Abstract

Background: To compare the prevalence of malaria parasitaemia and the mean parasite density in pregnant women at first antenatal visit with those of the control subjects at Nnamdi Azikiwe University Teaching Hospital, Nnewi.

Method: A case control prospective survey using a structured questionnaire to collect data from pregnant women attending antenatal clinic between 1 April and 30 September 2001 and matched controls at the GOPD during the same period. Peripheral blood smears were examined in 420 pregnant women at their first antenatal visit and 200 control subjects to compare the prevalence of malaria parasitaemia and mean parasite density in pregnant women and controls.

Results: The prevalence of parasitaemia was 79.3 percent (i.e. 333 of 420) for pregnant women and 31.5 percent (or 63 of 200) for the control. For both pregnant women and controls, an overall prevalence of 63.1 percent was observed. The study found the mean parasite density for the pregnant women to be 1978±1531 (Mean±SD), while that of the controls was 766±1923.

Conclusion: This study demonstrates the higher prevalence of malaria parasitaemia and mean parasite density in pregnant women when compared with the matched controls.

Key Words: Malaria Parasitaemia, Pregnancy, Mean Parasite density, Prevalence and Controls

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Introduction

Malaria continues to be one of the main public health problems in the world, especially in the majority of African countries. In Nigeria, malaria is the commonest cause of out patient visits and it ranks among the three most common causes of death. It accounts for about one million deaths in Africa and nine out of ten cases of malaria worldwide occur in Africa, south of the Sahara.

About 200 million people in the world are at risk from malaria and the disease kills about 2 million people every year.

Although anyone can get malaria, two groups of people are at a special risk from malaria such as children under five years of age and pregnant women. This is especially so during the first and second pregnancies. The direct effect of heavy placental parasitization by malaria parasites includes the reduction of placental blood flow and the available oxygen to the fetus. Consequently, this may manifest as low birth weight, abortion, intra uterine growth retardation, intra uterine death, premature labour, stillbirth, prenatal asphyxia, and neonatal asphyxia.

The effect of malaria on a population is dependent on whether malaria is stable or unstable. Stable malaria exists in regions where there is perennial transmission of malaria parasites. The intensity of attack in such areas remains constant throughout the year. In contrast, unstable malaria is found in areas with intermittent transmission of malaria parasites due to some natural phenomenon enhancing transmission at certain periods. In the unstable malaria community, immunity to malaria is variable and epidemics could occur with a very high incidence of severe malarial complaints including cerebral malaria.

The immunity to malaria is dependent upon a persistently low-grade parasitaemia, which could be natural or acquired and is passively transmitted through the placenta.

The acquired immunity is both humoral (antibodies to the parasites) and cellular (macrophages and phagocytes) and is provoked only by the asexual erythrocyte stage of the malaria parasites, not by the liver stages or by gametocytes. Although, gametocytes have some antigenic properties, provoking antibodies that are apparently not protective.

The increased susceptibility of pregnant women to malaria has been thought to be due either to...
sequestration of the parasites in the placenta or to
depression of selected components of the immune
system in association with the increased production of
several hormones or other proteins. The placenta
sequesters parasites that are able to cytoadhere to
chondroitin-4-sulfate, a glycosaminoglycan molecule
expressed by the placental syncytiotrophoblast, while
parasites from a non-pregnant host do not bind to
chondroitin-4-sulfate.
Parasites adhere to the surface of trophoblastic villi,
eliciting the accumulation of inflammatory leukocytes in
the intervillous space, and the necrosis of adjacent
placental tissue. Maternal malaria results in poor
pregnancy outcome, although the responsible
mechanisms have not been defined.

Patients and Methods
Study Design
The study design is a case control prospective study of
pregnant women as cases and non-pregnant women as
controls.

Location of Study:
This study was conducted at the antenatal clinic of
Nnamdi Azikiwe University Teaching Hospital, Nnewi.
The hospital is a Federal Government owned tertiary
health institution in Nnewi, a high population density area
of Anambra State. Nnewi has a projected population of
480,000 inhabitants in 2001 and is located in the South
Eastern zone of Nigeria. The climatic condition in this
zone include an environmental temperature between 20
and 28 degrees centigrade; mean monthly rainfall greater
than 10 centimetres, relative humidity greater than 60
percent; and topography with altitude less than 2000
metres above sea level.
The town has a rain forest type of vegetation and the area
is Holoendemic for malaria. The predominant malaria
vectors include Anopheles Gambiae, A. Funestus, A.
Darlingi, and A. Punctulatus. Plasmodium falciparum is
the parasite that is found in over 80 percent of malaria
patients while P. malaria causes 15 percent and P. ovale
causes less than 5 percent of malaria cases in Nnewi.
However, mixed infections with all three species of
plasmodium occur.
An average of five new booking of antenatal women was
enrolled daily on their own accord with a few referred from
nearby towns by private, missionary and general
hospitals. Most referrals were cases with complicated
pregnancy, labour, or puerperium. Antenatal services
have a backup of laboratory and blood bank, Ultra sound,
and other ancillary services.

Ethical precepts in relation to the research
The ethical committee of NAUTH, Nnewi approved this
study. Standard pre-test counselling for HIV screening
was first carried out and confidentiality in the handling of
the result of the screening was observed. The subjects
who declined were left out of the study while those who
accepted gave an informed consent for HIV screening;
haemoglobin genotype and blood smear investigations.
The subjects understood their rights not to participate in
the study if they so wished and could at any point opt out.
The subjects did not pay for the investigation.

Patients/subjects
All pregnant women attending their first antenatal visit at
the antenatal clinic, Nnamdi Azikiwe University
Teaching Hospital, Nnewi during the study period of 1
April to 30 September 2001 who met the inclusion
criteria were enrolled for the study. Four hundred and
twenty (420) consecutive pregnant women were
enrolled. The period coincided with the rainy season
when malaria transmission is most intense. These
women were predominantly Igbo and resided in Nnewi.

Controls
A comparison group was chosen among patients
attending the General Out Patient Department of the
same hospital in the same period. For every pair of
pregnant woman, one non-pregnant woman whose age
and parity matched was chosen as control.

Inclusion Criteria
All pregnant women on their first antenatal visit during
the study period of 1 April to 31 September 2001 after an
informed consent were obtained from them. These
pregnant women and the controls were normally
resident in Nnewi during the period of the study and at
least one year previously. They were HIV sero-negative
and were not on cytotoxic drugs or on any immuno
suppressive therapy. They did not have sickle cell
disease, were not recently transfused with blood and did
not have any debilitating illness.

Exclusion criteria
The subjects excluded were pregnant and non pregnant
women who were HIV sero-positive; sickle cell disease
as confirmed by Haemoglobin genotype screening; or
had a recent blood transfusion.

Data Collection
A well-structured questionnaire was used for data
collection by personal interview. The subjects' weight
was recorded in kilograms, while the height was recorded in metres. Thereafter, blood was withdrawn from the cubital vein (about 5mls) of each subject for the preparation of thick blood films, haemoglobin electrophoresis, and HIV screening.

**Method**

Sixty-five samples were excluded (HIV sero-positive samples (61), haemoglobin SS sample (1) and (3) of doubtful quality). Slides were prepared using the Gimsa method. After drying, the stained slides were examined in batches using (x 100 objective) lens in oil immersion. The parasite count per microlitre (1) of blood from the thick film was estimated by multiplying the average number of parasites per high power field (100 x objective) by 500.

The average number of parasite per high power field was established by examining 10-50 fields (depending on parasitaemia). The average number of trophozoites per high power field (HPF) was then multiplied by a factor of 500. The product gave the estimated number of parasites per microlitre of blood.

A control group of non pregnant women between the ages of 15 years and 45 years normally resident in Nnewi and sharing the same characteristics as those pregnant had their thick blood smears examined for malaria parasites using the same Giemsa staining technique and parasite density estimation method as that used with the pregnant subjects.

**Results**

A total of 620 subjects were studied between the period 1 April 2001 and 30 September 2001; made up of 420 consecutive pregnant women attending first ante-natal visit (booking) at NAUTH, Nnewi and 200 control subjects (non pregnant women) attending General Out Patient Department, after the exclusion of 64 observations; both groups satisfied the set criteria. An average of four pregnant women and two controls per day were studied.

Table I shows that the mean age for the pregnant women and controls were (mean±SD) 28.6±5.7 years and 28.5±5.18 respectively. Those below 20 years of age constituted 5.2% for pregnant women and 4% for the controls while the most frequent age group was 25 to 29 years in both pregnant women and controls. About 36.2% of pregnant women and 39.5% of the controls were in the 25-29 years age group.

About 124 of 420 or 29.5% of the study population and 53 of 200 or 26.5% of the controls werenulliparous while the least frequent parity (para 3) were 7.1% and 7.5% for pregnant subjects and controls respectively.

Table II shows that about 333 of the 420 pregnant women (study group) had malaria parasitaemia giving a prevalence rate of 79.3% while 63 of 200 controls had malaria parasitaemia i.e. a prevalence rate of 31.5%. P value 0.000 (P<0.05). This is statistically significant. The ratio of the prevalence rate of malaria parasitamia of pregnant to non pregnant women is 2.5: 1.

The mean parasite density of 1978 microlitre in the pregnant women is much higher than that found in the control subjects 762 microlitre. The ratio of mean parasite density of pregnant to non pregnant women is 2.6: 1. However, the spread as shown by the standard deviation is greater in the control than in the pregnant women.

Table I: Summary of the distribution of comparable selected socio-demographic characteristics of pregnant women and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pregnant Women</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (Mean±SD) years</td>
<td>28.6±5.7</td>
<td>28.5±5.18</td>
</tr>
<tr>
<td>No. of women aged less than 20 years</td>
<td>22 (5.2%)</td>
<td>8 (4%)</td>
</tr>
<tr>
<td>Most frequent age group 25-29 years</td>
<td>152 (36.2%)</td>
<td>79 (39.5%)</td>
</tr>
<tr>
<td>Nulliparous women</td>
<td>124 (29.5%)</td>
<td>53 (26.5%)</td>
</tr>
<tr>
<td>Literacy (secondary school leavers)</td>
<td>274 (62.5%)</td>
<td>130 (65%)</td>
</tr>
<tr>
<td>Least frequent parity (para 3)</td>
<td>30 (7.1%)</td>
<td>15 (7.5%)</td>
</tr>
<tr>
<td>Body Mass Index (BMI &lt; 25 kg/m²)</td>
<td>170 (40.5%)</td>
<td>68 (34%)</td>
</tr>
<tr>
<td>Occupation of spouse (middle class)</td>
<td>314 (74.3%)</td>
<td>148 (74.3%)</td>
</tr>
</tbody>
</table>

Table II: Prevalence of malaria parasitaemia in pregnant women and controls at NAUTH, Nnewi

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. with malaria Parasitaemia</th>
<th>Total studied</th>
<th>Percentage positive for parasitaemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREGNANT WOMEN</td>
<td>333</td>
<td>420</td>
<td>79.3</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>63</td>
<td>200</td>
<td>31.5</td>
</tr>
<tr>
<td></td>
<td>393</td>
<td>620</td>
<td>63.38</td>
</tr>
</tbody>
</table>

Table III: Comparison of the mean parasite density in pregnant women at booking and controls in NAUTH, Nnewi.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. with MP</th>
<th>No. studied</th>
<th>Prev. rate</th>
<th>MPPD per microlitre (mean±SD)</th>
<th>No. with MP</th>
<th>No. studied</th>
<th>Prev. rate</th>
<th>MPPD per microlitre (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1978</td>
<td>97.5</td>
<td>1797/1910</td>
<td>63</td>
<td>200</td>
<td>79.5</td>
<td>762/963</td>
<td>31.5</td>
</tr>
</tbody>
</table>

**MP** = Malaria parasitaemia.

**Prev.** = Prevalence.

**MPPD** = Mean Parasite Density
Discussion
This study revealed a high prevalent rate of malaria parasitaemia of 79.3 percent among pregnant women at their first antenatal visit in NAUTH, Nnewi and 31.5 percent among the control subjects studied. Overall, the prevalence for both groups together was 63.8 percent. The finding agrees with what has been reported in literature by researchers in other parts of the world. The prevalent rate of malaria parasitaemia in pregnant women was about 2.5 times of the non pregnant women, while the mean parasite density was about 2.6 times. These figures suggest that pregnant women are more susceptible to parasitaemia in terms of spread and severity.

In Central India, Singh et al observed high malaria prevalent rate among pregnant women (17.16%), when compared with non-pregnant women (7.56%). The mean parasite densities for both P. falciparum and P. vivax were significantly higher in the pregnant women than in the non-pregnant women.

Diagne et al working in Senegal observed an incidence rate of malaria attack that was about 4.2 times higher in pregnancy than during control period in the same women during the year which preceded or followed their pregnancy. The survey revealed that the parasite rate was significantly higher during pregnancy. Although, this study included women in their immediate postpartum period, which could affect the conclusion that pregnant women were more susceptible to malaria than their non-pregnant counterparts. However, some researchers have shown that the increased susceptibility to malaria continued into the early postpartum period.

Okonofua et al reported 72 percent parasite rate among pregnant women at Ile-Ife; although, the study was not done with controls.

The very high figure in this study may be accounted for by the fact that the study location, Nnewi is an area with perennial malaria transmission without organized mosquito or malaria control programmes. The period also coincided with the rainy season when water drains and gutters are usually blocked with refuse. The dirty stagnant water pools have generally provided breeding places for the mosquito vectors.

Susceptibility to malaria infection and the severity of clinical manifestation are determined by the level of pre-pregnancy immunity, which in turn depends largely on the intensity and stability of malaria transmission. The reason for the increased susceptibility of pregnant women to malaria parasitaemia has been a subject for many research. Tian et al found an increased population of young red cells in pregnant women and this he pointed out as contributing to the increased malaria susceptibility during pregnancy. Another probable reason is that the placenta provides an immunologically privileged site where the proliferation of the malaria parasites can proceed unbridled. It is more likely that many factors interplay to make the pregnant women more susceptible to malaria parasitaemia than the non-pregnant ones.

In conclusion, the prevalence rates and parasite density of malaria parasitaemia in pregnant women is more than twice the figures in their non pregnant counterparts at NAUTH, Nnewi.

For health workers in this environment, it is therefore recommended that all pregnant women be offered presumptive treatment and preventive regimen in line with WHO's recommendation. The authors agreed with the recommendation by Shultz et al that intermittent therapy be coupled with the tetanus vaccination as an antenatal policy. Full doses of Sulphadoxine/Pyrimethamine should be given to pregnant women at the beginning of second and third trimesters. Pregnant women should be counselled on the need to undertake individual malaria vector control measures such as sleeping under insecticide treated bed nets (ITBN), protective clothing, destruction of mosquito breeding places and prompt treatment of fevers.

Limitation of the Study
The number of the controls enlisted was fewer because of the caution exercised in matching controls with the pregnant subjects. Women of child bearing age (15 to 45 years) who attended the GOPD during the study period were fewer. However, the sample size is large enough to give the study a power greater than 80%.

Acknowledgement
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References: