (Chesbrough, 2002).
DISCUSSION

The prevalence of malaria among pregnant women by ABO blood group were A, 18(7.2%), B 10(4.0%) AB, 2(0.8%) and O, 72(28.8%) respectively and children A 41(16.40%), B 19(7.6%), AB 2(0.8%) and O, 60(24.0%) respectively. The result of severe malaria by ABO blood group were: A 10(4.0), B 5(2.0%), AB 0(0.00%) and O 2(0.8%), whereas for children A, 13(5.2%), B 7(2.8%), AB 0(0.00%) and O, 4(1.6%) respectively. The overall prevalence of severe malaria are pregnant women 17(6.8) and children 24(9.6) respectively as shown in Table 3.

The percentage occurrences of haemoglobin genotype among pregnant women was HbAA 198(79.2%), HbAS 52(20.8%) whereas for children HbAA, 197(78.8%) HbAS 53(21.2%) respectively. The prevalence of malaria by Hb-genotype was HbAA 34(33.6%) HbAS 18(7.2%) for pregnant women. For children HbAA 99(39.6%) and HbAS 23(9.2%) ; The prevalence severe malaria by Hb- genotype among pregnant women are HbAA 7.2%, HbAS 1.2% and children AbAA 8.0%, HbAS 1.6% respectively.

DISCUSSION

The prevalence of malaria parasitemia among 250 pregnant women and 250 children are 40.6% and 48.8%, the overall prevalence for both pregnant women and children was 40.8%. The species of malaria parasite (Plasmodium) identified was only Plasmodium falciparum. In similar studies on the prevalence of malaria among pregnant women, (Nwonnwu et al., 2009) reported prevalence of 42% in Ghana and ( Bououakot et al., 2003) recorded prevalence of 57.5% among pregnant women in Garan. In Nigeria, (Nduka et al., 2006) reported prevalence of 54% in Southeast Nigeria and in Osogbo Southwest Nigeria, (Adefioye et al., 2007) observed prevalence of 72% among pregnant women. Wogu et al., (2013) recorded prevalence of 26% among pregnant women in Port Harcourt. This was low compared to the values referenced above. The low prevalence was attributed to the Rivers State Government Roll Back Malaria Programme. Biolarvicide was used to destroy mosquito larvae, mosquito treated nets were given free and malaria drugs administered free to malaria infected individuals in Port Harcourt metropolis. The prevalence of malaria obtained in this study was similar to results obtained in other parts of Nigeria and West Africa. This prevalence is in line with the findings that malaria due to Plasmodium falciparum in pregnancy is a major contributing factor to maternal morbidity and mortality in Sub-Saharan Africa and about 47% of pregnant women are diagnosed with malaria in Nigeria (Stekete et al., 2001; USAID, 2005). The rate of prevalence of malaria among children was slightly high compared to that of pregnant women. Statistical analysis at p< 0.05 did not show significant difference in the prevalence of malaria among pregnant women and children. Data obtained showed that the prevalence of malaria by age group was slightly high among age group 40 – 49 yrs 46.8%, as compared with the others age groups. Statistical analysis at p<0.05 did not show significant difference in prevalence of malaria by age groups among pregnant women and children. The high prevalence of malaria among pregnant women might be attributed to frequent child birth which may reduced their haemoglobin level, reduced immunity associated with pregnancy and the environmental conditions in the rural areas that encourage breeding of mosquitoes. The children, aged group 0 – 4 yrs had high prevalence of malaria 43.2%, compared to other age groups.

Similar results were obtained by ( Nwaoug and Orajaka, 2011) who had a prevalence of 58.2% among children and age group 1-3yrs had prevalence of 71.2%. Okafor and Oke-Ose (2012) examining children in Benin City, Nigeria, had prevalence of 36.4% and children aged ½ -3 yrs had prevalence of 58.6%. Etusin et al., (2013) observed prevalence of 83.9% among children in Uturu, Abia State, Nigeria and age group 1-3 yrs had prevalence of 89.5%. Statistically, at p< 0.05 there was significant difference at in the prevalence of malaria between age group 0 – 4 yrs and other age groups. The high prevalence of malaria among children from age 0-4 yrs might be due to gradual loss of maternally derived antibodies as also observed by (Nwaorgu and Orajaka, 2011). These children from rural areas are exposed to mosquito bite from surrounding environment such as bushes, farms and gardens surrounding the houses, the environment encouraging mosquito breeding sites and the fact that mosquitoes in the area of study are both diurnal and nocturnal. The inability of children of age 0-4 yrs to ward off mosquitoes could also be predisposing factor. Considering the percentages of ABO-blood group, blood group O was highest in percentage occurrence 64.8% among pregnant women and 44.4% among children, as compared with non O blood groups.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Number sample</th>
<th>Severe malaria in group</th>
<th>Overall severe malaria in group</th>
<th>Number sample</th>
<th>Severe malaria in group</th>
<th>Overall severe malaria in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>51(20.4)</td>
<td>18(7.2)</td>
<td>102(40.6)</td>
<td>10(4.0)</td>
<td>33(13.2)</td>
<td>131(52)</td>
</tr>
<tr>
<td>B</td>
<td>30(12.0)</td>
<td>10(4.0)</td>
<td>40(16.0)</td>
<td>5(2.0)</td>
<td>12(4.8)</td>
<td>16(6.4)</td>
</tr>
<tr>
<td>AB</td>
<td>7(2.8)</td>
<td>2(0.8)</td>
<td>9(3.6)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
</tr>
<tr>
<td>O</td>
<td>162(64.8)</td>
<td>72(28.8)</td>
<td>250(98.8)</td>
<td>2(0.8)</td>
<td>4(1.6)</td>
<td>6(2.4)</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>102(40.6)</td>
<td>17(6.8)</td>
<td>18(7.8)</td>
<td>24(9.6)</td>
<td>24(9.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Haemoglobin Genotype</th>
<th>Number examined pregnant women</th>
<th>Number positive pregnant women</th>
<th>Severe malaria in group</th>
<th>Overall severe malaria in group</th>
<th>Number examined children</th>
<th>Number positive children</th>
<th>Severe malaria in group</th>
<th>Overall severe malaria in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAA</td>
<td>198(79.2)</td>
<td>84(33.6)</td>
<td>18(7.2)</td>
<td>18(7.2)</td>
<td>99(39.6)</td>
<td>20(10.2)</td>
<td>20(10.2)</td>
<td>20(10.2)</td>
</tr>
<tr>
<td>HbAS</td>
<td>52(20.8)</td>
<td>18(7.8)</td>
<td>2(1.2)</td>
<td>2(1.2)</td>
<td>122(48.8)</td>
<td>24(9.6)</td>
<td>24(9.6)</td>
<td>24(9.6)</td>
</tr>
</tbody>
</table>
The result was in agreement with those obtained by (Zerihum et al., 2011; Stekete and Petros, 2010; Otajevwo, 2013). The prevalence of malaria by ABO-blood group showed that pregnant women and children with O blood group had high prevalence rate of malaria 28.8% and 24.8%, compared with non O blood group. This was contrary to the results obtained by (Zerihum et al., 2011, Miggot-Nabias et al., 2000), they had low prevalence of malaria parasitemia among blood group O individuals; as opposed to high prevalence among blood group O subjects obtained in this study. Pregnant women and children with O blood group had less severe cases of malaria with clinical episode, compared with non O blood group as also noted by (Pathirana et al., 2005, Miggot-Nabias et al., 2000, Zerihum et al., 2011). Statistical analysis at P < 0.05 does not show significant difference in the prevalence severe malaria between blood group O and other ABO blood group, but there was significant difference at p < 0.05 between the prevalence malaria and severe malaria among blood group O subjects.

The reducing effect on blood group O on rosetting (Deepa et al., 2011, Row et al., 2007). Blood group O show deficiency of most adhesive molecules and contain disaccharide sugar molecules which reduce the rate, size, and stability of rosetting formed during Plasmodium falciparum infections (Van der Heyde et al., 2006, Daniels, 2005) are reasons for low clinical episode. The percentage occurrence of HBAA was 79.2, HBAS 20.8% among pregnant women and HBAA 78.8%, HBAS 21.2% among children. Comparing the variation in ABO blood group with that of haemoglobin genotype among pregnant women and children, the ratio of Hb-genotype seem to be more stable than ABO blood group in the population. In a related study (Otajevwo, 2012) observed HBAA was 68.1% and HBAS 24.4%, while (Otajevwo and Enbulele, 2013) had HBAA 66.9% and HBAS 27.5%. The result of the percentage occurrences of HBAA to HBAS in this study was similar to those obtained by other worker in Nigeria. HBAA subjects had high prevalence of malaria and severe malaria among the pregnant women and children examined, Table 4.

Statistical analysis at p<0.05 showed significant difference in the prevalence of malaria between HBAA and HBAS among pregnant women and children. The finding agrees with that of (Williams et al., 2005, Amala and Nwibani, 2015). HBAS have been shown to protect against symptomatic P. falciparum malaria, mild clinical malaria and low parasite densities during such episode. These were significantly lowered in HBAS compared with HBAA subjects. HBAS protects against serious clinical conditions like cerebral malaria and severe anemia. One of the reasons is the reduced parasite ability to grow and multiply in HBAS (Friedman, 1978). Protection against hospital admission for P. falciparum and severe malaria episode were also noted in HBAS individuals (Possell et al., 1978) which is attributed to early removal of erythrocytes from circulation by the immune system in HBAS subjects.

Parasite infected HBAS cells sickle 6 times more than unparasitized HBAS cells (Luzzato et al; 1970, Rott et al; 1978). This phenomenon may lead to intracellular parasite death, reduced oxygen tension and enhanced removal of infected cells from circulation by the immune system. Plasmodium species identified to be associated with malaria in this study was P. falciparum only. The protection offered by HBAS was found to P. falciparum specific (Williams et al., 2005). The pathogenesis of malaria related anemia involves both bone marrow suppression and acute haemolysis (Menendez et al; 2000, Weatherall and Abdulla, 1982). Individuals with HBAS benefit from two advantages, suffering few clinical episode of P. falciparum malaria which means their baseline haemoglobin level may be higher with lower parasite densities during infection (Aidoo et al; 2002). The above reasons may accounts for the difference in the level of malaria parasitemia and severe malaria in HBAA and HBAS subjects.

Conclusion
The prevalence of malaria among pregnant women and children are high, the prevalence malaria among children was slightly higher that of pregnant women. Children of age 0-4yrs are more predisposed to malaria. ABO blood group O protects against severe malaria, whereas HBAS individuals have low parasite densities and less clinical episode. The Plasmodium species associated with malaria was only P. falciparum.

REFERENCES


