Histopathology of Ovarian Tumours

Vitamin A and Malaria

Salt Hypertension and Amlodipine

Limb Gangrene from Uterine Fibroid

Which potent Oral antibiotic did you ask for?
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Vitamin A Supplementation as an Adjunct in the Treatment of Mild to Moderate Childhood Malaria: Effect on Haematological Indices

Olayemi SO', *Oreagba IA1, Alayo AS', Temiyi EO1, Nwoye E1, Bamiro BS and Gjobor PD1
Nigerian Medical Practitioner Vol. 69 No. 3-4, 2016 (32-38)

Summary
Vitamin A supplementation to preschool children is known to decrease the risks of mortality and morbidity from some forms of diseases such as diarrhea, measles, and human immunodeficiency virus (HIV) infection. These effects are likely to be the result of the actions of vitamin A on immunity. Therefore, the role of Vitamin A supplementation in the treatment of mild to moderate falciparum malaria in Nigerian children was investigated using haematological parameters as outcome indices. Eighty four (84) children between the ages of 5 months and 12 years with fever and a positive Malaria Parasite result using Rapid Diagnostic Test were randomized and divided into two groups. Fiftyfour (54) patients received vitamin A supplementation in addition to a complete regimen of Artemether/lumefantrine given to both groups. Clinical and laboratory parameters (Malaria Parasite Density, WBC and PCV) were measured on days 0, 3 and 28. On the third day, both groups of patients were found to have complete Malaria Parasite clearance irrespective of Vitamin A supplementation. Vitamin A supplementation significantly increased mean packed cell volume(PCV) from 23.44 ± 0.17 % to 28.60 ± 0.22 % (p<0.05); it also increased mean white blood cell count (WBC) from 7080.00 ± 693.56cells/mm3 to 8711.1 ± 629.78cells/mm3 however this was not statistically significant (p>0.05). After treatment (on day 28) the mean PCV and WBC were significantly increased in both groups of patients (p<0.05). At baseline there was a significant(r=0.46) linear relationship between Malaria parasite density (MPD) and WBC while patients PCV showed no significant(r=0.41) linear relationship with MPD. Vitamin A plays a significant role as supplementation in the treatment of mild to moderate malaria by improving PCV and WBC laboratory indices; However more comprehensive monitoring of Malaria Parasite Density within the first 48 hours of commencement of treatment is suggested.

Introduction
According to WHO, there are an estimated 214 million acute cases of malaria around the world in 2015, resulting in about 438,000 deaths. Approximately 90 percent of these deaths occur in Africa, mostly in young children.(1) Although an understanding of the influence of nutrition on malaria is far from complete, it is clear that nutrition strongly influences the disease burden of malaria.(2)

Vitamin A deficiency and malaria are both highly prevalent health problems in Africa. A low serum retinol concentration (a marker of vitamin A deficiency) is commonly found in children suffering from malaria,(3) but it is not certain whether this represents pre-existing vitamin A deficiency, a contribution of malaria to vitamin A deficiency, or merely an acute effect of malaria on retinol metabolism or binding.

The majority of those who die from malaria are young African children who do not get treatment as quickly as possible.(4) Clinical trials show that high-dose vitamin A supplementation reduces morbidity and mortality in children with acute measles infection.(5)

Vitamin A supplementation increases host resistance to malaria.(6) Furthermore in vitro studies have demonstrated the toxic effect of retinol (vitamin A alcohol) on Plasmodium falciparum,(7) and confirmed its antiparasomal activity across all stages of parasite development.(8) There is however limited information on the role of vitamin A in human malaria. The prevalence of VAD (defined as serum retinol <0.70 μmol/L or the presence of abnormal impression cytology) among African preschool children in the year 2000 was estimated to be 32%, affecting 33 million children (Micronutrient Deficiency Information System [MDIS] of the World Health Organization).(9)

Since retinol supplementation is most often used in children, its safety, simplicity and availability, justify the need to carry out a controlled trial of retinol as an adjunctive treatment for falciparum malaria. A previous RCT showed a decrease in mortality amongst children with cerebral malaria.(10) This study aimed to determine the effect of vitamin A supplementation as an adjunct in the treatment of mild to moderate malaria infection making use of haematological indices.

Materials and Methods
Study setting: The study took place at the Community Health Center, Makurdi, Benue State, South Western Nigeria. The Centre is one of the six Community Health Center under Makurdi Local Government. It's a four bedded Health Center. The centre provides maternal and infant health care in addition to maternity service.

Materials
Rapid Diagnostic Test kit (Abon Biopharm
(Hangzhou) Co., Ltd, China) Vitamin A supplements (Banner Pharmacaps Ltd, Canada). Each capsule contains 200000 IU (60mg) of Vitamin A (retinol palmitate). Other materials used were 2ml and 5ml needle and syringe, cannula, Giemsa stain and EDTA bottles.

**Study design:** This was a randomized placebo-controlled trial. Inclusion criteria were fever (body temperature > 37.5°C, with positive blood smear for P. falciparum asexual parasites and mother’s consent. Exclusion criteria included children recently immunized, and those on vitamin A supplementation.

Sample size was estimated at 5% significance and 80% power using the method of Campbell et al.(11)

**Procedure:** Eighty-four patients between the ages of six months and twelve years with various severity of malaria ranging from mild to moderate malaria were randomly assigned to receive either Artemether/Lumefantrine(AL) alone or AL plus vitamin A supplementation. Assigning each child to groups was done according to a pre-determined simple random list. They were then followed up accordingly.

**Drug administration:** All children were treated with Artemether/ Lumefantrine as antimalarial drug adhering to the national standard treatment guideline for malaria treatment. Children were observed for one hour after each drug administration. Treatments were re-administered if the child vomited within the observation period. Children who vomited the re-administered dose were withdrawn.

In the study group, vitamin A was administered immediately after AL. The small end of the vitamin A capsule was cut off and then gently squeezed into the child’s mouth with each capsule containing 200000 IU of Vitamin A while water was used as placebo for control.

The Vitamin A container was tightly closed after each use and protected from moisture and light and was stored in a refrigerator.

**Sample collection:** Blood samples of patients were collected three times within a period of 28 days on days 0, 3 and 28. Day 0 represents the day of first contact; patients were referred directly from the medical officer to a phlebotomist in the team for collection of blood samples and also administration of either vitamin A or placebo. All febrile patients were seen, however only those that were MP positive through a RDT were recruited. Patient’s bio data were recorded on a form which contained age, sex, body temperature, serial number, types of anti-malaria drugs given and other complaints. Samples were taken from the ante-cubital vein. One out of every four patients was used as control (that is AL with water and no Vitamin A administered). Body temperature was repeated using infra-red thermometer as digital device. Patient’s biodata including mother’s mobile phone number were collected.

**Follow up:** Patients were seen on the third day for follow up. Samples were collected and patients examined, neither vitamin A nor placebo was given on this visit. On day 28, patients were again seen for the last time in this study, blood samples were taken but no drug administered. Child and mother were then provided with incentive (bed nets) as promised during the first visit. Signs and symptoms and occurrence of adverse events were monitored throughout the study period. Adverse events were defined as any untoward medical occurrence, irrespective of its suspected relationship to study medications according to E2A International Conference on Harmonization (ICH) guidelines.(12)

**Laboratory investigation:** All samples were taken to the College of Medicine University of Lagos parasitology and haematology laboratory within 24 hours of collection. Malaria parasitaemia (expressed as parasites per microlitre of whole blood) was determined in Giemsa-stained thick blood films following WHO approved protocol for malaria diagnosis.(13) parasite density was determined by counting the number of asexual stage parasites relative to 200 white blood cells (WBC), and multiplied by the measured WBC count. Each slide was read independently by two microscopists and the average of two concordant (count < 25% and Pos/Pos films) readings recorded. Approximately 10% of all slides and discordant slides (count > 25% and Pos/Neg films) were read by a third expert microscopist who was independent of the study. Any discrepancies in the readings were resolved by the third microscopist who is the tie breaker. RDT for the histidine-rich protein 2 (Abon Biopharm (Hangzhou) Co., Ltd, China) were done according to the manufacturers’ instructions. The total WBC and differential counts were measured by means of an automated haematology analyzer.

**Data and statistical analysis:** Data were presented as means and simple proportions. Frequency of anti-malarial efficacy was presented in percentage. Relationship between malaria parasite density and haematological parameters such as PCV and WBC was assessed by Pearson’s correlation (r). Effects of antimalarial therapy was determined on patients’ PCV and WBC by student’s t-test while same tool (t-test) was also
used to determine the combined effect of antimalarial and vitamin A supplementation on PCV and WBC of same patients. The level of significance was determined at 95% (Such as $\alpha = 0.05$).

**Ethical approval:** Ethical clearance was obtained from the Research and Ethics Committee, Lagos University Teaching Hospital Iddi-araba Lagos. Permission for the study was also obtained from Sagamu Local Government South Western Nigeria. The details of the study were explained to mothers/caregivers of the subjects and written consents to participate was obtained.

**Results**

A total of ninety five patients were tested for malaria, eighty four patients tested positive while eleven tested negative. The ninety five (95) patients fulfilling the inclusion criteria of fever $>37.5^\circ C$, they were then randomized to vitamin A supplementation group ($n=57$) or vitamin A non-supplementation group ($n=38$) and evaluated on day 3. Three patients in the vitamin A group were lost to follow up on day 3 while eight in the vitamin A non-supplementation group were lost to follow up. The vitamin A supplementation group received 200000 IU supplementation with a full dose of antimalarial treatment while vitamin A non-supplementation group received only antimalarial treatment without vitamin A supplementation and thus served as control. The flow of subjects through the study is shown (Figure 1). A total of 84 children completed the study.

Patient seen were between the ages of six months and twelve years. Mean age, baseline admission clinical and laboratory parameters are shown in Table 1. The parasite clearance rate on days 1, 2 and 3 of treatment, usually determined by the parasite reduction ratios (PRR) was not calculated because samples were not collected at 6-hourly intervals as required. Haematological parameters were therefore used to measure malaria indices.

**Table 1:** Patient baseline admission parameters and demographics

<table>
<thead>
<tr>
<th>Baseline Parameter*</th>
<th>Vitamin A supplementation group $n=54$</th>
<th>Non supplementation group $n=30$</th>
<th>Vitamin A supplementation group $n=30$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>4.8 ± 0.62</td>
<td>6.5 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.6 ± 0.45</td>
<td>37.9 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Parasite Density (/μL)</td>
<td>82,419 ± 632</td>
<td>6563 ± 243</td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>36.37 ± 1.32</td>
<td>36.25 ± 1.75</td>
<td></td>
</tr>
<tr>
<td>WBC (cells/mm3)</td>
<td>7809 ± 575</td>
<td>7370 ± 620</td>
<td></td>
</tr>
</tbody>
</table>

*No significant difference between mean baseline admission parameters except for parasite density

At baseline there was a significant ($r=0.56$) linear relationship between Malaria parasite density (MPD) and WBC while patients PCV showed no significant ($r=0.41$) linear relationship with MPD. (Figure 2). Vitamin A supplementation significantly increased overall mean packed cell volume (from day zero to day 28) from 23.44 ± 10.57% to 28.60 ± 15.16% ($p<0.05$) and it also increased mean White blood cell count (WBC) from 7080.00 ± 693.56 cells/mm$^3$ to 8711.11 ± 629.78 cells/mm$^3$ however this was not statistically significant ($p>0.05$). Table 2

**Table 2:** Effects of Vitamin A supplementation on mean Haematological Indices

<table>
<thead>
<tr>
<th>Antimalarial</th>
<th>N</th>
<th>WBC (cells/mm3)</th>
<th>PCV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL plus Vitamin A</td>
<td>54</td>
<td>8771.11 (629.78)</td>
<td>28.60 (8.62)</td>
</tr>
<tr>
<td>AL only</td>
<td>30</td>
<td>7080.00 (693.56)</td>
<td>23.44 (10.57)</td>
</tr>
</tbody>
</table>

* Overall mean ±SEM from day 0 to day 28

WBC= White Blood Cells. PCV= Packed cell volume. AL= Artemether/Lumefentrine
Table 3: Mean PCV and WBC at specified follow up days

<table>
<thead>
<tr>
<th>DAYS</th>
<th>DAY 0 Mean (SEM)</th>
<th>DAY 3 Mean (SEM)</th>
<th>DAY 28 Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean PCV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL plus vitamin A supplementation</td>
<td>28.00 (1.93)</td>
<td>28.78 (1.44)</td>
<td>36.37 (1.32)</td>
</tr>
<tr>
<td>AL only</td>
<td>23.60 (1.34)</td>
<td>22.73 (1.90)</td>
<td>26.25 (1.75)</td>
</tr>
<tr>
<td><strong>Mean WBC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL plus vitamin A supplementation</td>
<td>8970 (611)</td>
<td>7661 (746)</td>
<td>7809 (575)</td>
</tr>
<tr>
<td>AL only</td>
<td>6500 (715)</td>
<td>6771 (656)</td>
<td>7370 (620)</td>
</tr>
</tbody>
</table>

AL = Artemeter / Lumefantrine

Figure 1: Trial profile of malaria patients on vitamin A supplementation

After antimalarial treatment, the mean PCV was significantly increased in both groups of patients (p<0.05) while their mean WBC was also significantly increased. (Table 2, Table 3 shows mean PVC and WBC at specified follow up periods.

Mean PCV increased from day 0 to day 28 in both groups while mean WBC decreased from day 0 to day 3 but increased on day 28 in the vitamin A group while it increased progressively in the control group.

Figure 2: A linear correlation graph of baseline WBC and Parasite Density of patients before treatment with antimalarial.

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Discussion

The study of the interactions between nutrition and malaria may provide insight to protective mechanisms and result in nutrient-based interventions as low-cost and effective adjuncts to current methods of malaria prevention and treatment. This study addresses the rationale behind nutritional supplementation of antimalarial treatment with cheap, affordable and locally available food supplements for children with malaria infections in Nigeria.

Our results showed a significant (p<0.05) decrease in the mean PCV of all patients at baseline (both groups). This aligns with a previous study showing that children with malaria parasitaemia had a significantly lower haemoglobin and packed cell volume than children without parasitaemia.(14)

So many factors can contribute to anaemia during malaria infections. Malaria parasite depletes folic acid in the body. During episodes of malaria infection there is loss of appetite and thus reduction in intake of micronutrients. Oxidative stress during malaria may also contribute to haemolysis and anemia.(15) Excessive removal of non-parasitized erythrocytes, immune destruction of parasitized red cells and impaired erythropoiesis as a result of bone marrow dysfunction are few of the different mechanisms through which malaria may cause anaemia.(16)

These findings confirm the inverse relationship existing between malaria parasitaemia and haematological parameters such as PCV and haemoglobin. Therefore, the main cause of anaemia in malaria infection is the erythrocytic phase of malaria parasite cycle. This is more severe in P. falciparum that affect the red blood cells in all the age groups.

When malaria is treated promptly with highly effective antimalarial drugs like the ACTs, this rapidly reduces parasite density and eventually clears parasites from the blood, allowing erythrocyte numbers to be restored(16-18) and hence increasing PCV.

Haematological indices of malaria were thus used to evaluate the effect of vitamin A supplementation on malaria outcomes. Our findings revealed a significant (p<0.05) increase in PCV in the group that had Vitamin A supplementation over the control group that received only antimalarial drug. This correlated well with improved clinical outcomes. Thus Vitamin A supplementation may enhance patient recovery from anaemia caused by effects of malaria parasite infection. Previous controlled trials of either vitamin A or zinc supplementation showed that these nutrients can substantially reduce clinical malaria attacks.(2) In children with cerebral
group). Thus it was not possible to conclude on the possible effects of vitamin A supplementation on malaria parasite clearance time. Despite this limitation, the study demonstrated a favorable effect on malaria outcomes in the vitamin A group compared with the control group using haematological indices.

Conclusions
Vitamin A supplementation has a significant role as an adjunct in the treatment of mild to moderate childhood malaria by improving haematological indices. A relationship exists between these indices and parasite density. This study adds to the growing evidence that routine vitamin A supplementation during mild to moderate childhood malaria will likely reduce morbidity and mortality and improve clinical outcomes. However, knowledge gap still exists for its effect on parasitological outcomes and therefore needs to be filled by further studies.

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