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# LEUCOCYTE PHAGOCYTOSIS IN CHILDREN WITH URINARY SCHISTOSOMIASIS AND ASYMPTOMATIC MALARIA PARASITEMIA

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**In the participants considered for this study, leucocyte migration, neutrophil candidacidal activity and ability to generate reactive oxygen were determined as percentage migration index (%MI), candidacidal phagocytic index (%CI) and bacterial stimulated nitroblue tetrazolium (NBT) dye reduction index (%NBT) respectively. Also, malaria density was counted from thick blood film of glass slide stained with Giemsa stain. The participants were 54 school children having urinary schistosomiasis without malaria parasites (USS-M), 18 children with both urinary schistosomiasis and malaria parasites (USS+M), 46 children with malaria parasites without urinary schistosomiasis (M-USS) and 29 controls. The mean %MI was least while %NBT index was highest in USS+M subjects but M-USS subjects had least %CI. Malaria density was higher in M-USS subjects than USS+M subjects. The results of this study showed that low prevalence and reduced severity of malaria parasites in children with urinary schistosomiasis may be due to adequate production of LMIF and reactive oxygen species.**

**Keywords: Leucocyte phagocytosis, malaria, schistosomiasis, Nigeria**

## INTRODUCTION

The epidemiology of malaria coincides geographically with that of schistosomiasis in many region of the world. However, immune correlates of resistance during schistosomiasis or malaria remains unclearly defined (1). Also little is known about the immune responses of individuals with concurrent infections of schistosome or malaria parasites with other parasites/pathogens. Co-infection of malaria parasite with certain pathogens/parasites was found to result in both susceptibility and protection against cerebral malaria.

Protection against cerebral malaria was observed in CBA/J mice inoculated with attenuated third stage larva of *Brugia pahangi* (2) and in C57BL/6 mice infected with both *Plasmodium berghei* and murine leukemia virus (3). In contrast, others (4, 5) reported increased mortality of mice infected with *Leishmania mexicana* and *Plasmodium yoeli* or *Plasmodium inuei* and hepatitis B virus.

Also, the outcomes of co-infection in schistosoma parasite with other pathogens/parasites are diverse. Susceptible AKR mice acquired the capacity to resolve *Trichuris muris* infection when co-infected with *Schistosoma mansoni* (6) whereas increased parasitaemia of *Plasmodium chabauddi* was seen in malaria resistant C57BL/6 mice concurrently infected with both *S. mansoni* and *P. chabauddi* (7) Marshall *et al* (8) concluded that *S. mansoni* infection caused a significantly higher mortality in *Toxoplasma gondii* infected C57BL/6mice.

Before now, co-existence or immunological studies in humans infected with both *Plasmodium spp* and *Schistosoma spp* was not considered. This is important considering increased treatment failure being recently experienced in malaria and schistosomiasis subjects. Therefore, the present study determined leucocyte migration, ability to kill *Candida* and to produce reactive oxygen intermediates in

Nigerian school children having urinary schistosomiasis (USS) with or without malaria parasites. Such knowledge will be beneficial in rational design of treatment programme and optimization of vaccination protocols.

## MATERIALS AND METHODS

### *Subjects*

The study was approved by the Parent Teachers Association of St John's Primary School, Mokola, Ibadan, Nigeria where the subjects were recruited. Informed consent was obtained from the pupils, parents/guardians of the pupils, teachers and head-teachers before sample collection. All subjects (n=147, age 6-14 years) gave consents of participation and were grouped as:

1. Children with USS only (USS-M) (n=54)
2. Children with both USS and asymptomatic malaria (USS+M) (n=18)
3. Children without USS but with asymptomatic malaria (M-USS) (n=46)
4. Non- infected and apparently healthy controls (n=29).

### *Diagnosis of USS*

USS was diagnosed by identification of terminally spined eggs of *S. haematobium* in urine sediments following centrifugation at 2000 rpm for 5 minutes. The urine sample was obtained from each subject in a clean 50 ml plastic tube with the assistance of the class teachers between 10.00hrs-12.00hrs after brief exercise. The sediment was examined using 40 X objective lens of a binocular microscope (Wild Heerbrugg).

### *Diagnosis/classification of malaria*

The thick blood films on glass slides were stained with 4% Giemsa stain and examined for 100 high-power fields under oil-immersion objective lens. All stages of malaria parasites seen were counted and the densities recorded as number of parasites per 200 white blood cells (WBCs). Children with no malaria detected in their blood samples and without the eggs of *S. haematobium* in their urine samples were considered as controls.

### *Exclusion criteria*

Children with HbSS or HbAS blood genotype as confirmed by the method of Marengo-Rowe (9). Also excluded were those on antimalaria or anti-schistosoma chemotherapy between 1-30 days to the time of sample collection.

### *% Leucocyte migration index (%MI)*

The method described by Hudson and Hay (10) was followed. Five ml of heparinized blood was mixed with equal volume of 3% dextran solution. The capillary tubes filled with packed white blood cells were anchored into migration chamber containing either medium (15% foetal calf serum) or antigen (PPD)-medium solution (1:20 dilution). A drop of streptomycin was added to each well and incubated at 37°C in $CO_2$ for 18 hours. The percentage MI due to the antigen was calculated thus: % MI=T/C X 100, where C is the area of migration in the medium alone and T is the area of migration in antigen solution. The migration index value of 80% or less was taken as positive (11).

### *Preparation of neutrophil suspension*

Neutrophil suspension was prepared from fresh unclotted whole blood as described by Strober (12). Five ml of blood was mixed with equal volume of 6% dextran in Kreb Ringers solution at 20°C for 45 minutes. The leucocyte-rich plasma was gently layered on 5ml Ficoll-Hypaque

solution and spun at 1400rpm for 40 minutes leaving neutrophil / red blood cells pellet. The RBCs were lysed with 10ml cold 0.2% NaCl for 30 seconds, after which 10ml ice cold 1.6% NaCl was added to restore the isotonicity. The neutrophil number was adjusted to 5 X $10^6$ cells / ml.

***Percentage Candidacidal Index (%CI)***

The percentage CI was determined using the ability of neutrophils to kill *Candida albicans* as previously described (14). A saline-wash concentrated suspension of a 24-hr culture of *C. albicans* was made in Krebs Ringer solution. This was adjusted to 5 x $10^6$ cells/ml of Krebs Ringer solution and the viability of the cells was confirmed to be 95% by the Trypan blue dye. To a mixture of 0.25 ml of autologous plasma, 0.25ml of 5 x $10^6$ neutrophil suspension, and 0.25 ml of Krebs Ringer solutions; Candida (0.25 ml at 5 X $10^6$/ml of Krebs Ringer solution) was added.

A similar set up was made for the control tube except that neutrophil suspension was omitted. The tubes containing the mixture were incubated for 1hr with shaking at every 15 minutes. At the end of this period, 0.25ml of 2.5% sodium deoxycholate was added to each mixture to lyse neutrophil but not *Candida*. Four ml of 0.01% of methylene blue was added for 10 minutes to stain dead *Candida*. This was carefully removed leaving about 0.5ml to resuspend the organism. The % of dead *Candida* was determined using haemocytometer.

***Nitroblue tetrazolium (%NBT) dye reduction***

The method described by Strober (14) was followed. A drop of 10g/ml Phorbol myristate acetate was spread and dried on a glass coverslip. A drop of non-coagulated blood was added, incubated at 37°C for 45 minutes in humidity chamber until clot and 2 drops of NBT were added for 20 minutes. This was fixed with 100% methanol for 1 minute and counterstained with Safranin for 10 minutes. The % NBT positive cells in a total 200 neutrophils were determined using microscope at 40 X objective. Neutrophils that had undergone respiratory burst showed a granular blue black cytoplasmic formazan staining were taken as NBT-positive. NBT-negative neutrophils exhibited no such cytoplasm staining.

***Cell viability test***

Cell viability was determined by mixing 10µl of cell suspension with 10µl of 0.5% Trypan blue and 180 µL of Krebs Ringer solution. The number of cells excluding the stain was expressed as a percentage of the total number of cells counted. Cell suspension used in this study had viability greater than 90%.

***Statistical analysis***

Statistical methods employed in the analysis of data generated include mean, standard deviation, Student's t-test and Chi-square (2 X 2 Contingency) test.

**RESULTS**

The overall prevalence of subjects with co-infection of urinary schistosomiasis and asymptomatic malaria (USS+M) in the study population was 12% while the prevalence of subjects with asymptomatic malaria without urinary schistosomiasis (M-USS) was 31%. Malaria parasite numbers were lower in USS+M subjects (23-2924 malaria parasites numbers per µl blood) compared with M-USS subjects (63-4942 malaria parasites per µl blood). Statistical analysis showed significant difference. The most prevalence specie of *Plasmodium*

among M-USS or USS+M subjects was *P. falciparum* followed by *P. malariae*. Few of the M-USS subjects had *P. ovale* only (4%) or *P. ovale* + *falciparum* (2%). None of the USS+M subjects had *P. ovale* only, *P. falciparum* + *P. ovale* or *P. falciparum* + *P. malariae* (Table 2). Table 3 shows that mean % MI was significantly reduced in M-USS subjects, USS-M and USS+M compared with the controls. % MI was least in USS+M subjects, NBT dye reduction index was similar in M-USS subjects and the controls, but the value in USS+M was the highest (Table 3). % CI was comparable in USS-M, USS+M and the controls but the value was least in M- USS subjects (Table 3).

**Table 1: The prevalence of asymptomatic malaria, USS or both among pupils attending St John's Primary School, Ibadan, Nigeria**

| | | USS | | |
|---|---|---|---|---|
| | | **With** | **Without** | **p** |
| **Asymptomatic Malaria** | **With** | 18(12%)<br>109 (23-2624)* | 46(31%)<br>319(63-4942)* | <0.001 |
| | **Without** | 54 (37%) | 29(20%) | <0.001 |

* = Mean and (range) of malaria parasite number per µl of blood

**Table 2: The prevalence of different species of Plasmodium in M-USS and USS+M subjects**

| Subjects | *P. falciparum* | *P. malariae* | *P. ovale* | *P. falciparum* + *ovale* | *P. falciparum*+ *malariae* |
|---|---|---|---|---|---|
| M-USS | 33(72%) | 5(11%) | 2(4%) | 1(2%) | 5(11%) |
| USS+M | 14(77%) | 4(23%) | 0(0%) | 0(0%) | 0(0%) |

$X^2 = 1.82$, $p > 0.10$

**Table 3: Leucocyte migration indices (%MI), Candidacidal index (%CI) and NBT dye reduction index (%NBT) in M-USS, USS-M, USS+M compared with the controls**

| Subjects | n | %MI | % CI | % NBT index |
|---|---|---|---|---|
| Controls | 29 | 69+/-28 | 26.2+/-6.2 | 86.9+/-10.4 |
| M-USS | 46 | 54+/-20.3* | 20+/-36* | 80.1+/-18.9 |
| USS-M | 54 | 55± 29.4* | 24.8± 5.9[b] | 88.0± 20* |
| USS+M | 18 | 48+/-15.9*[b] | 24.3+/4.1[b] | 89+/-17.3*[b] |

* Significantly different from the controls ($p<0.005$), [b]Significantly different from M-USS ($p<0.05$), *Significantly different from USS-M ($p<0.05$)

## DISCUSSION

The result of the present study indicates that malaria density is less in USS+M subjects (23-2624 malaria parasites/µl blood) compared with M-USS subjects (63-4942 malaria parasites/µl blood). Moreso, co-existence of *Plasmodium spp* and *S. haematobium* in same host alters leucocyte migration, neutrophil candidacidal activity and ability to generate reactive oxygen intermediates. This contradicts the results of Lewinsohn (13) and Lwin *et al* (14) that observed no change in malaria parasitaemia and activities of CD4+ T lymphocytes in mice infected with both *S. mansoni* and *Plasmodium* parasite.

In the present study, leucocyte migration was significantly reduced in all test subjects (particularly USS+M) compared with the controls. This may be a result of increased production of leucocyte migration inhibitory factor (LMIF). LMIF that inhibit random migration of leucocytes is produced by activated lymphocytes. Increased LMIF production retards leucocyte migration with resultant reduced % MI. This is supported by a report that LMIF has the ability to retain leucocytes at the site of infection or inflammation *in vivo* (11). From the present results, it could be proposed that lymphocytes in malaria or / and USS subjects produced excess LMIF and that leucocytes from them respond appropriately.

The percentage CI were similar in USS-M, USS+M and the controls indicating that *S. haematobium* infection do not alter candidacidal activity which is based on production of reactive oxygen species. Nitric oxide production has been proposed as important mechanism of protective immunity to malaria (14, 16) and schistosomiasis (17). In this study, the efficiency of superoxide generation as shown by % NBT index was significantly higher in USS+M compared with either the controls or M-USS subjects. The ability USS+M subjects to generate adequate amount of superoxide might have interfered with the growth of *Plasmodium*, thus low malaria parasite density in USS+M subjects. Adult worms of schistosome attract RBCs to cover their surfaces (18). These RBCs are lysed so as to release intracellular malaria parasites for phagocytosis. This is in support of low malaria parasitaemia in USS+M subjects.

The present study revealed that leucocyte phagocytosis is not adversely affected during co-infection of malaria with schistosomiasis but *Plasmodium* infection alone reduces neutrophil Candidacidal activity by reducing the production of reactive oxygen species.

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# THE PATTERN OF PACKED CELL VOLUME, PLASMA ELECTROLYTES AND GLUCOSE LEVELS IN PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM*

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**Fifty-two patients (27 males, 25 females aged 25 ± 18.4 years) with *Plasmodium falciparum* infection and 53 healthy control subjects (27 males, 26 females aged 28.3 ± 19.2 years) were recruited for the study. Plasma electrolytes ($Na^+$, $K^+$, $Cl^-$), glucose and $HCO_3^-$ were respectively analyzed colorimetrically and biochemically. There was an observed lower significant mean value of packed cell volume, $Na^+$, $HCO_3^-$ and glucose in *Plasmodium falciparum* infected subjects than the values obtained from the normal control subjects with $P < 0.05$. Higher significant mean value of $Cl^-$ and $K^+$ was observed in the test than the control subjects ($P < 0.05$). Significantly lower packed cell volume, $Na^+$, $Cl^-$, glucose and higher significant $K^+$ levels were observed in the test subjects aged 1-10 years than test subjects aged 11-72years with $P < 0.05$. This study further affirms the effects of *Plasmodium falciparum* infection on the pattern of packed cell volume, plasma electrolytes and glucose concentrations.**

**Keywords: Electrolytes, *Plasmodium falciparum*, Glucose, Packed Cell Volume**

## INTRODUCTION

*Plasmodium falciparum* is a mosquito-borne haemoprotozoan parasite causing falciparum, malignant tertian or subtertian malaria (1-3). The parasite invade all ages (old and young) of erythrocytes indiscriminately so that very high infection rates may occur and anaemia is most pronounced in falciparum infections with extensive and rapid destruction of red blood cell (1, 3). Haemolysis in malaria infection may be due to antigen-antibody plus complement reaction and excessive destruction of erythrocytes by the invading parasite (1). Haemolysis may also arise from autoimmune response or opsonization of the infected erythrocytes in *Plasmodium falciparum* (1).

Loss of potassium (the major intracellular fluid cation) from red cells to the extracellular fluid will raise the plasma level of potassium and promote extracellular acidosis (4). Low urinary concentration of chloride in the infected subjects have also been reported (1). This study was designed to measure the effect of *Plasmodium falciparum* infection on the pattern of packed cell volume (PCV) and the electrolytes ($Na^+$, $K^+$, Cl and $HCO_3^-$) in the extracellular fluid (plasma) of the infected subjects.

## MATERIALS AND METHOD

### *Subjects/Study area*

Fifty-two patients (27 males, 25 females aged 25 ± 18.4 years) with *Plasmodium falciparum* infection and 53 healthy control subjects (27 males, 26 females aged 28.3 ± 19.2 years) were recruited from the Medial Outpatient Department of Baptist Medical Center, Saki, Oyo State, Nigeria, for the study.

### *Sample collection/Materials*

Six to 10 milliliters of venous blood was collected from each subject and divided into 2 parts. One part was collected into Lithium heparinized tubes and used for blood film examination for malarial parasite and analysis of plasma electrolytes. The other part was preserved

in Fluoride-Oxalate anticoagulated bottles for the analysis of glucose.

***Method***

Thin and thick blood film Field's stain technique described by Cheesbrough (5) was used to identify *Plasmodium falciparum* in the blood film.

Plasma was extracted from the blood by centrifugation at 1500 rpm for 5 minutes. Plasma potassium was estimated by colorimetric method of Terri *et al* (6). Plasma sodium was estimated by colorimetric method of Maruna (7) and Trinder (8) using the kit of Teco Diagnostics (4925E, Hunter Ave, Anachem, CA 92807). Plasma chloride was analyzed by the colorimetric mercuric thiocyanate – iron III nitrate method using the kit of Quimica Clinica Applicada (SA, Amposta, Spain). Plasma bicarbonate was analyzed by the method described by Van Slyke (9).

Packed cell volume was determined by capillary tube method described by Cheesbrough (10) and plasma glucose was estimated by colorimetric glucose oxidase method of Trinder (11).

**RESULT**

The observed mean values of this study are as shown in Tables 1-3. The mean values of the PCV (28.1± 10.0%), $Na^+$ (137.7±5.5 mmol/L), $HCO_3^-$ (27.9±4.7 mmol/L) and glucose (86.8 ± 6.0 mg/dL) observed in the *Plasmodium falciparum* infected subjects were significantly lower than the mean values obtained from the control/normal subjects of 34.8 ± 5.2%, 140.5 ± 4.7mmo/L, 29.8 ± 1.2mmo/L and 92 ± 8.3 mg/dL, respectively ($p < 0.05$).

A higher significant mean value of $K^+$ (5.5 ± 0.83 mmol/L) and $Cl^-$ (109.3 ± 6.0 mmol/L) were observed in the *Plasmodium falciparum* infected subjects than the mean values of $K^+$ (4.1 ± 0.38 mmol/L) and $Cl^-$ (99.0 ± 1.43 mmol/L) observed in the normal control with $p < 0.05$ (Tables 1 and 2).

Considering Tables 1 and 3, there was an observed lower significant mean values of PCV (15.5 ± 4.9%), $Na^+$ (133.5 ± 6.0 mmol/L), $Cl^-$ (105.1 ± 6.5 mmol/L) and glucose (81.33 ± 3.4 mg/dL) in Plasmodium infected subjects aged 1-10 years compared with the mean values of PCV (33.3 ± 6.4%) and glucose (89.1 ± 5.3 mg/dL) obtained from the Plasmodium infected subjects aged 11-72 years with $p < 0.05$. A higher significant mean value of potassium ($K^+$) was observed in the *Plasmodium falciparum* infected subjects aged 1-10 years than infected subjects aged 11-72 years (6.4 ± 0.49 mmol/L versus 5.2 ± 0.7 mmol/L) with $p < 0.05$.

These was no statistical significant difference in the mean values of $HCO_3$ in *Plasmodium falciparum* infected subjects aged 1- 10 years compared with the infected subjects aged 11-72years with $p > 0.05$ ($HCO_3^-$ = 28.1 ± 4.9 versus 30.2 ± 3.4 mmol/L).

**Table 1: The mean ± standard deviation of PCV, plasma levels of electrolytes and glucose in *Plasmodium falciparum* infected patients and normal subjects.**

| | | PCV | $K^+$ | $Na^+$ | $HCO_3^-$ | $Cl^-$ | Glucose |
|---|---|---|---|---|---|---|---|
| Test subjects (n = 52) | X ± SD | 28± 10.0 | 5.5± 0.83 | 137.7± 5.5 | 27.9± 4.7 | 109.3± 6.0 | 86.8± 6.0 |
| Control-subjects(n=53) | X± SD | 34.8± 5.2 | 4.1± 0.38 | 140.5± 4.7 | 29.8± 1.2 | 99.0± 1.4 | 92.0± 8.3 |
| *P. falciparum* infected 1-10 years (n = 15) | X± SD | 15.5± 4.9 | 6.4± 0.49 | 133.5± 6.0 | 28.1± 4.9 | 105.1± 6.5 | 81.3± 3.4 |
| *P. falciparum* infected 11-72 years (n = 37) | X± SD | 33.3± 6.4 | 5.2± 0.7 | 139.4± 4.3 | 30.2± 3.4 | 111.5± 4.1 | 89.1± 5.3 |

**Table 2: The values observed in the test subjects versus control**

| | PCV | $K^+$ | $Na^+$ | $HCO_3^-$ | $Cl^-$ | Glucose |
|---|---|---|---|---|---|---|
| "T" value | 4.1 | 10.8 | 2.8 | 4.0 | 12.1 | 2.6 |
| "P" value | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |

**Table 3: The observed values in test subjects aged 1-10 years versus the mean values for those aged 11-72 years**

| | PCV | $K^+$ | $Na^+$ | $HCO_3^-$ | $Cl^-$ | Glucose |
|---|---|---|---|---|---|---|
| "T" value | 6.6 | 7.0 | 3.4 | 1.52 | 3.5 | 6.3 |
| "P" value | < 0.05 | < 0.05 | < 0.05 | > 0.05 | < 0.05 | < 0.05 |

## DISCUSSION

The observed significantly lower packed cell volume (PCV) and higher potassium ($K^+$) in the *Plasmodium falciparum* infected subjects than the normal subjects can be attributed to the massive destruction of the infected erythrocytes by the organism. This will in effect lower the PCV and increase the level of $K^+$ in the plasma as a result of the influx of this major intracellular cation from the intracellular to the extracellular fluid (1, 3, 4, 5, 12).

Significantly lower $Na^+$, $HCO_3^-$ and higher $Cl^-$ levels in the test subjects than the normal control are attributable to the fact that high level of $K^+$ can directly stimulate the release of aldosterone from adrenal gland and also increases the delivery of $HCO_3^-$ to the cortical collecting duct by inhibiting $HCO_3^-$ reabsorption in the proximal tube thereby lowering the $HCO_3^-$ / $Na^+$ level (13).

The lower significant mean sodium level obtained from the test subjects than the control can also be attributed to vomiting and haemolysis which causes raised plasma potassium level thereby forcing more $Na^+$ back to the cell to maintain electrochemical neutrality (2). The observed higher chloride level in the test than the control can also be attributed to the fact that the urinary concentration of chloride is always low in *Plasmodium falciparum* infection, which may be due to $Cl^-$ retention (1).

Glucose level observed in the Plasmodium infected subjects, which was lower than the normal control, is consistent with the reports of Maruna (7) and Warrell *et al* (12), in which low

glucose level is associated with the infected subjects.

The observations in this study in the subjects' aged 1-10years considering the lower significant packed cell volume and glucose, and higher potassium level compared to the test subjects aged 11-72years, agrees with the reports of Terri and Sesin (6), Maruna (7), and Warrell *et al* (12) that the disease is more severe in children, who usually present with anaemia and hypoglycaemia.

This study in essence has affirmed the observations of previous workers of the effects of *Plasmodium falciparium* infection on the packed cell volume, plasma electrolytes and glucose, in patients seen in this locality.

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# PREVALENCE OF MALARIA PARASITAEMIA IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT JOS UNIVERSITY TEACHING HOSPITAL, NIGERIA

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**The prevalence of malaria parasitaemia in 200 pregnant women attending the antenatal clinic (ANC) of Jos University Teaching Hospital (JUTH) between April and June 2003 was determined. Geimsa-stained thick and thin blood films were examined microscopically for malaria parasites; the parasite densities were determined on the thick films. Eighteen (9%) of the women were positive for malaria parasites and only *Plasmodium falciparum* was encountered in the study. Pregnant women in the 15-20 year age group recorded the highest prevalence of 16%, closely followed by the age group 21-25 years with 15.2%. The 26-30, 31-35, 36-40 and 41-50 year age groups recorded 6.7%, 4.5%, 4.1% and 0% prevalence rates respectively. Women in their first trimester recorded 13.3% as against 10.2% and 3.8% for the second and third trimester respectively. The primigravidae had a prevalence of 12.9% as against 7.2% for multigravidae. Most of the women with malaria parasitaemia (89%) had parasite densities of less than 1000/μL of blood. The low prevalence of malaria parasitaemia in the ANC women is attributed to the regular prophylactic malaria therapy and the impacts of the health talks normally given to pregnant women during routine antenatal visits**

**Keywords: Malaria, pregnancy, prevalence, prophylaxis**

## INTRODUCTION

Malaria and pregnancy are two conditions which have an impact on one another. Globally, malarial infection during pregnancy is a major health problem, and the management is becoming increasingly difficult and controversial as multiple-drug resistance is emerging. The hormonal and immunological changes brought about by pregnancy aggravate malaria. The increased nutritional needs and the immunodepressant action of certain hormones (mainly cortisol) lead to a state of immunodepression (1, 2), which may affect the functions of T-lymphocytes (3).

Plasmodium infection is a very common occurrence in Africa and several studies have reported the relationship between malaria and pregnancy and low birth weight, anaemia, splenomegaly and congenital transmission (4, 5). Low birth weight is a major determinant of infant mortality. In endemic areas, pregnant women seem to lose their immunity against malaria parasite and are more likely to develop heavy parasitaemia towards the end of the second trimester. This is more frequent during the first and second pregnancy and rare in multigravidae (4).

Susceptibility to infection and the severity of clinical manifestations are determined by the level of immunity during pregnancy which in turn depends on the intensity and stability of malaria transmission (6). It has been reported by several researchers that primigravidae usually have a higher prevalence of malaria infection when compared to multigravidae (7-10).

There have been numerous trials of chemoprophylaxis in pregnancy in malaria holoendemic areas in an attempt to reduce the incidence of small for gestation age (SGA) infants (11-13). The choice of the

agent for treatment of malaria and prophylaxis will depend on the drug resistance pattern in an area and safety in pregnancy. In general, the preferred agent for therapy in pregnancy is chloroquine if the parasites are sensitive. For chloroquine resistant malaria, quinine combined with clindamycin is the treatment of choice in pregnancy. Other drug combinations should be considered as recommended by the CDC (14).

This study determined the prevalence of malaria parasitaemia in pregnant women in order to assess the effect of routine malaria prophylaxis given in ANC of JUTH.

**SUBJECTS AND METHOD**

A multi-stage sampling method was used to select 200 pregnant women attending ANC of Jos University Teaching Hospital from April to June 2003. Information with respect to age, gestational age and parity were obtained from each of the studied subjects.

Two milliliters of blood was collected from each subject by venipunctures and dispensed into EDTA bottles. Thick and thin blood films were made on the same slide, labelled and stained appropriately using Giemsa's staining method (15). The stained films were examined systematically for malaria parasites and 200 leucocytes were counted before declaring the film negative for malaria parasite. A count of both the sexual and asexual forms of the parasite was done for the estimation of parasite density (16).

**RESULT**

The overall prevalence of 9% for malaria parasitaemia was recorded among the pregnant women in Jos University Teaching Hospital as shown in Table 1. The prevalence in relation to age groups of the women is also shown in Table 1.

**Table 1: Prevalence of malaria parasitaemia in relation to the age of women attending ANC in JUTH**

| Age group | No Examined | No positive | % positive |
|---|---|---|---|
| 15-20 | 25 | 4 | 16.0 |
| 21-25 | 46 | 7 | 15.2 |
| 26-30 | 60 | 4 | 6.7 |
| 31-35 | 44 | 2 | 4.5 |
| 36-40 | 24 | 1 | 4.1 |
| 41-45 | 1 | 0 | 0.0 |
| **Total** | **200** | **18** | **9.0** |

The 15-20 year age group recorded the highest prevalence of 16%, closely followed by the age group 21-25 years with 15.2%. The 26-30, 31-35, 36-40 and 41-50 year age groups recorded 6.7%, 4.5%, 4.1% and 0% prevalence rates respectively.

**Table 2: Prevalence of malaria parasitaemia in relation to gestational period of women attending ANC in JUTH**

| Gestation period | No examined | No positive | % positive |
|---|---|---|---|
| 1st trimester | 30 | 4 | 13.3 |
| 2nd trimester | 117 | 12 | 10.2 |
| 3rd trimester | 53 | 2 | 3.8 |

Table 2 shows the prevalence in relation to gestation period. Women in their first trimester recorded 13.3% as against 10.2% and 3.8% for the second and third trimester respectively. Primigravid women had a prevalence of 12.9% as against 7.2% for multigravidae (Table3).

**Table 3: Prevalence of malaria parasitaemia in relation to parity of women attending ANC in JUTH**

| Parity | No examined | No positive | % positive |
|---|---|---|---|
| Primigravidae | 62 | 8 | 12.9 |
| Multigravidae | 138 | 10 | 7.2 |

The prevalence in relation to malaria parasite density is shown in Table 4. Most of the women with malaria parasitaemia (89%)

had parasite densities of less than 1000/μL of blood.

**Table 4: Malaria parasite densities in women with parasitaemia**

| Parasite density (/μL) | No positive | % positive |
|---|---|---|
| < 1000 | 16 | 89.0 |
| 1000-2000 | 1 | 5.5 |
| 2000-3000 | 0 | 0.0 |
| 3000-4000 | 1 | 5.5 |

## DISCUSSION

This study has shown a relatively low prevalence of malaria parasitaemia in women attending antenatal clinic of Jos University Teaching Hospital. This low prevalence may be due to the fact that most of the pregnant women registered early for antenatal care and were immediately placed on chemoprophylaxis. They were also usually enlightened during routine antenatal visits on how to prevent the scourge of malaria during pregnancy. Most of them therefore took the prophylactic drug meticulously; and most use mosquito nets and chemical sprays, which reduced the man-vector contact.

The study also confirmed that as women get older, their resistance to malaria becomes higher due to improvement in host immunity. Women in the first trimester had the highest prevalence and this implies that pregnant women should register early for ANC, so that associated complications of malaria in pregnancy can be reduced. The primigravids had higher malaria parasitaemia than the multigravids and this is probably due to the suppressive action of hormones on cell-mediated immunity (17). Most of the patients with positive parasitaemia had low parasite density, an indication of good compliant with the prophylactic drugs. The two patients with high parasite densities had clinical malaria at the time of blood sample collection and were not yet on any antimalaria therapy.

From the result of the study, there is a good evidence to show that the effort at reducing the mortality and morbidity associated with malaria in pregnancy at JUTH is yielding good result. However, majority of these groups of women live in rural areas where access to qualitative ANC care is lacking and where man-vector contact is high. Treatment and prophylaxis for malaria in pregnancy should, as a matter of concern, be free at all levels to reduce the scourge of this disease in pregnancy.

In conclusion, malaria represents additional risk in pregnancy especially in non-immuned or partially immuned women. The disease is particularly severe in the pregnant women, and can as a result of transplacental transmission, cause congenital malaria, which is associated with high neonatal mortality. The economic burden on household resulting from illness or death of a mother is devastating and the need for prompt diagnosis and effective treatment is a high priority for this high-risk group. Unfortunately, most of the newer antimalarial drugs are contraindicated in pregnancy because of possible toxicity to the fetus or their teratogenic potentials.

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# SPLEEN WEIGHT, LIVER WEIGHT AND LEVELS OF CIRCULATING IMMUNE COMPLEXES IN VITAMIN DEFICIENT MICE INFECTED WITH *PLASMODIUM BERGHEI*

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**Three groups of mice viz: well fed mice, vitamin deficient mice and vitamin deficient *Plasmodium berghei* infected mice were studied. In these groups of mice, the weights of the liver and spleen were determined using a weighing balance and the levels of circulating immune complexes measured spectrophotometrically using polyethylene glycol precipitation method. The mean spleen weight, liver weight and CICs of vitamin deficient mice or vitamin deficient *P. berghei* infected mice were reduced compared with those of well-fed mice. However, the reduction in spleen weight was significant in vitamin deficient mice from day 15-post vitamin deficiency compared with well-fed mice. Also, the reduction in liver weight was significant in vitamin deficient mice at day 5- and day 10-post vitamin deficiency compared with well-fed mice while the reduction in liver weight was significant in vitamin deficient *P. berghei* infected mice at day 5-, day 10-, day 15- and day 20- post *P. berghei* infection compared with well-fed mice. The reductions in the levels of CICs were significant in both vitamin deficient mice and vitamin deficient *P. berghei* infected mice compared with well-fed mice from day 5-post *P. berghei* infection or day 5-post vitamin deficiency. The observed decreased CICs in vitamin deficient mice accompanied by reduction in liver and spleen weights showed that vitamin is essential in mounting effective immune response against malaria.**

## INTRODUCTION

Malaria infection has been associated with great morbidity and mortality with severe consequential effect on children under five year of age and pregnant women (1) living in malaria endemic regions of the world. In 1999, it was estimated that there were some 261 million cases of malaria and 870,000 deaths in malaria endemic areas (2).

Several investigators have provided evidence that cell-mediated immunity and humoral immunity act in concert or sequentially to control and clear a blood-stage malaria infection (3, 4). The prolonged release of different types of antigens in large quantity into the circulation induces a massive immunological response. The endo-erythrocytic stage of malaria parasite when present in numbers induces a lot of immunological responses. This commences with proliferation of phagocytes of the reticuloendothelial system particularly in the spleen, liver and bone marrow. These cells phagocytose parasitized and unparasitized red blood cells, free malaria parasites and malaria pigments. The action is controlled by thymus-derived lymphocytes (4, 5).

During the early phase of infection, both reactive oxygen and reactive nitrogen metabolites produced by non-specific immune cells participate in controlling the primary parasitaemia (6). This initial CD4+ Th1 cell response is followed by a switch to Th2 cytokine production, stimulating antibody-dependent mechanisms involved in the final control and clearance of the parasite (3, 4, 5). Malaria antigen causes proliferation of splenic lymphocytes that leads to macrophage proliferation and active immunoglobulin synthesis (3, 4).

Transient splenomegaly occurs with acute malaria in children and non-immune adults. In malaria endemic areas, a chronic

form of splenomegaly can be found in children (7). Persons with hyper-reactive malaria splenomegaly are immune adults in malarious areas who have gross and chronic splenomegaly, with an overproduction of IgM, high levels of malaria antibody and circulating immune complexes, and a moderately enlarged liver with hepatic sinusoidal lymphocytosis (8).

Resistance to malaria depends on nutritional status of the host among others factors. Most studies on nutritional effects on malarial severity were concentrated on protein energy malnutrition (9). There are scarce literature on vitamins and malaria severity. Deficiency of vitamin A results in reduced weight of thymus and decreased lymphocyte proliferation in response to mitogens (20-22). Feeding diets deficient in vitamin B1, or B2 decreases antibody responses in rodents. Vitamin B6 or vitamin C deficiency results in marked reduction in cell-mediated immune responses and decrease in production of thymic inductive factors. Vitamin E is associated with reduction in T cell, NK cell and phagocyte responses (11, 12).

The importance of liver and spleen in controlling *Plasmodium* infection and the involvement of vitamins in disease control cannot be underrated. This study is designed to find out the effects of vitamin deficiency on the immune system of experimental animal (mice) infected with *P. berghei*. This was carried out by determining the levels of soluble circulating immune complexes and weights of spleen and liver of normal mice, vitamin deficient and vitamin deficient *P. berghei* infected mice.

## MATERIALS AND METHOD

Two hundred and twenty-five albino mice (6-10 weeks of age and weighing 9-14kg) were distributed into well-fed mice (Group 1), vitamin-deficient mice (Group 2) and vitamin-deficient *P. berghei* infected mice (Group 3). The animals in Group 1 were fed with complete normal diet while animals in Groups 2 and 3 were fed with vitamin deficient diet. The diet composition is as shown below.

| Ingredients | Normal complete diet | Vitamin deficient diet |
|---|---|---|
| Maize starch | 71.8g | 71.8g |
| Casein | 18g | 18g |
| Palm oil | 5g | 5g |
| Mineral mixtures | 5g | 5g |
| Vitamin mixtures | 0.2g | nil |

Vitamin deficiency in the mice of Groups 2 and 3 commences on weaning (4 weeks post birth). 3.0 x $10^8$ *P. berghei* parasitized red blood cells were inoculated intra-peritoneally at the volume of 0.2ml into experimental mice. Organs (liver and spleen) and blood samples were obtained from etherized dissected mice on days 1, 5, 10, 15, 20 post *P. berghei* infection and/or vitamin deficiency. The weights of the spleen and liver were taken using a sensitive weighing balance while the level of circulating immune complexes were determined as previously described using polyethylene glycol precipitation method (13).

## RESULTS

Table 1 compared the spleen weights in the 3 groups of mice. There was no significant reduction in the spleen weights of vitamin deficient *P. berghei* infected mice compared with well-fed mice. Significant reduction in the weight was noted in vitamin deficient mice compared

with well-fed mice from day 15-post vitamin deficiency. The weights of the liver of vitamin deficient mice showed significant reduction only at days 5 and 10 post vitamin withdrawal while the liver of vitamin deficient *P. berghei* infected mice showed significant reduction from day 5 to the end of the end of the study when compared with well-fed mice (Table 2). In Table 3, the levels of CICs were reduced in both of vitamin deficient mice and vitamin deficient *P. berghei* infected mice compared with well-fed mice.

**Table 1: Comparison of spleen weights in well-fed mice, vitamin deficient mice and vitamin deficient-*P. berghei* infected mice.**

| Groups | n | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 |
|---|---|---|---|---|---|---|
| 1 | 5 | 0.06±0.23 | 0.06±0,32 | 0.07±0.03 | 0.07±0.52 | 0.07±0.35 |
| 2 | 5 | 0.06±0.001 | 0.05±0.002 | 0.05±0.002 | 0.05±0.30 | 0.05±0.30 |
| 3 | 5 | 0.06±0.20 | 0.05±0.33 | 0.05±0.35 | 0.06±0.50 | 0.05±0.22 |
| t-, p-values[a] | | 0.19, >0.20 | 0.49,>0.02 | 0.30,>0.20 | 2.30,<0.05 | 2.2,<0.05 |
| t-, p-values[b] | | 0.56, >0.20 | 1.06,>0.02 | 0.93,>0.20 | 0.80,>0.02 | 1.2,>0.10 |

a = Well-fed mice compared with vitamin deficient mice.
b = Vitamin deficient-*P. berghei* infected mice compared with normal mice.
Group 1 = Well-fed mice.
Group 2 = Vitamin deficient mice.
Group 3 = Vitamin deficient-*P. berghei* infected mice.

**Table 2: Comparison of liver weights in well-fed mice, vitamin deficient mice and vitamin deficient *P. berghei* - infected mice**

| Groups | n | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 |
|---|---|---|---|---|---|---|
| 1 | 5 | 0.76±0.26 | 0.70±0.61 | 0.72±0.11 | 0.75±0.11 | 0.79±0.11 |
| 2 | 5 | 0.66±0.03 | 0.53±0.51 | 0.51±0.10 | 0.67±0.05 | 0.59±0.24 |
| 3 | 5 | 0.66±0.02 | 0.50±0.53 | 0.45±0.15 | 0.56±0.10 | 0.55±0.12 |
| t-, p-values[a] | | 0.00,>0.20 | 2.89,<0.02 | 3.30,<0.01 | 1.30,>0.2 | 0.87,>0.2 |
| t-, p-values[b] | | 0.56, >0.20 | 4.06,<0.01 | 4.93,<0.01 | 3.80,<0.01 | 3.42,<0.01 |

a = Well-fed mice compared with vitamin deficient mice.
b = Vitamin deficient-*P. berghei* infected mice compared with normal mice.
Group 1 = Well-fed mice.
Group 2 = Vitamin deficient mice.
Group 3 = Vitamin deficient-*P. berghei* infected mice.

**Table 3: comparison of circulating immune complexes in well fed mice, vitamin deficient mice and vitamin deficient *P. berghei* -infected mice**

| Groups | n | Day 1 | Day 5 | Day 10 | Day 15 | Day 20 |
|---|---|---|---|---|---|---|
| 1 | 5 | 5.70 ± 0.57 | 6.00 ± 2.3 | 6.0 1± 3.2 | 6.50 ± 3.1 | 6.60 ± 4.1 |
| 2 | 5 | 5.21 ± 1.93 | 2.70 ± 3.2 | 0.5 ± 0.5 | UD | UD |
| 3 | 5 | 5.11 ± 1.54 | 2.80 ± 3.5 | 1.5 ± 0.5 | UD | UD |
| t-, p-values[a] | | 0.59,>0.20 | 3.20,<0.01 | 4.20,<0.01 | UD | UD |
| t-, p-values[b] | | 0.66, >0.20 | 3.60,<0.01 | 3.93,<0.01 | UD | UD |

a = Well-fed mice compared with vitamin deficient mice.
b = Vitamin deficient *P. berghei* infected mice compared with normal mice.
Group 1 = Well-fed mice.
Group 2 = Vitamin deficient mice.
Group 3 = Vitamin deficient *P. berghei* infected mice

**DISCUSSION**

Malnutrition and malaria affects both cellular and humoral immune systems (5, 11, 12). Malnutrition has been known to occur in many children especially in areas endemic for malaria (9). Inhibition of protein synthesis, disturbance of normal function of T lymphocytes and macrophages are possible methods by which *P. berghei* suppresses the immune system (3, 4). The present study gives an insight to the effects of vitamin deficiency and malaria infection on liver weight, spleen weight and levels of circulating immune complexes in mice. Sowunmi (7) reported increase spleen weight during malaria.

The result of the present study showed decrease in spleen weight in vitamin deficient mice or vitamin deficient *P. berghei* infected mice compared with well-fed mice. Therefore in vitamin deficiency vital organs like spleen are readily affected. Suskind (14) previously observed this. This observation may be accounted for by the fact that vitamins are essential for the synthesis of proteins and are needed for maintainance and growth of body tissues. The clearance of antigens from the blood has been considered indicative of non-specific activity of spleen. Also, spleen is the site of contact between antigens and immunocompetent cells (15).

Malaria parasite destroys red blood cells that are usually removed from circulation by spleen; therefore the weight of the spleen is expected to be increased in *P. berghei* infected mice. This is corroborated by WHO (16) report that treatment of malaria infection results in return of the spleen size to normal.

The study also showed that in vitamin deficient *P. berghei* infected mice, the reduction in the weight of the liver was significant earlier (day 5 and day 10) than of the spleen (day 15). Withdrawals of dietary vitamins cause reduction in the synthesis of proteins by the liver because some vitamins are co-factors for the synthesis of proteins that are use for the repair of body tissues.

Reduced circulating immune complexes (CICs) observed in this study could be due to reduced formation but not rapid clearance. CICs are macromolecules consisting of immunoglobulins bound to different antigens. Malaria infection results in reduced bone marrow function (17), and vitamin deficiency cause reduced DNA synthesis (10, 12), lymphocyte proliferation and B lymphocyte function (10, 11). Since bone marrow produces B lymphocytes plasma cells that synthesize immunoglobulins, the effects of both vitamins and malaria could lead to reduced levels of immunoglobulins. Therefore low levels of antibodies will lead to reduced CICs formation.

This study revealed that vitamin deficiency causes reduction in CICs formation, spleen weight and liver weight in experimental animals and that these are aggravated by *P. berghei* malaria. It is therefore recommended that vitamin supplement be given during treatment of malaria.

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**ORIGINAL ARTICLE**

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# USE OF INJECTABLE ANTI-MALARIALS AMONG PATIENTS IN SELECTED HEALTH FACILITIES IN ILORIN, NIGERIA

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**Irrational use of injectable antimalarial is commonplace in developing countries. This descriptive survey was conducted to determine the prevalence of injectable antimalarials use and factors related to this practice in selected health facilities in Ilorin, Nigeria. A total of 356 outpatients were interviewed in the selected health facilities and available clinical records checked. Awareness of both oral and injectable antimalarials was fairly high among the respondents. Injectable antimalarial was the most preferred form by the patients. Request for injectable antimalarial was significantly more among educated patients and those attending private clinics and health centers. Among respondents, 90.3% had ever used injectable antimalarial. Use of injectable antimalarial irrespective of clinical indications was common practice. Rational practices in the prescription of antimalarial and promotion of oral therapy need to be widely encouraged among health workers in developing countries. This will reduce the hazards associated with unnecessary injections and also reduce cost.**

**Key words: Injectable antimalarial, use, health facilities.**

## INTRODUCTION

Malaria is an endemic disease in Nigeria like other sub-Sahara African countries. It accounts for highest morbidity and mortality in most health facilities in Africa (1-4). Because of the high burden of malaria, use of antimalarials in homes and health facilities is very frequent. Antimalarial drugs are available in oral, injectable and rectal preparations. Oral and injectable preparations are more frequently used for treating patients.

Rational use of injectable antimalarials is however a concern particularly in developing countries in view of the implications of injections in this part of the world. This study examines the prevalence of use of injectable and oral antimalarials and factors responsible for this practice among patients seen in selected health facilities.

## MATERIALS AND METHOD

This is a descriptive cross-sectional survey conducted in selected health facilities in Ilorin, Nigeria. The selected health facilities were the University of Ilorin Teaching Hospital, Children specialist hospital, Okelele health center and Temitope private clinic to represent the various health facilities available in Ilorin.

Proportionate random sampling was used to select 356 outpatients attending the selected health facilities. In the selected health facilities, systematic sampling was used to select respondents until the desired sample size in the facility was obtained. The selected patients were interviewed using semi-structured questionnaires designed to collect patients' demographic information, awareness, preference and use of antimalarials. Clinic records of the patients were examined where available to determine

probable reason for prescription of injectable antimalarial.

The data obtained was entered and analyzed with EPI-INFO version 6.04 computer software to produce frequency distribution and Chi-square analysis. Level of significance used was $p < 0.05$.

**RESULT**

A total of 356 patients were interviewed. One hundred (28.1%) were from General Hospitals, 46 (12.9%) from Private clinics, 51 (14.3%) from Primary Health Centres and 159 (44.9%) from Teaching Hospital. More than half (56.4%) of them had primary education and 27.3% had no formal education. Sixty percent of those interviewed were females and 40% were males. Patients' age ranged from 1-75 years, and mean age was 26 ± 15.71 years.

About three-quarters (74.0%) of those interviewed were aware of oral antimalarials while about two-third (65.7%) of the patients were aware of injectable antimalarials. About half of the respondents preferred the injectable antimalarials, 29.6% preferred oral form, 23.9% were satisfied with either oral or injectable forms. Significantly higher proportion of females than males knew about oral and injectable antimalarials and the awareness was also more with educated patients ($p < 0.05$).

About one-third (34.8%) reported ever requesting the doctor to prescribe injectable antimalarials for them. Request for injectable antimalarial was significantly higher among respondents with at least secondary education ($p < 0.05$). Preference for injectable antimalarial was least among respondents seen in General Hospitals (22.0%) and highest among those seen at health centres (68.9%).

Also, preference for injectable antimalarial was higher among those seen in private clinics (60.0%) than those seen at Teaching Hospital (50.3%). However, request for injectable antimalarial was significantly higher ($p < 0.05$) among respondents interviewed at health centers (70.0%) and private clinics (85.6%) than those in Teaching Hospital (31.6%) and General Hospital (8.1%).

Among the respondents, only 5.4% had never had antimalarials prescribed for them and 4.7% had never used antimalarials. Most of the respondents (91.1%) had ever used oral antimalarials and 90.3% had ever been given injectable antimalarials. Sixty percent of those

**Table 1: Awareness of antimalarials**

| Factor | Awareness of antimalarial | | | | | |
|---|---|---|---|---|---|---|
| | Oral antimalarial | | | Injectable antimalarial | | |
| **Sex** | **Yes** | **No** | **Total** | **Yes** | **No** | **Total** |
| Female | 162(83.1) | 33(16.9) | 195 | 140(86.5) | 50(13.5) | 190 |
| Male | 77(61.1) | 49(38.9) | 126 | 74(58.7) | 52(41.3) | 126 |
| **Total** | **239** | **82** | **321** | **214** | **102** | **316** |
| | $X^2 = 19.36$ p = 0.00001 | | | $X^2 = 7.73$ p = 0.0054 | | |
| **Level of Education** | | | | | | |
| None | 37(39.8) | 56(60.2) | 93 | 36(38.3) | 58(61.7) | 94 |
| Primary | 39(69.6) | 17(30.4) | 56 | 35(62.5) | 21(37.5) | 56 |
| Secondary | 47(85.5) | 8(14.5) | 55 | 39(76.5) | 12(33.5) | 51 |
| Tertiary | 128(93.4) | 9(6.6) | 137 | 115(86.5) | 18(13.5) | 133 |
| **Total** | **251** | **90** | **341** | **225** | **109** | **334** |
| | $X^2 = 86.9$ p < 0.0001 | | | $X^2 = 60.72$ p < 0.0001 | | |

interviewed currently suffer from malaria. Among them, equal proportion (45.7% each) had injectable and oral antimalarials prescribed and 8.7% did not know the form of antimalarial prescribed.

**Table2: Reason for prescribing injectable antimalarial**

| Reasons | No (%) |
|---|---|
| **Perceived reason For injection** (N=122) | |
| Acts faster | 76 (62.3) |
| For treatment | 32 (26.2) |
| Can not swallow | 2 (1.6) |
| Vomiting | 1 (0.8) |
| Others | 7 (5.7) |
| **Probable indication for Injectable** (N=149) | |
| Vomiting | 43 (28.9) |
| Severe Malaria | 15 (10.1) |
| Itching from tabs | 3 (2.0) |
| Difficulty in swallowing | 4 (3.0) |
| Others | 14 (9.4) |
| No reason | 78 (52.3) |

Almost two-third of those who gave perceived reasons why injectable antimalarial was prescribed, felt it was because it acts faster (Table 2). From the clinical records, the probable indication for prescribing injection was found in only 71(47.7%) out of 149 respondents. The educated respondents significantly prefer the use of injectable antimalarial than the non-educated or respondents with primary education, while those with no formal education were less likely to have preference for a particular form of antimalarial ($p < 0.05$). At least 80% of respondents across the various health facilities had ever had injectable antimalarial prescribed for them (Table 3).

**Table 3: Factors related to type of antimalarials used**

| **Factor** | **Antimalarial preference, request and use** | | | | |
|---|---|---|---|---|---|
| **Level of** | **Preferred form of antimalarials** | | | | |
| **Education** | **Both** | **Injectable** | **Oral** | **Total** | |
| None | 48(52.7) | 23(25.3) | 20(22.0) | 91 | |
| Primary | 13(25.5) | 21(41.2) | 17(33.3) | 51 | $X^2 = 71.3$ |
| Secondary | 5(9.8) | 26(51.0) | 20(39.2) | 51 | $p < 0.0001$ |
| Tertiary | 11(8.2) | 81(60.4) | 42(31.3) | 134 | |
| **Total** | **63** | **151** | **99** | **327** | |
| **Facility type** | | | | | |
| Gen. Hospital | 45(49.4) | 20(22.0) | 26(28.6) | 91 | |
| Private Clinic | 6(13.3) | 27(60.0) | 12(26.7) | 45 | $X^2 = 64.3$ |
| Health Centre | 4(8.9) | 31(68.9) | 10(22.2) | 45 | $p < 0.0001$ |
| Teaching Hosp | 26(16.5) | 79(50.3) | 52(33.1) | 157 | |
| **Total** | **67** | **157** | **100** | **338** | |
| | **Ever requested injectable antimalarials** | | | | |
| **Level of education** | **Yes** | **No** | **Total** | | |
| None | 21(22.6) | 72(77.4) | 93 | | |
| Primary | 10(17.9) | 46(82.1) | 56 | $X^2 = 23.72$ | |
| Secondary | 20(36.4) | 35(63.6) | 55 | $p < 0.0001$ | |
| Tertiary | 66(47.8) | 72(52.2) | 138 | | |
| **Total** | **117** | **225** | **342** | | |
| **Sex** | | | | | |
| Female | 66(33.5) | 131(66.5) | 197 | $X^2 = 0.11$ | |
| Male | 40(31.7) | 86(68.3) | 126 | $p = 0.743$ | |
| **Total** | **106** | **217** | **323** | | |
| **Facility type** | | | | | |
| Gen. Hospital | 8(8.1) | 91(91.9) | 99 | | |
| Private Clinic | 30(65.2) | 16(34.8) | 46 | $X^2 = 77.86$ | |
| Health Centre | 35(70.0) | 15(30.0) | 50 | $p < 0.0001$ | |
| Teaching Hosp | 50(31.6) | 108(78.4) | 158 | | |
| **Total** | 123 | **230** | 353 | | |
| | **Ever used injectable antimalarials** | | | | |
| **Facility type** | **Yes** | **No** | **Total** | | |
| Gen. Hospital | 80(83.3) | 16(16.7) | 96 | | |
| Private Clinic | 37(80.4) | 9(19.6) | 46 | $X^2 = 17.3$ | |
| Health Centre | 46(93.9) | 3(6.1) | 49 | $p = 0.0000$ | |
| Teaching Hosp | 152(96.2) | 6(3.8) | 158 | | |
| **Total** | **315** | **34** | **349** | | |

## DISCUSSION

Awareness of oral and injectable antimalarials was fairly high in the study population. This is expected in view of endemic nature of malaria in Nigeria. Given the high risk to malaria attack in this country and the fact that more than 90% of the respondents had ever had oral and injectable antimalarial prescribed for them, awareness of antimalarials should be much higher than found in this study. The fact that some adults do not know about antimalarials may be related to poor communication between patients and health workers concerning their ailments (5).

Over a third of respondents specifically request for injectable antimalarial when they were to receive treatment for malaria and the practice was found to be more prevalent among educated patients and those seen in private clinics and health centers. It appeared that patients attending private clinics and health centers communicate more with health workers there and therefore feel free to request their choice of antimalarial. The educated patients have also been known to communicate more with the health care workers and are also more likely to make request for preferred choice (5).

The most preferred form of antimalarial by the respondents was injectable antimalarial and the main reason for this was the desire for fast action. In this study, among the respondents currently suffering from malaria, equal proportion of the patients were prescribed injectable and oral antimalarial, however very few were found to have clinical indications like severe malaria or vomiting for injectable antimalarial. Over 80% of the patients had ever been prescribed injectable antimalarial. This suggests that prescribers tend to prescribe the form of antimalarial irrespective of clinical indication and probably to satisfy requests from their patients. Health workers do not rationalize the use of intramuscular injections (6). A study on the rationale for antimalarial prescription among prescribers in this setting is therefore desirable.

Frequent use of injectable antimalarial without clinical indications need to be discouraged in view of hazards related to injections particularly in developing countries (7-9). Intramuscular injection is a common reason for paralysis of the legs in African children. Although the practice of the use of the same needles for many patients has decreased, using the same syringe for many patients, with only rapid washing between, is still commonplace. Little attention to asepsis/antisepsis, lack of safety precautions and abuse of prescriptions also contribute to the transmission of diseases like hepatitis and HIV (6). The cost of using injectable antimalarials is certainly higher than with use of oral antimalarials. Cost consideration is also important in developing countries where affordability of health care by patients is relatively low (10, 11).

Rational practices in the prescription of antimalarials and promotion of oral therapy need to be widely encouraged among health workers in developing countries. This will reduce the hazards associated with unnecessary injections and also reduce cost. Patients understanding on the benefit of oral antimalarials use should also be promoted to reduce perceived benefits of injectable antimalarials and

ultimately reduce demand by patients for injectable antimalarials.

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**REVIEW ARTICLE**

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# EPIDEMIOLOGY OF MALARIA IN AFRICA

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**Malaria is a life threatening parasitic disease transmitted by female anopheles mosquitoes. There are four types of human parasites; *Plasmodium vivax, P. malariae, P ovale* and *P. falciparum*. *P. falciparum* and *P. vivax* are the most common and *P. falciparum*, the most deadly type of infection, is most common in sub-Saharan Africa. A large number of environmental factors affect the distribution, seasonality and transmission intensity of malaria. Rainfall provides breeding sites for mosquitoes and increases the humidity, which enhances their survival. While malaria is largely endemic in Africa, varying proportion of countries in the continent are at risk of endemic malaria. Today, approximately 40% of the world population, mostly those living in the world's poorest countries, is at risk of malaria. This is mostly in the tropical and sub-tropical regions of the world. There are at least 300 million acute cases of malaria each year globally resulting in more than a million deaths, around 90% of these occur in Africa, mostly young children. In areas of stable malaria transmission, very young children and pregnant women are the population at highest risk for malaria morbidity and mortality. The populations most at risk of epidemics are those living in highlands, arid and desert-fringe zones and those living in areas where successful control measures have not been consolidated or maintained.**

**Keywords: Epidemiology, Malaria, Africa**

## INTRODUCTION

Malaria is a life threatening parasitic disease transmitted by mosquitoes. It was once thought that the disease came from fetid marshes, hence the name 'mal aria' (bad air). In 1880, scientists discovered that the parasite was transmitted from person to person through the bite of female Anopheles mosquito. The agent transmitted was found to be a one-celled parasite called plasmodium.

There are four types of human malaria, *Plasmodium vivax, P. malariae, P. ovale* and *P. falciparum*. *P. falciparum* and *P. vivax* are the most common and falciparum the most deadly type of infection. *P. falciparum* is most common in sub-Saharan Africa (1). Malaria parasite enters the human host when an infected Anopheles mosquito takes a blood meal. Inside the human host, the parasite undergoes a series of changes as part of its complex life-cycle. Malaria symptoms appear 9-14 days after the bite of an infectious mosquito.

## EPIDEMIOLOGY

The disease was once widespread globally but it was successfully eliminated from many countries with temperate climates during the mid-twentieth century. Today, approximately 40% of the world population, mostly those living in the world's poorest countries, is at risk of malaria. This is mostly the tropical and subtropical regions of the world.

The vast majority of deaths occur in sub-Saharan Africa where malaria also presents major obstacles to social and economic development. There are at least 300 million acute cases of malaria each year globally resulting in more than a million deaths, about 90% of these deaths occur in Africa, mostly in young children (2, 3).

Malaria is Africa's leading cause of under-five mortality and contributes 10% of

the continent's overall disease burden. It accounts for 40% of public health expenditure, 30-50% of in-patients admissions and up to 50% of outpatient visits in areas with high malaria transmission (2, 4-6). In all malaria-endemic countries in Africa, 25-40% (average 30%) of all outpatient clinic visits are for malaria (with most diagnosis made clinically). In these same countries, between 20% and 50% of all hospital admissions are a consequence of malaria (7).

In Africa, most cases of malaria are diagnosed on the basis of clinical symptoms and treatment is presumptive rather than based on laboratory confirmation. Moreover, malaria parasitaemia is common among clinic attendees in many endemic cases, so that a positive laboratory result does not necessarily mean that the patient is ill with malaria. Routine reports of the number of malaria cases and deaths have limited value for comparison of the malaria burden between countries because of the variation in timeliness and completeness of reporting (7).

About 90% of all malaria deaths in the world today occur in Africa south of the Sahara. This is because the majority of infections in Africa are caused by *P. falciparum* (1, 8), the most dangerous of the four human malaria parasites. It is also because the effective malaria vector, the mosquito *Anopheles gambiae*, is the most widespread in Africa and most difficult to control (7, 9).

**PATTERN OF TRANSMISSION**

A large number of environmental factors affect the distribution, seasonality and transmission intensity of malaria. Rainfall provides breeding sites for mosquitoes and increases the humidity, which enhances their survival. Temperature affects the transmission cycle of malaria. At temperature below 22°C, the determining factor is the number of mosquitoes surviving the parasite incubation period, which takes 55 days at 18°C and ceases at around 16°C (10).

Malaria affects the lives of almost all people living in the area of Africa defined by the southern fringes of the Sahara desert in the North, and latitude of about 28° in the South. Most people at risk of the disease live in areas of relatively stable malaria transmission; infection is common and occurs with sufficient frequency that some level of immunity develops (7).

While malaria is largely endemic in Africa, varying proportion of countries in the continent are at risk of endemic malaria. Malaria is endemic in some of the offshore islands to the west of mainland Africa; Sao Tome and Principe and São Tiago Island of Cape Verde. In the East, malaria is endemic in Madagascar, in the Comoro islands (both the Islamic Federal Republic of the Comoros and the French Territorial Collectivity of Mayotte), and on Pemba and Zanzibar (6). The risk of endemic malaria in some African countries is as follows; Gambia 100%, Nigeria 97.0% and 39.7% in Ethiopia (3).

Endemic areas are defined as "areas with significant annual transmission, be it seasonal or perennial" (10). Where prevalence is greater than 75%, malaria is holo-endemic; where prevalence is between 51 and 75%, malaria is hyper-endemic; where prevalence is between 11 and 50%, malaria is meso-endemic, and where prevalence is less than 10%, malaria is hypo-endemic. In areas of stable malaria,

the amount of transmission is high without any marked fluctuations over the years, although seasonal fluctuations may exist. In unstable malaria, the amount of transmission varies from year to year. In areas of stable malaria, immunity is high and epidemics are unlikely and in unstable malaria, immunity of the population is low and epidemics are possible (11). In North Africa, a combination of high temperatures with rapid onset of a short duration of rainfall allow for a limited transmission of less than 3 months (10).

Malaria kills an African child every 30 seconds. Many children who survive an episode of severe malaria may suffer learning impairments or brain damage. Pregnant women and their unborn children are particularly vulnerable to malaria, which is a major cause of perinatal mortality, low birth weight and maternal anaemia (3).

In areas of stable malaria transmission, very young children and pregnant women are the population groups at highest risk for malaria morbidity and mortality. Most children experience their first malaria infections during the first year or two of life, when they have not yet acquired adequate clinical immunity, which makes these early years particularly dangerous. Ninety percent of all malaria deaths in Africa occur in the young children. Adult women in areas of stable transmission have a high level of immunity, but this is impaired especially in the first pregnancy, with the result that risk of infection increases (7).

Over 40% of the world's children live in malaria endemic countries. Each year 30-50 million infections leads to over 1 million deaths, of which over 75% occur in Africa children under 5 years infected with *P. falciparum*. It is estimated that African children have between 1.6 and 5.4 episodes of malaria fever each year.

In areas with stable malaria transmission, *P. falciparum* infection during pregnancy is estimated to cause as many as 10,000 deaths each year, 8% to 14% of all the low birth weight babies and 3% to 8% of all infants' deaths. Adult women in areas of stable malaria transmission have high levels of immunity, but this is impaired especially in the first pregnancy, with the result that risk of infection increases (2, 7).

Poor people are at increased risk both of becoming infected with malaria and of becoming infected more frequently. Child mortality rates are known to be higher in poorer households and malaria is responsible for a substantial proportion of these deaths (12, 13). This is mainly because poor families live in dwellings that offer little protection against mosquitoes and are less able to afford insecticide-treated nets. Poor people are also less likely to be able to pay either for effective malaria treatment or transportation to a health facility.

The rapid increase in the world's urban population has major implications for the epidemiology of malaria. A review of malaria transmission in sub-Saharan African cities shows the strong likelihood of transmission occurring within these sprawling cities, whatever the size or characteristics of their bioecological environment (14).

**MALARIA EPIDEMICS**

Epidemics can occur when malaria attacks vulnerable populations with little or no immunity. In such situations, people of

all age groups are at risk of death or severe malaria. The populations most at risk of epidemics are those living in highlands, arid and desert-fringe zones and those living in areas where successful control measures have not been consolidated or maintained (2). Epidemic areas are defined as "areas prone to distinct inter-annual variation, in some years with no transmission taking place at all" (10). A smaller proportion of people live in areas where risk of malaria is more seasonal and less predictable, because of either altitude or rainfall patterns. People living in the peripheral areas north or south of the main endemic area or bordering highland areas are vulnerable to highly seasonal transmission and to malaria epidemics (7).

Two factors precipitate malaria epidemics; i. natural factors such as climatic variations and natural disasters and ii. man-made factors such as conflicts and war, agricultural activities, dam construction, mining, logging and failure of control measures (7, 8). These factors make the physical environment suitable for mosquitoes to transmit malaria.

In Mauritius, malaria has been well controlled since 1950s, but occasional outbreaks of vivax malaria occur, the last in association with a cyclone in 1982. Since that year, there has been a steady decrease in cases and risk is now extremely low (7). Only about 0.02% of Nigerians are at risk of epidemic malaria. In Ethiopia, 23.9% of the population is at risk of malaria epidemics.

Malaria has been well controlled or eliminated in the five northernmost African countries; Algeria, Egypt, Libya Arab Jamahiriya, Morocco and Tunisia. In these countries, the disease was predominantly caused by *P. vivax* and transmitted by mosquitoes that were easier to control than those of the ones in Africa south of the Sahara (7). High altitudes in East Africa, Horn of Africa and among the arid deserts at the juncture of Kenya, Ethiopia and Somalia are unstable for malaria transmission (10)

**MALARIA IN NIGERIA**

In Nigeria, 37 Anopheles species of mosquito have been documented. The main agent of malaria is *P. falciparum.* Transmission occurs in the entire country and it is all year round in only small part of the southern part of the country. In the remaining parts, duration of transmission is 3-10 months (months of February to December). The risk of endemic malaria is 97% and epidemic risk 3%. Malaria is highly endemic in Nigeria and is one of the major causes of ill-health and death (15).

About 50% of Nigerian population experience at least one episode of malaria each year (16). In a survey of selected health facilities in 2001, 36% of deaths among children under 5 years of age were attributed to malaria, with 7.2% case fatality rate and 31.7% attributable malaria morbidity (3).

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# RECENT ADVANCES IN THE LABORATORY DIAGNOSIS OF MALARIA

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**Malaria is a global health problem with about 2-4 billion people at risk, about 200-300 million cases occurring annually resulting in about 1 million death, 90% of which occur in the sub-Saharan Africa. Prompt and accurate diagnosis is the key to effective disease management, one of the main interventions of the Global Malaria Control Strategy. The "gold standard" test for the diagnosis of malaria, blood film microscopy, has in recent times come under some criticism. Apart from being cumbersome and time consuming, the reliability of the test depends on the competence of the microscopist and the test is not sensitive when parasitaemia is less than 100 parasites/μl. of blood, a situation usually seen in non-immune subjects. Several new innovative malaria diagnostic tests called Rapid Diagnostic Tests (RDT) have been developed to circumvent these limitations. The application of these new techniques in clinical laboratories is limited by cost, variable sensitivities, spill-over antigenaemia and false positive reactions in some cases. Although the polymerase chain reaction (PCR) assays for malaria diagnosis is extremely sensitive and specific, and has been suggested to replace blood film microscopy as the gold standard, the long time, high cost and technical expertise required is limiting the usefulness of these techniques, especially in Africa. This communication provides information on the available malaria diagnostics and the recent advances in the laboratory diagnosis of malaria.**

**Key words: Recent advances, malaria diagnostics**

## INTRODUCTION

Malaria is a global health problem with about 2-4 billion people at risk. Worldwide, about 200-300 million cases occur annually with about 1 million deaths, 90% of which occur in the sub-Saharan Africa (1). Prompt and accurate diagnosis is the key to effective disease management, one of the main interventions of the Global Malaria Control Strategy (2).

Unfortunately, poor diagnosis has continued to hinder effective malaria control especially in the developing countries where high prevalence of asymptomatic infection and non specific clinical presentation occurs, and where resources are lacking and access to heath care providers and health facilities are insufficient, thereby encouraging widespread practice of self-treatment for clinically suspected malaria.

The "gold standard" for the laboratory diagnosis of malaria is the microscopic examination of stained blood film for malaria parasites (2, 3). Although this is a sensitive and specific method of diagnosis when correctly performed, it can be unreliable and wasteful when poorly executed. It is also labour intensive and time-consuming taking at least 60 minutes for result to be available, a situation that may make clinicians take decisions on treatment without the benefits of the result.

These limitations have led to the development of alternative methods of diagnosis such as the nucleic acid detection and the Rapid Diagnostic Tests (RDTs) over the past 15 years. Most of the RDTs for malaria use immunochromatographic technique to detect malaria parasite antigens in peripheral blood, many of which have been tested in clinical and field

situations with comparable sensitivities, specificities and accuracies with the blood film microscopy.

This review highlights the various diagnostic methods available for diagnosis of malaria and discusses the benefits and limitations of the RDTs and other newer methods in developing countries.

**BLOOD FILM MICROSCOPY**

The conventional light microscopy examination of the stained blood film is the "gold standard" method with which other diagnostic methods are compared. Samples that can be analyzed by microscopy include peripheral blood (venous), finger prick blood (capillary), buffy coat, bone marrow aspirates, cord blood or placenta impression smear and postmortem smears of grey matters (capillary/post capillary venous blood). Examination is done usually under the oil immersion light microscope

**Thick blood film**

The thick film concentrates malaria parasites and makes this method very sensitive. It is mainly used for making a diagnosis of malaria and estimation of parasite density. When examination is done by a competent microscopist, the sensitivity approaches 5-10 parasites/μL of blood or 0.0001-0.0002% parasitaemia (3), but under general field conditions, the detection capabilities may be realistically placed at 100 parasites/μL of blood (4). 3% Geimsa stain is the commonly employed stain without any need for fixation, but Field stains (A and B) can equally be used.

The level of parasitaemia is essential in *Plasmodium falciparum* infection and this is better achieved with a thick film, which is usually in excess of 50, 000 parasites/μL of blood. A number of methods can be used to estimate parasite density. The most reliable is counting the malaria parasites in relation to number of white blood cells (WBC), usually 200 WBC (or 500 WBC when the number of parasites is less than 10 per 200 WBC counted) and multiplying this by the average of the total WBC count in such individual (5). An average of 8,000 leucocytes per μL is usually taken as standard WBC count but in our environment; a value of 6,000 leucocytes per μL appears more appropriate (6, 7).

Another method by Earle and Perez (5) employed counting asexual parasites per known volume (usually 5 μL) of blood spread as thick film. This is however time consuming and generally employed only in research studies. The 'plus' system is an alternative but less precise method commonly used in our environment.

Because the stains destroy the red blood cells, it is difficult to identify the species of plasmodium in thick film.

**Thin blood film**

The thin film allows the observation of details about the species of plasmodium and the blood film morphology, which enable speciation of parasites, hence, it is used mainly for speciating the parasites. The blood film preparation is carried out on moisture and grease free slides and staining is better done with 10% Geimsa stain following an initial 1-2 minutes methanol fixing step, to preserve the red cells. The modified Field stain is rapid but does not stain Schuffner's dots and is useful only as a screening method. The Leishman's stain is methanol based and is useful only in thin film. It is however superior to other stains in demonstrating details of malaria parasites, therefore aiding better speciation.

Examination of thin film is preferably done in the lower third of the smear where erythrocytes are just overlapping but the tail of the film demonstrate better the matured *Plasmodium ovale*, *P. vivax* and gametocytes of *P. falciparum*. A good film should reveal erythrocytes as pale straw or light grey and the leucocytes as cell with dark blue or purple nuclei with lighter cytoplasm and platelets as blue or purple. The size of the erythrocytes and presence of stipplings as well as the size and number of the different forms of the parasite's life cycle are important in arriving at the species of the parasite.

In *P. falciparum* infection, the erythrocytes are usually of normal size. The ring forms (early trophozoites) of the parasites are usually small and multiple (> 2) in the erythrocytes while the late trophozoite forms have moderate, compact and granular pigment with irregular large, red mauve dots called Maurer's cleft. Schizonts of *P. falciparum* are rare in peripheral circulation as most of them are sequestered in the microcirculation because of their adhesive nature. The gametocytes are crescent (boat shaped) with single nucleus.

In *P. vivax* infection, the erythrocytes are usually larger than normal; the ring trophozoites are large and < 2 within the erythrocytes while the late trophozoites are large amoeboid with fine pigment and the red cells have numerous fine stipplings called Schuffner's dots. The erythrocytes in *P. ovale* infection are also larger than normal; the ring form appear compact and < 2 per cell and the late trophozoites are small, oval, non-amoeboid with coarse pigments with some erythrocytes containing numerous stipplings called James' dots. In *P. malariae* infection, the erythrocytes are smaller than normal; the ring forms are compact and < 2 per cell while the late trophozoites are small, compact (and band like) with coarse pigments but erythrocyte usually show no stippling. The gametocytes of *P. vivax*, *P. ovale* and *P. malariae* appear spherical and compact with single nucleus, which is diffuse and coarse, though in *P. vivax* and *P. malariae*, this appear smaller and less diffuse.

Conventional blood film light microscopy offers several advantages. It is sensitive, detecting between 5-10 parasites/μL of blood (4) when done by a competent microscopist and about 100 parasites/μL under general field conditions. It gives precise information about the species of plasmodium involved and quantification can be done to demonstrate hyperparasitaemia associated with severe malaria and to assess parasitological response to chemotherapy. It is also relatively inexpensive with estimated cost in endemic areas ranging between US $ 0.12 – US $ 0.40 per slide examined (8). This cost per test however increases with low utilization or if microscopy in the health facility is used only for malaria diagnosis. Blood film microscopy also provides permanent record (smears) of the diagnostic findings and can be subjected to quality control.

However, it is labour intensive and time consuming, normally requiring at least one hour from specimen collection to result availability. It is exacting and depends absolutely on good technique, reagents,

microscopes and most importantly well trained and supervised technicians. Unfortunately, these conditions are often not met, particularly at the more peripheral levels of the health care systems and in some developed countries where competent microscopists may not be available. In these circumstances, microscopic diagnosis risks becoming an unreliable tool that use up resources for doubtful results. These shortcomings have led to the development of alternative rapid and robust methods of diagnosis.

**Fluorescent microscopy**

Fluorescent microscopy on blood films can be achieved by using fluorochromes such as acridine orange either on the blood smears (9) or on centrifuged blood specimens called quantitative buffy coat (QBC) technique (10). Although this is more sensitive than conventional microscopy, it is expensive and requires special equipment and supplies such as centrifuge; centrifuge tubes, ultra-violet light source and filters, and is therefore not commonly used for routine diagnostic purposes but for research.

**MALARIA RAPID DIAGNOSTIC TESTS (RDTs)**

Rapid Diagnostic Tests (RDTs) for the diagnosis of malaria are based on the detection of antigens derived from malaria parasites in lysed blood using immunochromatographic or enzyme-linked immunosorbent assay methods. The results of the tests are available within 5-15 minutes of specimen collection. The introduction of the first RDTs was based on the work of Howard *et al* (11) who described the production of histidine-rich protein II (HRP II), a water soluble protein, by trophozoites and young (but not matured) gametocytes of *P. falciparum* and secreted from infected erythrocytes. A rapid technique, Parasight® F, (12) was then developed by Becton Dickinson Tropical Diseases Diagnostics, Sparks, Maryland, United States of America.

Today, several commercial test kits are available with most frequently employing dipsticks bearing monoclonal antibodies directed against the parasite target antigens. Antigens targeted include , histidine-rich protein II antigen (11) which is available from only *P. falciparum*, and parasite lactate dehygrogenase (pLDH) (13) enzyme produced by asexual and sexual (gametocytes) stages of malaria parasites. Available kits detect pLDH from all four human plasmodium species and can distinguish *P. falciparum* from non-falciparum species but cannot reliably distinguish between *P. vivax*, *P. ovale* and *P. malariae*. Other antigens that are present in all four plasmodia species are also targeted in kits that combine HRP II antigen of *P. falciparum* together with that of an, as yet unspecified, pan- malarial antigen of other species.

**Principle of RDTs**

The methods that detect the HRP II or pLDH, which include several commercial immunochromatographic kits such as Parasight F (12), ImmunoChromatographic Test (ICT) malaria (14), and Diamed OptiMAL test kit (15), employ the use of a nitrocellulose or glass fibre strip. Test-specific reagents are pre-deposited on the strip during manufacture and these include a line of capture antibody specific for the antigen under investigation (several lines are used if several antigens are being investigated) and a procedural control line,

with an antibody that will capture the labeled antibody.

A finger prick (2-50 µL) blood sample collected using a microcapillary tube or anticoagulated blood or plasma, is mixed with a buffer solution that contains a haemolysin compound and specific antibody that is labeled with a visually detectable marker such as colloidal gold or sulpho-rhodamine, in a well or separate test tube or on a sample pad. In some kits, the labeled antibody is pre-deposited during manufacture onto the sample pad or in the well or tube, in which case only a lysing and washing buffer are added to the blood. If the blood sample contains the antigen under investigation, antigen-antibody complex migrates up the test strip by capillary action towards test-specific reagents. A washing buffer is then placed either in a well from which it migrates up the strip or it is added directly on the strip or the entire strip is washed in a test tube of buffer solution to remove the haemoglobin and permit visualization of any coloured line on the strip.

The labelled antigen-antibody complex is immobilized at the pre-deposited line of capture antibody and is visually detectable. Whether or not the blood contains antigens, the control line will become visible as labeled antibody is captured by the pre-deposited line of antibody directed against it. The complete test run time varies from 5 to 15 minutes.

While the method that detect HRP II is only available for *P. falciparum* which will show only 2 visible lines on the strip (test and control), the method that detect pLDH can differentiate *P. falciparum* from non-falciparum malaria parasites but not *P. vivax* from *P. ovale* or *P. malariae*. A negative blood will therefore show visibility only at the control line on the strip, non falciparum malaria as two visible lines while pure *P. falciparum* or mixed infection will show three visible lines on the strip.

**Merits of RDTs**

RDTs are simple to perform and interpret, and they do not require electricity, special equipment or training to perform. Peripheral health workers and other health care providers as well as community volunteers can be taught the procedure in a matter of hours with good retention of skills over one-year period (8).

They are also relatively robust and test performance and interpretation vary relatively little among individual users and the kits can be shipped and stored under ambient conditions. This makes them especially useful in the developing countries. Because they detect circulating antigens, they may detect *P. falciparum* infection even when they are sequestered in the deep vascular compartments and thus undetectable by microscopic examination of peripheral blood smear. Also in women with placenta malaria as demonstrated by placenta impression smear, RDT have detected circulating HRP II even though blood smears were negative due to sequestration of *P. falciparum* in the placenta (16).

The test performance of RDTs has been assessed in diverse clinical settings in both malaria endemic and non-endemic countries. In Nigeria, few researchers have investigated the use of HRP II antigen based RDTs, with reported sensitivity (compared to gold standard thick blood film (TBF) microscopy) of > 80% and specificity of >

90% for parasitaemia of > 100 parasites/μL of blood (6, 17, 18, 19). Others have reported lower sensitivity of 70% or less (7, 20). In some of these studies (7, 20, 21), the limited number of subjects employed does not allow conclusive inferences to be drawn. However, nearly all the investigators reported a spill-over antigenaemia effect lasting for 7-14 days after appropriate malaria therapy. This has affected the use of this diagnostic kit in monitoring response of patients to antimalaria therapy and detecting resistant infections.

Detection methods based on pLDH enzymes have equally been investigated. Agomo *et al* (22) reported a sensitivity of 63.95% for "OptiMAL 1" test kit (based on pLDH) in a study of 240 subjects with clinically diagnosed malaria in Ogun State of Nigeria. Ujah *et al* (21) also reported that sensitivity of OptiMAL test kit was higher (though not statistically significant) than TBF microscopy in a study of 62 patients with clinically diagnosed malaria in Jos, Nigeria. However, these rapid tests can only become useful in the diagnosis of malaria in the tropics, where malaria is endemic, when there is correlation between parasitaemia levels (at which the kit become positive) and clinical manifestations of malaria.

Studies of RDTs in Zimbabwe (23), Kenya (14), Tanzania (12), Cameroon (16), Thailand (24), India (25, 26), Brazil (27), East Indonesia (28), Kuwait (29), Canada (30) and the United States of America (15) have reported high sensitivity and specificity of the kits for the diagnosis of malaria (when compared to TBF microscopy or polymerase chain reaction (PCR) method as gold standard), especially in returned travelers from malaria endemic areas (15, 30) and in placenta malaria (16), but their usefulness is limited by inability to differentiate the different species of plasmodium, persistent antigenaemia and false positive reactions seen in some cases. In the assay based on pLDH, false positive reactions can occur in a situation where gametocytes, which are not the infective forms of the parasite, are the only forms present in the blood.

Some manufacturers of the HRP II antigen kit have attempted to incorporate a "pan malaria antigen" in the kit to differentiate falciparum from non falciparum malaria especially *P. vivax*. Although the sensitivity and specificity of these types of kits for *P. falciparum* is 85-95% and > 95% respectively, the sensitivity for *P. vivax* can be as low as 60% in some cases (29) and generally less than 80%, though the specificity approaches 100% (28, 29).

At present, the RDTs can only complement the TBF microscopy in the diagnosis of malaria in the tropic, especially in areas where microscopy may be unavailable and in certain conditions such as placenta malaria or cerebral malaria where sequestration of parasites occur in the placenta or brain micro-capillaries. Before they can gain widespread use, the cost of the kit must be drastically reduced. The cost per test at the moment varies from $0.60 to $2.50 and possibly more depending on the marketing area (8). The kit must also be able to differentiate the different species of plasmodium especially in areas where two or more species co-exist and issue of false positivity and spill-over antigenaemia must be addressed, so that they can be useful in disease monitoring and detecting early resistance.

**PLASMODIUM NUCLEIC ACID DETECTION**

**DNA hybridization method**

The earliest DNA probe described by Barker *et al* (5) was specific for *P. falciparum* and detects 20-25 parasites/μL of blood. The probe was a $^{32}$P-labelled DNA (Pf 14 DNA probe) containing 1 kb of *P. falciparum* DNA made of tandemly arranged degenerate 21 bp repeats. It is only useful for large number of samples and screening blood donors for malaria in endemic countries. It is very expensive, requiring high technical expertise and is also time consuming.

**Polymerase chain reaction detection method**

The polymerase chain reaction (PCR) method employed the use of oligonucleotide primers with *P. falciparum* sequences. The earliest primers constructed were similar in sequence to those of DNA probes of Barker *et al* (5). Amplified products following PCR are detected by Southern blotting or dot blot hybridization with chemiluminiscent substrates following blot transfer onto hybridization membrane or nitrocellulose. Sensitivity can be as high as 10 parasites/μL of blood.

In recent times, the sequence of a small subunit rRNA (SSUrRNA) or 18S rRNA genes of plasmodium have been found to be highly stable and conserved (31, 32, 33). These subunits contain both genus-specific and species-specific sequences. PCR primers directed against these subunit sequences have allowed for detection of the different human plasmodium species involved in single or mixed infections (31-37) and assays to detect them have displayed no cross reactions to human DNA or other human pathogen DNA or RNA including non-human plasmodium species.

Evaluation of the traditional "gold standard" PCR assay, the nested PCR, for the detection of plasmodium species in human infection has shown a higher sensitivity and specificity than the TBF microscopy (38), especially at parasite count of < 100/μL. This assay is however a lengthy procedure that requires specialized and costly equipment and reagents, as well as laboratory conditions that are often, not available in the field. Because it takes a long time (about 8 hours) for the result to be available to the health care physicians, the routine use of this assay in clinical laboratory is limited. Several innovative PCR assays are now available (39, 40, 41, 42) with result made available within 2 hours of specimen collection. For example, the "Real-Time" PCR assay for detection of malaria parasites provide qualitative (39, 41) or both qualitative and quantitative (40, 42) estimates of parasitaemia and are particularly useful in returned travelers with low parasitaemia and in patients with mixed infections. The results are also available in less than 45 minutes (39) or within 2 hours (40-42).

Knowledge of several other gene sequences of the different stages of malaria parasites have been used to construct primers for identification of genetic variants of the different plasmodium species (43). Such gene sequences include cysteine protease (44) and mitochondrial cytochrome b gene sequence (45) of trophozoites and ookinete surface protein gene sequence (46).

At present, nucleic acid based method of laboratory diagnosis of malaria is restricted to returning travelers from endemic areas and for mixed infections. The application of this technology in Africa is

limited to large research institutes and until issues of cost and technical expertise are resolved, it will have no place in routine laboratory diagnosis in this part of the world.

**OTHER LABORATORY DIAGNOSTIC METHODS**

**Antibody detection by serology**

This detection method measures antibodies that are produced by the body following exposure to the parasite and not specifically current infections (47). Antibodies to the asexual blood stages appear a few days after malaria parasites invade erythrocytes and rise in titres over the next few weeks. They may persist for months or years in semi-immune patients in endemic areas where re-infection is frequent. However, in non-immune patient treated for a single infection, antibody levels fall more rapidly and may be undetectable in 3-6 months. Re-infection or relapse leads to secondary response with high and rapid rise in antibody titres.

Various protocols for detecting these antibodies include indirect fluorescent antibody technique (IFAT) with malaria antigen for the assay prepared from peripheral blood of infected patients (all types of plasmodia) or by continuous *in vitro* culture of *P. falciparum* or adapted growth of *P. falciparum* in primates. Enzyme linked immunosorbent assay (ELISA) for the detection of antimalaria antibody to *P. falciparum* is also available in kit forms (48). Recently, a test kit "SD Bioline" with recombinant *P. falciparum* circum-sporozoite proteins (CSP) and merozoite surface protein (MSP) as antigens, was compared with "OptiMAL 1" test kit (22) in Nigeria. The performance of the "SD Bioline" was dismal with only 54.84% sensitive and 42.9% specific, while the "OptiMAL 1" was 63.95% sensitive and 92.2% specific when compared to TBF microscopy.

Antibody testing in endemic area is not useful and is only helpful in prospective screening of blood donors and retrospective confirmation of malaria in residents of non-endemic areas recently treated empirically overseas. It may be useful in the investigations of cases of tropical splenomegaly syndrome (TSS) now called hyper immune malaria splenomegaly (49).

**Depolarized monocytes/Pseudoreticulcytosis**

Some researchers have attempted the use of "Cell-Dyn 4000 Haematology Analyzer", a new generation automated instrument that has found widespread use in routine haematology laboratories in developed countries (50), to diagnose imported malaria. The principle employed is based on unexpected abnormalities in differential white blood cell plots and reticulocyte histogram seen in patients with malaria.

Monocytes that have ingested malaria breakdown product, haemozoin, can be differentiated from normal monocytes because haemozoin depolarizes laser light used for routine differentiation of eosinophils. Also, nuclear materials of intra erythrocytic malaria parasites can be detected by fluorescent nucleic acid dye used for routine quantification of reticulocytes. The presence of infected erythrocytes leads to a distinct fluorescent spike in reticulocyte histogram referred to as "pseudoreticulocytosis".

Using these two parameters, Wever *et al* (50), found 62% sensitivity for imported malaria (all species) at parasite density of ≥ 0.5% when compared to expert microscopy

and 96% sensitivity for *P. falciparum* infection alone. This novel method may be a useful addition to conventional microscopy in the diagnosis of imported malaria, especially when expert microscopists are not available. It is an expensive test however, requiring the use of expensive novel equipment and generally not applicable in the developing countries.

**Laboratory culture of plasmodium**

*In vitro* cultivation of plasmodium in the laboratory is usually not for routine diagnosis but for purposes of antigen preparation for serology and for malaria research.

**CONCLUSION**

Malaria remains a major public health problem in the tropic. Prompt diagnosis is essential for timely initiation of appropriate therapy. At present, blood film microscopy remains the "gold standard" and the most applicable method of malaria diagnosis in the tropics. Although the rapid diagnostic techniques have a role to play in certain situations and in certain clinical conditions, the high cost prohibits their widespread use. The place of the polymerase chain reaction based method in malaria diagnosis in most parts of Africa is even less certain.

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# MORPHOLOGICAL CHANGES IN MALARIA

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**Malaria remains a global health problem. Several organs of the body are affected by the Plasmodium species which parasitized erythrocytes. The small blood vessels of all the major organs of the body are usually filled with parasitized red cells and this represents the major morphological changes seen in malaria. Other common findings include hyperaemia and congestion and deposition of haemozoin at various sites in these organs and scattered small haemorrhages in various organs of the body. More organ-specific findings include fatty infiltration of the liver, hyaline membrane formation in the lungs, fatty degeneration of myofibrils and brown atrophy in the heart and "Durck" granuloma in the brain. This is a review of the various morphological changes seen in malaria.**

**Key words: Morphological changes, Malaria**

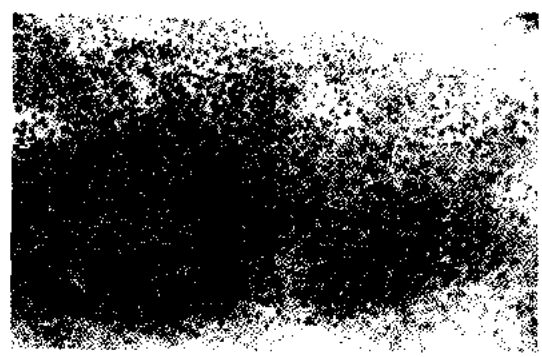
## INTRODUCTION

Many organs show morphological changes in both the acute and chronic stages of malaria. Parasitization is greatest in descending order in the following organs: brain, heart, liver, lung, kidney and blood (1-3). Secondary changes can occur in all the other major organs, depending on the type and severity of the infection. We review the morphological changes in malaria in all the major organs of the body.

### Spleen

The spleen is the first organ to show morphological changes (4). These changes can be seen as early as two weeks after infection. In the acute stage, there is splenomegaly. The cut surface shows congestion and is slaty greyish with the Malpighian corpuscles prominent (3). Microscopy demonstrates the presence of parasitized red cells in the blood vessels, Billroth cords and sinusoids (5).

The parasitized and unparasitized red cells and haemozoin are seen in the pulp histiocytes and sinusoidal lining cells (5) (Fig 1).



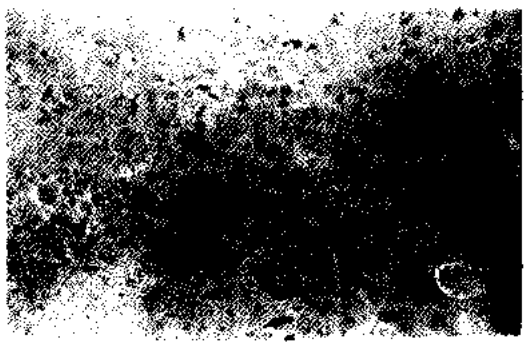
Haemozoin is an iron-porphyrin complex that is phagocytosed and processed by the macrophages. The pigment is seen as crystalline clump of dark-green material that polarizes under polarising light (5). Degeneration of the endothelial cells of splenic vessels may be seen resulting in thrombosis, haemorrhage and infarction (3).

### Liver

The histopathological changes due to malarial involvement of liver are specific (5-7). During the acute stage of an attack, the Kupffer cells demonstrate hypertrophy and hyperplasia (5, 6). The liver is congested

with a grey or black pigmentation as a result of accumulation of haemozoin (5). Microscopically, in acute malaria, there is a pronounced hyperaemia with dilatation of all capillaries (7). Parasitized red cells may be attached to endothelial of the vessel and Kupffer cells may contain parasitized red cells (7).

Following survival of an acute attack, haemozoin gradually migrates from the parenchyma to portal areas (5, 7) (Fig 2).



Fatty infiltration may be seen throughout the liver, but particularly around the centrilobular vein (3, 8). Focal hepatocyte necrosis may also be seen and these two changes are usually attributable to poor nutritional status (8). Malaria is not considered to be precirrhotic (7).

**Kidneys**

In severe malaria, there is gross congestion of the vessels with parasitized red cells, especially in the capillaries of the glomerular tuft (3). Scattered small haemorrhages may be seen in the cortex and medulla (9). The histological changes are those of acute tubular necrosis due to reduced cortical perfusion (10).

Pigments are widely seen in blood vessels and interstitial tissue and occasionally in the epithelial cells of the tubules and within phagocytes in the capsular spaces (10). Hyaline, epithelial and granular casts may be present in the tubules (10). *Plasmodium malariae* causes a nephropathy of immune complex origin with microscopical patterns ranging from minimal change to membranous glomerulonephritis (9).

**Lungs**

The small blood vessels of the lung are packed with parasitized red cells and small haemorrhages may be present (11). There may also be hyaline membrane formation, thickened alveolar septa and areas of alveolar haemorrhages (11). The alveoli are congested with pigment-laden macrophages, plasma cells, neutrophils and parasitized red cells.

**Cardiovascular system**

The vessels are congested with parasitized red cells, pigment-laden macrophages, lymphocytes and plasma cells (3). There may be small subendocardial haemorrhages (3) and fatty degeneration of the myofibrils and brown atrophy may also be seen.

**Adrenal glands**

The changes in the adrenal glands are variable (3). Degenerative and necrotic changes are seen in the inner zone of the cortex with loss of lipid. The more usual findings are gross congestion and haemorrhage (3).

**Bone marrow**

The bone marrow is greyish red, soft and hyperaemic and there is hyperplasia in the long bones (12). In the acute stages, the vessels are full of parasitized red cells and haemozoin is present in the reticulo-endothelial system and monocytes. There is marked normoblastic hyperplasia, even in the absence of peripheral reticulocytosis and there is myelocytic proliferation (12).

**Gastrointestinal system**

There is congestion with capillary stasis, necrosis, mucosal ulceration and haemorrhage (3).

**Central nervous system**

Although changes have been reported in the spinal cord and peripheral nerves, the most marked changes are seen in the brain itself (13). The central neuropathological feature of cerebral malaria is the preferential sequestration of parasitized red cells in the cerebral microvasculature (14). The meninges are grossly congested with the smaller vessels packed with parasitized red cells. The brain may show gross congestion only but it is usually leaden in colour (13). Gross congestion of the vessels is a constant finding and in the majority of instances, numerous petechial haemorrhages are evident in the white mater of the cerebrum, brain stem and cerebellum (14). Haemorrhages are not usual in the grey mater.

Histologically, the capillaries and arterioles are packed with parasitized red cells and ring haemorrhages are prominent (3). Ring haemorrhages consist of a central vessel, which is usually an arteriole, containing an agglutinated mass of parasitized red cells surrounded by brain tissue and then by a ring of extravasated red cells.

Malarial or "Durck" granuloma may be seen in older haemorrhages. This consists of necrosis of the midzonal brain tissue and a peripheral reaction of small glial cells. Immunohistochemical and electron microscopy would demonstrate widespread cerebral endothelial cell activation, in addition to endothelial cell damage and necrosis (13).

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# RECENT ADVANCES IN CHILDHOOD ANTIMALARIA CHEMOTHERAPY

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**As malaria continues to kill many people in our world and spreading into areas that were never known to have it before, it becomes necessary to make occasional reviews of what therapeutic measures are effective in areas of malaria endemicity. There is a global concern as to reducing malaria morbidity and mortality worldwide. Malaria eradication had been viewed as impossible with the mechanisms used against it and the world has settled for just a control. One of the critical areas of this control is effective case management. As it was the case with tuberculosis, leprosy and bacterial infections, there is a paradigm shift from the monotherapy that have been used for nearly three centuries (Quinine) and nearly 60 years after other drugs were discovered (Chloroquine, since early 1940s and subsequently others) with no remarkable drop in the global morbidity and mortality. The World Health Organization (WHO) now advocates combination therapy, which are mainly Artemisinin-based. We in this article made an extensive review of the combination chemotherapeutic possibilities and advocacy for it to achieve increase survival, reduced disease burden through effective parasitaemic clearance with reduced chance of early recrudescence. A necessary overview has been made of the life cycle and clinical presentation of malaria which has not changed significantly over the years. Also the combination chemotherapy including Artemisinin-based, Sulphadoxine/Pyrimethamine-based and the non-Artemisinin non-Sulphadoxine/Pyrimethamine-based chemotherapy have all been reviewed and concluded that their use will lead to effective case management and reduced mortality. We therefore advocate for a therapeutic paradigm shift to these combination therapy.**

**Keywords: Combination chemotherapy, antimalaria, childhood, review**

## INTRODUCTION

About 300-500 million clinical episodes of malaria occur each year globally, resulting in about one million deaths (1). Figure 1 shows the global malaria distribution. Majority of the deaths occur among the people who did not intervene, intervened incorrectly/ wrongly or intervened too late that such intervention could not salvage their lives. More than 90% of the deaths occur in the sub-Saharan Africa and greater percentage being children.

In 1998, the World Health Organization (WHO) launched the Role Back Malaria (RBM), with the goal to halve malaria deaths worldwide by 2010 (2). To achieve this requires prevention interventions (ITNs, Household spraying) and appropriate case management, which is directly linked with death prevention. To achieve mortality reduction, malaria cases must be promptly and accurately diagnosed and well treated with effective antimalaria drugs. Presently, progress on effective treatment seems so inadequate that RBM seem to be failing to reach its targets. The reason given for this is the fact that RBM is acting on the background of increasing global malaria burden (3).

One of the major reasons why malarial mortality may increase is case management failure. Management failure can either be due to no treatment or treatment failure. Treatment failure will usually be due to inadequate treatment, fake drug syndrome, delayed treatment or multi-drug resistance of the deadly species, *Plasmodium falciparum*.

Presently, WHO said the global malaria control is being threatened on an unprecedented scale by the continued use of outdated drugs such as chloroquine which was said to be ineffective in most part of Africa and Sulphadoxine/Pyrimethamine (SP) which is also becoming less useful (4). For instance, in West Africa, a 12 year community-based study showed that the

onset of chloroquine (CQ) resistance doubled childhood malaria mortality risk and that in some sites, the risk increased 11-fold especially in young children (5, 6).

In the East and Southern African countries, the childhood mortality from malaria double as CQ and later SP resistance started from 1980s to the 1990s even when mortality from other causes declined. In all other African countries, the proportion of admission to hospital increased significantly and the deaths attributed to malaria increased by 2 to 4 folds (7). The link between drug resistance, treatment failure and deaths from malaria are well established and WHO agrees that CQ resistance is the most likely reason why there is increased mortality in Africa (2).

Sequel to the non-usability status of CQ and SP due to treatment failure, it becomes necessary to find alternate drugs for treating malaria. Artemisinin class combination therapy readily comes up as the best option for treatment. A large meta-analyses of close to 6000 patients showed that combining existing antimalaria drugs with an artemisinin reduces patient's risk of treatment failure by 75% and reduces the pool of infectious parasites that transmit the disease. Between year 2000 and now, studies (9, 10, 11) have been conducted in virtually every continent of the world which showed that artesunate combination therapy successfully treated greater than 90% of patients, hence its recommendation by the WHO.

The present approach to malaria control has been the implementation of various strategies. The strategies can be broadly classified into improved case management and disease prevention. The improved case management is our major concern here.

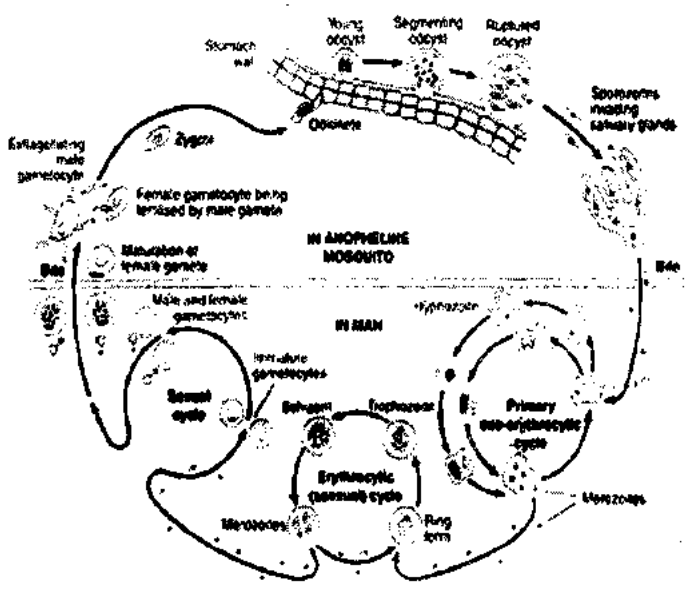


**Diagram depicting the life cycle of human malaria** ***(Asexual phase in human body and sexual phase in the mosquito)***

## LIFE CYCLE OF MALARIA PARASITE

### Asexual phase in the human host

#### *Pre-erythrocytic (Tissue) schizogony*

This phase starts with the inoculation of the parasite (sporozoites) into the human blood by the bite of a female anopheles mosquito. Some of these reach the liver within half an hour, and invade the liver cells. In the liver cells, the sporozoites multiply asexually (exoerythrocytic schizogony). Eventually, invaded liver cells rupture, releasing thousands of merozoites into the bloodstream.

The time taken for the completion of the tissue phase is variable, depending on the infecting species; (8 - 25 days for *P. falciparum*, 8 - 27 days for *P. vivax*, 9 - 17 days for *P. ovale*, 15 - 30 days for *P. malariae*) and this interval is called pre-patent period. In case of *P. vivax* and *P. ovale*, some sporozoites may go into hibernation, the cryptobiotic or dormant phase, in which they are called as hypnozoites. They can lie dormant for months or years and on reactivation cause clinical relapse.

#### *Erythrocytic schizogony*

The merozoites released from the liver cells attach onto the red blood cell (RBC) membrane and by a process of invagination, enter the red cell. Within the RBC, the asexual division starts and the parasites develop into trophozoites. After a period of growth (ring forms, early and late schizont), the trophozoites divide and develop, eventually forming between 8-24 merozoites. The merozoites then invade fresh erythrocytes and another generation of parasites develops in the same manner. This process occurs repeatedly during the course of infection and is called erythrocytic schizogony.

The intra erythrocytic cycle takes about 48 hours in *P. falciparum*, *P. vivax*, and *P. ovale* infections and 72 hours in case of *P. malariae* infection. It occurs synchronously and the merozoites are released at approximately the same time of the day. The contents of the infected cell that are released with the lysis of the RBC stimulate the release of tumor necrosis factor (TNF) and other cytokines, which results in the characteristic clinical manifestations of the disease. A small proportion of the merozoites undergo transformation into gametocytes - male and female. Mature gametocytes appear in the peripheral blood after a variable period and enter the mosquito when it bites an infected individual.

### Sexual phase in the mosquito (Sporogony)

The male and female gametes fuse and form into a zygote. This transforms into an ookinete which penetrates the gut wall and becomes an oocyst. The oocyst divides asexually into numerous sporozoites which reach the salivary gland of the mosquito. On biting a man, these sporozoites are inoculated into human blood stream. The sporogony in the mosquito takes about 10 - 20 days and thereafter the mosquito remains infective for 1 - 2 months.

## CLINICAL PRESENTATION OF MALARIA

#### *Uncomplicated or simple malaria*

This is malaria that has no life threatening manifestations. It presents as fever, rigors, vomiting, malaise, diarrhoea and generalized weakness or other non-life threatening manifestations.

#### *Complicated or severe malaria*

This is defined by the demonstration of asexual forms of *P. falciparum* in a patient with a potentially fatal manifestation: clinical or laboratory features, as depicted below (12).

**Severe manifestations of *P. falciparum* in children**

- Prostration *(i.e. generalized weakness or inability to sit, stand or walk without support)*
- Impaired consciousness *(confusion or drowsiness or coma)*
- Respiratory distress *(difficulty in breathing, fast deep breath)*
- Multiple convulsions *(>2 generalized seizures in 24 hrs with regaining of consciousness)*
- Severe anaemia *( Hb <5 gm/dl)*
- Circulatory collapse *(shock)*
- Pulmonary oedema *(respiratory distress / radiology)*
- Abnormal bleeding *(disseminated intravascular coagulopathy)*
- Jaundice *(yellow discoloration of the eyes)*
- Haemoglobinuria *(Coca-Cola coloured urine)*
- Hyperparasitaemia *Density of asexual forms of P. falciparum in the peripheral smear exceeding 5% of erythrocytes (more than 250,000 parasites per µl at normal red cell counts)*
- Hyperpyrexia *(Rectal temperature above 40°C)*
- Hypoglycaemia *(Blood glucose <2.2mmol/l)*

**Figure 1: Showing the global distribution of Malaria**

**GOALS OF MALARIA MANAGEMENT**

There are malaria management expectations at various levels of health care based on the available facilities and staff capabilities.

Level I include all dispensaries and health posts. The cadre of staff at this level includes junior community health extension workers and village health workers and a syndromic approach focusing on disease identification, initiation of appropriate treatment and urgent referral of severe malaria is expected.

Level II include health centres with or without laboratory facilities and most private hospitals without intensive care with staff including medical officers, general practitioners, nurses and community health officers for in-patient care. Basic laboratory support for confirming diagnosis and monitoring of patients is expected at this level.

Level III include teaching, specialist, general hospitals and some private hospitals with specialist physician and highly skilled senior physician. In addition to quality total care, it is expected to provide intensive care for severe disease.

**COMMON ANTIMALARIA DRUGS**

Antimalarial drugs include several chemical groups (13) and the following gives a brief summary of common anti-malarial drugs.

**Quinine**

Quinine has been used for more than three centuries and was the only effective medication until the mid 1930's. It is one of the four main alkaloids found in the bark of the Cinchona tree and is the only drug which over a long period of time has remained largely effective for treating the disease. It is now only used for treating multidrug resistant and severe falciparum malaria partly because of undesirable side effects.

**Chloroquine**

This was one time a very effective 4-amino-quinoline both for malaria treatment and prophylaxis. It was first used in the 1940s shortly after the Second World War and was effective in curing all forms of malaria, with few side effects when taken in the dose prescribed and it was relatively cheap.

Unfortunately, most strains of falciparum malaria are now resistant to chloroquine and more recently, chloroquine resistant vivax malaria has also been reported. Presently it is not useful as antimalarial in most country of the world. The few countries that were still using it have recently discontinued its use.

**Proguanil**

This is a biguanide antimalarial first synthesized in 1946. It has a biguanide chain attached at one end to a chlorophenyl ring and is very close in structure to pyrimethamine. It is a folate antagonist that destroys the malarial parasite by binding to the enzyme dihydrofolate reductase in much the same way as pyrimethamine. It is still used as a prophylactic in some countries especially in Africa.

**Malarone**

Malarone is a combination of proguanil and atovaquone released in 1998. Atovaquone became available 1992 and was successfully used for the treatment of *Pneumocystis carinii*. When combined with proguanil, there is a synergistic effect and the combination is at the present time a very effective antimalarial drug. The drug combination

has undergone several large clinical trials and has been found to be 95% effective in otherwise drug resistant falciparum malaria. It has been claimed to be largely free from undesirable side effects but it should be noted that proguanil is an antifolate. At present it is a very expensive drug.

**Maloprim**

This is a combination of dapsone and pyrimethamine. Resistance to this drug is now widespread and its use is no longer recommended.

**Lapdab**

This is combination of dapsone and chlorproguanil. Its use has been reduced due to the serious complications of severe haemolysis and anaemia requiring transfusion.

**Fansidar**

This is a combination drug containing sulphadoxine and pyrimethamine in ratio 20:1 in each tablet. It acts by interfering with folate metabolism. Resistance to Fansidar is now widespread and clinical confidence now dropping.

**Mefloquine (Lariam)**

First introduced in 1971, this quinoline methanol derivative is related structurally to quinine. The compound was effective against malaria resistant to other forms of treatment when first introduced and because of its long half life was a good prophylactic, but widespread resistance has now developed and this together with undesirable side effects have resulted in a decline in its use. It is not widely approved for use in many African countries.

**Halofantrin (Halfan)**

This belongs to a class of compound called the phenanthrene-methanols. It is an effective antimalarial introduced in the 1980s, but due to its short half life of 1 to 2 days, is therefore not suitable for use as a prophylactic.

Unfortunately, resistant forms are increasingly being reported and there is some concern about side effects. Halofantrin has been associated with neuropsychiatric disturbances and arrythmias. It is contraindicated during pregnancy and is not advised in women who are breastfeeding. Abdominal pain, diarrhoea, pruritus and skin rash have also been reported. Its use is now restricted in some quarters.

**Coartem**

This is a combination of Lumefantrin and Artemether. It has proven very effective. However anecdotal experiences are showing early parasitaemic recrudescence.

**Artemisinins**

This is derived from a Chinese herbal remedy and covers a group of products. The two most widely used are artesunate and artemether. While they are widely used in Southeast Asia, they are not licensed in most of the Western World including Australia. It is a well tolerated drug with little or no side effects.

However due to some treatment failures, it is being combined with several other antimalaria drug for the treatment of falciparum malaria. It has become the base drug for which WHO wants others to be combined for use in the presence of widespread resistant strains of *Plasmodium falciparum* infection.

**ANTIMALARIA TREATMENT POLICY (ATP)**

Antimalaria Treatment Policy (ATP) is a set of recommendations and regulations concerning availability and rational use of antimalarial drugs in a country. It provides evidence based recommendations and gives clear guidelines for providing

early diagnosis and prompt treatment appropriate to the local context.

**The aims or purpose**

1. To provide rapid and long lasting clinical cure for individual malaria patients.

2. To prevent the progression of uncomplicated malaria to severe disease and death.

3. To shorten clinical episodes of malaria and reduce the occurrence of malaria related anaemia in the population in Endemic areas.

4. To reduce the unfavourable effects of malaria in pregnancy through chemoprophylaxis or preventive intermittent therapy.

5. To minimize the likelihood and rate of development of drug resistance.

**What should be the contents of ATP?**

A well written ATP will usually contain information relating to decision on whether a sick patient requires antimalarial treatment or not and the following must be well spelt out;

1. The first and second line antimalarial drugs with their indications and age appropriate dosages for treatment of both uncomplicated and severe malaria.

2. Chemoprophylaxis for various at-risk groups such as children, pregnant women and non-immuned individuals.

3. Distribution and drug delivery.

4. The criteria for changing treatment choice.

**How is a National ATP made?**

Information available on malaria parasite drug resistance, the current drugs and their roles in malaria management are considered. Using the criteria below the antimalaria drugs are then selected.

1 Efficacy and proven effectiveness against prevalent malaria species
2. Safety profile of drug
3. Simplicity of dosage and route of administration
4. Registered in the country of use
5. Cost

**The process of changing ATP policy** (14)

The key evidence for the need to change treatment policy is usually

1. The failing therapeutic efficacy of the antimalarial drugs in use assessed by standard WHO protocols.
2. Consumer and provider dissatisfaction with the failing medicines
3. Reports of increasing malarial morbidity and mortality.

**Treatment Policy Antimalaria classification**

First line drugs are drugs of primary intention for treatment of uncomplicated malaria for example chloroquine. Second line drugs are used when there is clinical failure or intolerance to first line example Fansidar.

Combination therapy, the present focus of discussion, is used for a more efficient case management. Example is Coartem. Adjuvant drugs are those that have antimalaria activities but are not used solely or alone for the treatment of malaria.

**Combination therapy (CT)**

***Principle***

The principle was derived from the treatment of tuberculosis, leprosy and bacterial infections where drugs are combined for synergism and reduction in the risk of resistance development

***Definition of CT***

CT simultaneously uses two or more blood schizonticidal drugs with

independent mode of action and different biochemical targets in the parasite. Multi-drug therapies that include the use of a non-antimalarial drug to enhance the antimalarial effect of blood schizonticidal drug are not included. Also fixed dose combination drugs are operationally considered as single drugs example SP.

***Classification***

**a. Artemisinin based combinations**

1. Artesunate+ amodiaquine
2. Artesunate + mefloquine for areas with low transmission
3. Co-artemether (Artemether 20mg + Lumefantrine 120mg/tablets
4. SP + Artemether or Artesunate

**b. SP based combinations**

1. SP + CQ as separate tablets
2. SP + Amodiaquine as separate tablets
3. SP + Quinine as separate tablets
4. SP + Mefloquine

**c. Non-artemisinin/SP based combinations**

This combines all other different antimalaria drugs other than Artemisinin or SP. Not necessarily combined in formulation but in drug administration example

1. Quinine + Tetracycline
2. Atovaquone+ Proguanil (Malarone)
3. Dapsone + Pyrimethamine (Use has been discontinued)
4. Dapsone + Chlorproguanil (Haemolysis is a major side effect)

The current WHO recommended treatment policy include all the Artemisinin combination therapies and the combination of Amodiaquine plus Sulfadoxine/Pyrimethamine especially for areas where efficacy of both drugs remain high and mainly in West Africa.

As at March 2004, 32 countries have adopted one of these WHO combination therapies. About 14 countries in Africa have adopted the Artemisinin as either first line or second line. As at now Kenya, Ghana, Equatorial Guinea, Cameroon, Sao Tome and Principe, Comoros, Gabon, Burundi, Zambia, Zanzibar and South Africa moved from using Chloroquine/SP to using Artemisinin. Also, Rwanda, Mozambique and Senegal moved from Chloroquine alone to Amodiaquine/SP. However, Ethiopia, Uganda, Eritrea and Zimbabwe moved from using Chloroquine alone to Chloroquine/SP. Democratic Republic of Congo and Cote d'Ivoire change from using Chloroquine to SP/ Amodiaquine (14). Several changes are taking place in Nigeria and several combinations are been used in different places but still awaiting official policy changes.

**CONCLUSION**

The effectiveness of the combination therapy has been proven in many countries of the world. These countries wasted no time in making needed changes in their national anti-malarial policies after following standard WHO protocol. The rate at which malaria parasites are becoming resistant to many drugs with increasing treatment failure and increasing case fatality, therapeutic paradigm shift from monotherapy to combination therapy is likely going to become a bride to be embraced by many more countries in the very near future. It is hoped that this will help reduce mortality to the minimum as we anticipate the discovery of effective vaccine for malaria in the near future.

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# VACCINE FOR MALARIA – HOW FAR?

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**This is a review of the progress made so far in the effort to produce a malaria vaccine. The problems that have made it impossible to get an effective vaccine for malaria are discussed. Also examined are the current efforts to produce the vaccine and the prospects for an effective vaccine in the future.**

**Key words: Vaccine, malaria, review.**

## INTRODUCTION

Vaccines are one of the most cost-effective and logistically feasible means of disease control, and have remarkable success in the control of many infectious diseases [1]. Examples include the eradication of smallpox and the near-eradication of polio [2]. It is surprising that a licensed potent vaccine for malaria has not arrived. The difficulties on the way of production of this vaccine have been identified for a long time but every advance towards the resolution of this problem, like chasing a mirage, has resulted in illusion.

Yet, the increasing resistance of the *Plasmodium* species to chemotherapeutic agents and the increasing resistance of the vectors, *Anopheles* mosquitoes, to insecticides [3] pinpoint the critical need for an effective vaccine against this infection. Although protective immunity is conferred by naturally acquired infection and by irradiated sporozoite immunization, no subunit malaria vaccine candidate so far has proven sufficiently efficacious for commercial development [4].

Hope for a potent malaria vaccine was raised when an international body of genome scientists and funding agencies was formed in 1996 [3]. This hope was on course with the publication of the genomic sequence of *Plasmodium falciparum* in 2002 [5] and simultaneous publication of the *Plasmodium falciparum* proteome representing stage-specific sporozoites, merozoites, trophozoites and gametocytes [6, 7].

Also completed is the gene expression profile of the parasite during the different stages of its life cycle [8]. The hope is that this knowledge will be exploited to identify and prioritize antigens and epitopes that may be targets of anti-malarial protective immunity. However, identification of the most effective epitopes is difficult because the genome of *Plasmodium falciparum* is large.

## PROBLEMS HINDERING THE PRODUCTION OF AN EFFECTIVE MALARIA VACCINE

Despite intense research efforts over close to half a century now, which has resulted in clinical trials of many candidate vaccines, few humans have been protected from malaria through vaccination [4]. Unlike in the development of vaccines against bacteria and viruses, developing a vaccine against the malarial parasite is complicated

by the complexity of the parasite and that of the host's response [3].

The identification of antigens that will stimulate the most effective immunity against *Plasmodium* is problematic because of: (a) the multistage parasitic life cycle, (b) a large genome encoding more than 5,300 proteins, and (c) distinct proteins expressed at different stages of the parasite. Added to these problems is the poor understanding of what constitutes the protective immune mechanisms that target the different parasite stages; and sequence polymorphism of identified target epitopes.

It is well established that immunization of humans with radiation-attenuated *Plasmodium* sporozoites confers sterile protective immunity [9-12]. Irradiated sporozoites could invade hepatocytes and undergo limited development but could not mature into blood-stage parasites [13] thus eliminating clinical symptoms of the disease and transmission of the parasite. However, immunization with heat-killed, formalin-inactivated or lysed sporozoites was not effective [3], making sporozoite-based vaccine production difficult. These observations seem to emphasize the requirement for live sporozoites targeting the liver. Even then the targets of cellular immunity, induced by irradiated sporozoites are largely unknown, and correlates of protection after immunization are not clearly elucidated.

A dilemma is suggested by the possibility that the protective immunity induced by irradiated sporozoite immunization is due to the summation of many immune responses of low magnitude against multiple targets which result from low density of epitopes [3]. Thus, responses against characterized hepatic-stage antigens recognized by sporozoite-induced cellular immune responses are not as potent as those induced by subunit vaccination [14].

Repeated natural infections confer immunity against severe infection but this immunity is normally species and strain specific; and it is dependent on continuous boosting and is usually short-lived. A potent vaccine based on this type of natural immunity will then be required to generate "super-natural" immunity [15].

**CURRENT APPROACHES TO MALARIA VACCINE DEVELOPMENT**

Presently, most candidate malaria vaccines are designed to protect against pre-erythrocytic and/or erythrocytic stage antigens. Transmission blocking vaccines are formulated to protect the entire community by inducing protective antibodies directed at sexual stage antigens [3]. These vaccines have been developed with potency against a single or a few key antigens, such as *Plasmodium falciparum* circumsporozoite protein (CSP) and merozoite surface protein-1 (MSP1), by immunizing with synthetic peptides or recombinant proteins in an adjuvant [15]. Results of trials with these subunit vaccines [16] leave us with the question of whether all the antigens are the same and it is the vaccine delivery system that matters. It is also not clear yet whether key protective antigens have already been identified or not.

Another approach to malaria vaccine development focuses on all of the currently known promising candidate antigens to give rise to a multivalent and multistage vaccine; produced mainly through the technology of DNA-based vaccines [17, 18]. First generation DNA vaccines were found suboptimal but

immune enhancement strategies, such as the use of adjuvants, show promise [19-21]. This approach to vaccine production faces limitations regarding the size of antigens that can be included in a given vaccine delivery system and the number that can be formulated for simultaneous administration without inducing antigenic competition.

Despite the challenges facing malaria vaccine production, there are lines of evidence showing that a vaccine is feasible. These include the age-related acquisition of immunity against severe clinical malaria in endemic regions [22, 23] and the ability of passively transferred antibodies from immune adults to protect against natural and challenge infections with *Plasmodium falciparum* [24-26]. Blood-stage vaccines against the parasite are aimed at preventing complications of the infection, such as cerebral malaria and anaemia.

Both *Plasmodium falciparum* and *Plasmodium vivax* can cause severe anaemia but only *Plasmodium falciparum* causes the many complications of cerebral malaria: hypoglycaemia, metabolic acidosis and respiratory distress [15]. Most effort has been devoted to the production of *Plasmodium falciparum* vaccines because it is responsible for majority of deaths from malarial infections.

Among the recombinant blood-stage antigens that have been proposed for development as candidate vaccines, the leading erythrocyte stage antigens, merozoite surface protein 1 (MSP1) and apical membrane antigen 1 (AMA-1) are expressed in all species of *Plasmodium* [15]. A phase 1 trial of a vaccine based on $MSP1_{19}$ fused to CD4 T cell epitopes from tetanus toxoid concluded that the vaccine was immunogenic but had a high rate of adverse reactions [27]. $MSP1_{42}$ formulated in ASO2 adjuvant has gone into phase 1 trials in the United States and Kenya [28-30]. Also, *Escherichia coli*-produced $MSP1_{42}$ and RTS, S combined with $MSP1_{42}$ has been phase 1 tried in the United States [31].

It is likely that many recombinant blood-stage vaccines will undergo safety and immunogenicity studies in malaria endemic regions soon. The short time of exposure of the merozoites to antibodies between release from one infected red blood cell and attachment and entry into another dictates that very high antibody levels are required to block entry [32]. Such levels of antibody may not be achieved with alum; therefore, a lot of effort is focused on the testing and development of new adjuvants for asexual blood-stage antigens.

The choice of adjuvants for use in man includes alum, MF 59, Montanide ISA 720, Montanide ISA 51. These are combined with immuno-stimulators, which include MPL, QS 21 and CpG. [15]. A number of human phase II trials have been carried out using SPf 66 [33, 34], a multi-component vaccine which includes a blood-stage antigen.

**PROSPECT FOR A POTENT MALARIA VACCINE**

Will an effective vaccine against malaria parasite eventually emerge? Advances in the fields of genomics, proteomics and molecular immunology offer new hope for the development of a malaria vaccine. However, algorithms that can effectively identify the targets of protective immune effectors for malaria from genomic data are just being developed [35]. Doolan *et al* [3] have indicated data showing that

protective immune responses induced by immunization with irradiated sporozoites are directed against multiple epitopes on multiple antigens, with variable potency. Thus, a multi-antigen vaccine that induces cell-mediated immune responses against antigens of the liver stage may be required to mimic irradiated sporozoite-induced protection. A vaccine that prevents blood stage infection and clinical disease is likely to emerge if the antigenic targets of irradiated sporozoite-induced immunity are identified and packaged in a vaccine formulation that is immunogenic and suitable for manufacture and administration.

Evidence that natural exposure to *Plasmodium falciparum* results in acquisition of anti-malarial immunity in humans include the observation of decrease in the incidence of infections, reduction in prevalence and density of parasitaemia and the lowering of morbidity and mortality associated with repeated infection [36-38]. The existence of naturally acquired immunity and the fact that erythrocytic stage immunity can be induced by natural exposure [36, 37] to repeated blood stage infection provide a strong rationale for the identification of the antigenic targets of naturally acquired immunity and the development of vaccines designed to include high levels of antibody responses against these antigens.

**CONCLUSION**

A malaria vaccine that is potent and protective against the infection is likely to emerge in the near future but disappointing experiences of the past counsels caution concerning a time-frame prediction. It is difficult to say now whether such a vaccine will target pre-erythrocytic stage parasite or blood stage infection or both.

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# PROSPECT AND PROGRESS OF MALARIA VACCINE DEVELOPMENT

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**Malaria kills one child every 30 seconds in Africa. The development of a safe vaccine remains an urgent unmet need which could greatly control and even lead to the eradication of the disease. The success recorded in the recent vaccine trials have given some ray of hope that a safe and effective vaccine against malaria will soon be produced. In this article, we bring together important published information on the status of malaria vaccine development and reviewed some field trials and the obstacles as well as prospect for effective malaria vaccination.**

**Keywords: Malaria vaccine, prospect, review**

## INTRODUCTION

Malaria causes more deaths than any other parasitic disease and is probably the most important pathogen for children under 5 years of age in sub-Saharan Africa. It is estimated that every 30 seconds one child in Africa dies from malaria (1). About 4 billion people in approximately 90 different countries are at risk of developing the disease and up to 500 million cases of malaria occur each year (2). Most disease and deaths are due to *Plasmodium falciparum*, although *P. vivax* is important throughout Asia and Latin America (3).

One strategy that could prove very effective in the control of malaria but is taking some time to come to fruition is a malaria vaccine (3). Four species of malaria infect humans, which raise an initial question of how many vaccines are needed (4). Although practical consideration of both development and production favour a single vaccine for *P. falciparum*, the different risk groups and vaccine requirements have generated at least three approaches for this species alone (4). An anti-infection vaccine aimed at protecting malaria-naive travelers or residents of low endemic areas from becoming infected; an anti-disease/anti-mortality vaccine aimed at children, pregnant women and migrants living in endemic areas; and an anti-mosquito-stage vaccine aimed at preventing the transmission of malaria vaccine from one person to another (5).

Successful vaccination against the malaria parasite entails overcoming special hurdles, because the parasite can establish a chronic infection within an immunocompetent host. Host genetic determinants, for example, specific polymorphisms in immune response genes, may profoundly influence the immunity towards individual antigens. (6). Despite these challenges, there are several lines of evidence supporting the feasibility of a vaccine.

## TYPES OF MALARIA VACCINE

### *Pre-erythrocyte vaccines*

A pre-erythrocytic vaccine would either prevent invasion of hepatocytes by sporozoites or kill parasites within infected

hepatocytes (2). They are designed to target sporozoites or schizont-infected liver cells and thus prevent the release of primary merozoites from infected hepatocytes (7). Research has also focused on the liver stage; a synthetic peptide representing region I bound specifically to hepatoma cells in vitro and antibodies to the region inhibited invasion of hepatoma cells by sporozoites (8). Thus a sequence in the CSP has been identified as a binding ligand and is being examined along with a second sporozoite surface protein (SSP2), which has sequence homologous to region II in CSP (9).

Naked DNA is also being developed as a delivery strategy for malaria as well as other diseases and may be particularly useful as a means to induce $CD8^+$ T cells (10). In 1994 protection from malaria in mouse model was demonstrated with a DNA vaccine encoding *P. yoelii* (11).

**i. RTS, S/AS02A**

Currently, this pre-erythrocytic stage vaccine is based on the CS protein of the 3D7 clone of *P. falciparum* and it is the malaria vaccine candidate for which clinical development is most advanced. (7). While many candidate malaria vaccines have been developed over the past 25 years, the RTS,S/AS02A vaccine is the first that has demonstrated a significant capability to protect human adult volunteers against an experimental infection with the malaria parasite (7).

The recent results also indicated that the vaccine induces protection against malaria in children one to four years old in Africa. Although the 57.7% reported vaccine efficacy against severe disease is less than that of classical childhood vaccines, which is often greater than 80%, the outcome of the trial is very encouraging for the future of malaria vaccines because it is the first demonstration of any efficacy against severe malaria in children (12).

**ii. ICC-1132 CS/hepatitis B core particle**

This is a CS-based particle vaccine that uses the highly immunogenic hepatitis B core antigen (HBcAg) as a delivery platform (13). Three Phase 1 clinical trials in healthy, malaria-naive adults are underway. Phase 1 trials in the United Kingdom and Germany have shown the vaccine to be safe and well tolerated (7).

**iii. Pf CS 282-383 long synthetic peptide**

This clinical grade vaccines based on CS and other protein were produced as a result of advances in the manufacturing of long synthetic polypeptides antigens (7). Based upon promising pre-clinical studies in mice and *Aotus* monkeys immunized with formulations alum or Montanide ISA 720, the vaccine formulations were well tolerated and induced antibodies that reacted with intact sporozoites, especially in the volunteers that received the Montanide ISA 720 formulation. A new dose and adjuvant finding Phase 1 study of Pf CS 282-303 formulated with GSKBio AS02A or Montanide ISA 720 adjuvants is currently underway (7).

**iv. Multi-epitope TRAP**

Investigators constructed this novel vaccines consisting of plasmid DNA or recombinant attenuated live viral vectors (14). Trial studies have been conducted in healthy malaria-naive adults in the United Kingdom to identify prime-boost vaccination regimens that optimize the cellular-mediated immune response and generate protective immune responses (7). Some of the tested regimens provided a prolongation of pre-

patent period, while other regimens also completely protected a portion of volunteers against heterologous strain sporozoite challenge. The safety of these vaccine candidates was investigated in adults and children in The Gambia (2).

v. **Plasmid DNA vaccines**

Investigators have developed a vaccine platform that uses combinations of plasmid DNA vaccines that permit targeting of one or more *P. falciparum* antigens (15). The Phase 1 study of the CS construct when injected as a single vaccine showed it to be safe and well tolerated, but poorly immunogenic for antibody production (none detected), while most subjects developed cell-mediated immune responses, including antigen-specific cytolytic T cell responses and $CD8^+$ ELISPOT IFN- production (16).

vi. **Peptide CS vaccine for *P. vivax***

This vaccine is composed of three long peptides representing different fragments of the central and flanking regions of the common type *P. vivax* CS protein (17). A Phase 1 clinical trial in 69 malaria-naive healthy adult showed the vaccines were safe and well tolerated. Immune responses are being evaluated to assist in the design of Phase 2 (9).

***Blood stage vaccines***

A second strategy for the development of a malaria vaccine is to target immune responses against the asexual stage (blood stage) of the parasite. Effective vaccination with a blood stage vaccine would either prevent invasion of red cells or prevent complications, such as cerebral malaria, anaemia, renal failure and all the manifestations of severe malaria in pregnancy (18).

These complications, which occur only in *P. falciparum* infection, are likely due to cytoadherence of PfEMP-1 expressed on the surface of infected red cells, to CD36 or intercellular adhesion molecule-1 (ICAM-1) on microvascular endothelial cells or chondroitin sulphate A or other glycosaminoglycans in the placenta (19). Although PfEMP1, which was cloned in 1995 (20), is the prime target for an 'anti-complication' vaccine, creating such a vaccine is a very difficult challenge due to its extraordinary variation and clonal switching, the characterization of which has now begun (21).

The principal target of current asexual stage vaccine development is the merozoite, the stage that is initially released from the infected hepatocyte and rapidly invades and replicates in circulating red blood cells (9). Erythrocyte invasion is an energy-dependent, active process that requires the merozoite to contact, adhere, and orient to the red blood cell membrane. During invasion, the membranes fuse, thereby permitting the parasite to become internalised without otherwise damaging the red blood cell (7).

This complex process is rapid (taking only seconds) and involves a number of parasite proteins that are located on the surface of the merozoite and that are thus transiently accessible to circulating antibodies (8). The most well studied antigens include merozoite surface protein 1 (MSP-1), MSP-2, MSP-3, and apical membrane antigen 1 (AMA-1). Antibodies to these molecules are reported to block invasion of merozoites, except for MSP-3, in which they trigger a monocyte-mediated effect. MSP-1, AMA-1, and MSP-3 have been

produced as candidate malaria vaccines and have been shown to protect non-human primates from uncontrolled asexual stage parasitaemia when administered with Freund's complete adjuvant (9).

**i. Merozoite surface protein**

Investigators have produced a C-terminal *P. falciparum* MSP-1 vaccine as a lyophilized recombinant antigen expressed in *Escherichia coli* (7). The safety and immunogenicity of the vaccine has been assessed in two clinical trials conducted in malaria-naive individuals and has been successfully completed, the vaccine is scheduled to begin Phase 1 studies in Kenyan children 1–4 years old living in a malaria-endemic region of western Kenya (22). A phase I trial in Washington DC produced sero-conversion in 9 out of 16 volunteers immunised with the higher dose, but with hypersensitivity problems after the third dose (23).

**ii. Apical Membrane Protein (AMA-1)**

While AMA-1 is an attractive candidate antigen, there is considerable antigenic polymorphism that could limit its impact in the field (7). Data from animal studies indicate that antisera raised against one form of the molecule that efficiently block the growth of the homologous parasite are less efficient at blocking the growth of heterologous parasites. This has led to the production or clinical grade antigens from two variants of the *P. falciparum* AMA-1: AMA-1(3D7) and AMA-1(FVO) (24).

**iii. AMA-1/MSP-1 chimera**

This combination contains conserved portions of both AMA-1 and MSP-1 proteins in an attempt to avoid the problems associated with sequence polymorphism (25). A Phase 1 trial in malaria-naive healthy adults is underway with a Montanide ISA 720 formulation (7).

**iv. Merozoite surface protein III**

A remarkable feature of this vaccine is the full conservation of the MSP-III critical epitopes. These epitopes were selected on the basis of data indicating that they were targets of human cytophilic antibodies that interact with monocytes to mediate antibody-dependent cell-mediated parasite killing (26). A Phase 1 dose ranging study was recently completed and showed that the formulations were safe; well tolerated with alum, and mildly reactogenic with Montanide ISA 720 (7).

**v. Glutamate-rich long synthetic peptide**

Glutamate-Rich Protein (GLURP) is a 128- amino acid synthetic peptide vaccine produced using the partial sequence from GLURP (7). A Phase 1 trial in normal healthy malaria-naive volunteers has been recently concluded using vaccine formulated with alum and Montanide ISA720. Local reactogenicity was noted with both preparations including swelling at the contralateral injection site with subsequent injections (7).

**vi. SPf66**

This is the only malaria vaccine to undergo extensive field trials in Latin America, Africa, and Southeast Asia. It a synthetic peptide cocktail developed in Colombia (27). In field trials, the vaccine, under conditions of high as well as low malaria transmission, gave partial protection in some studies and none in others (28). An analysis of all published trials to date in various age groups and epidemiologies concluded that SPf66 had a combined efficacy of 23% (29).

***Transmission-blocking vaccines***

Preclinical studies conducted over the past decade have clearly demonstrated that antibodies directed against several sexual stage antigens are capable of preventing the development of infectious sporozoites in the salivary glands of *Anopheles* mosquitoes, thereby suggesting that so-called transmission blocking vaccines (TBV) might be an effective weapon against malaria (7).

More than half a dozen prefertilization target antigens have been discovered some notable ones include Pf230, Pf48/45, and Pf11.1 (9). Studies have suggested that antibodies to Pfs230 may be complement dependent, and therefore inclusion of such an antigen in a vaccine may require an appropriate adjuvant to induce antibodies of the right isotype (9). A caveat is that in *P. vivax, transmission*-blocking antibodies, produced as a result of natural infection, may enhance transmission when present in low dilution (30).

**i. Plasmodium vivax s25 and P. falciparum s25**

The purification and characterization of this candidate vaccine has just recently been achieved. (7). A formulation adjuvanted with aluminum hydroxide is currently in a Phase 1 clinical trial. Similar preclinical data have been obtained with Pfs25 expressed in *P. pastoris*, setting the stage for a Phase 1 trial with this vaccine candidate (7).

***Multistage, multivalent vaccines***

There is a good case that malaria vaccines should be multistage and multivalent. As already discussed, a vaccine directed against one stage, if not fully effective, has no activity against later stages in the life cycle, hence, a malaria vaccine is expected to be more effective if it contains antigens against more than one stage of the parasite.

**i. NYVAC-Pf7**

This is a genetically engineered, attenuated vaccinia virus, multistage, multicomponent *P. falciparum* vaccine that includes the transmission-blocking Pfs25; the pre-erythrocytic antigens CSP, SSP2/TRAP, and liver-stage antigen 1; and the asexual blood-stage antigens MSP-1, AMA-1, and SERA. (9) This recombinant vaccine has been through phase I-IIa safety and efficacy studies, but the results in terms of protection were disappointing.(31).

**OBSTACLES TO VACCINE DEVELOPMENT**

Antigenic variation, diversity, and immune evasion represent one of the greatest hurdles to the development of both natural immunity to malaria and an effective subunit vaccine. (3). It is known, that a monoclonal antibody specific for one allelic variant of *P. yoelii*, MSP-119, does not recognize other variants (32). One vaccine strategy to overcome this obstacle would be to combine all the known allelic sequences together in a vaccine. The success of such a strategy would depend on how many epitopes would need to be combined and whether the immunogenicity of the individual epitopes would be preserved when mixed with many other epitopes. Such strategies are being attempted for malaria as well as for other pathogens (3).

The parasite protein on the erythrocyte surface (PfEMP1) performs the critical function of binding the parasite to the endothelium to protect the infected erythrocyte from sequestration in the spleen (19). Epidemiological data have demonstrated that anti P*f*EMP – 1 antibodies

provide protection against disease (33). However despite this apparent role in the development of antimalarial immunity, the use of P*f*EMP – 1 in vaccine development is hampered by the extensive polymorphism in the var gene family (34).

**PROSPECTS**

The foregoing gloomy picture of malaria and the armory of methods to combat its ravages, has led to conclusion that vaccination against *P. falciparum* and *P. vivax* is the method of intervention with the greatest potential to reduce the morbidity and mortality associated with severe malaria in areas of intense transmission (9). As with antimalarials, there is little commercial incentive for industry to invest in development of vaccines against any human parasite, including malaria (4). A malaria vaccine represents a major challenge, even with unlimited resources to devote to the task.

To have a large impact, a vaccine must be cheap, safe effective and easy to administer. The slow progress to date is an indication of the magnitude of the task and the lack of industrial interest to invest in development of vaccines against any human parasites, including malaria (9). In the decades after 1950, several dominant ideas set the stage for vaccine research. First it was argued that antibody was the mechanism of immunity, but now both cellular and humoral immunity, are now known to control the immune response and that both play critical roles. Another major development was the ability to culture *P. falciparum* parasite in vitro, making malaria research possible in any laboratory in the world.

Over the past five years, there has been increased funding from public-private partnerships and the result has been encouraging. There has been a remarkable increase in the quality and number of candidate vaccines entering clinical trials and considerable optimism that major progress is being made. Indeed, there are currently three vaccine candidates (RTS, S/AS02A, MVA-ME TRAP, and MSP-1/AS02A) being studied. The recent success recorded in the phase 2b testing of RTS, S/AS02A among 1-4 year old children in Maputo Province, southern Mozambique is a welcome development (12). There is increasing involvement on the part of industry that brings crucial, pre-clinical experience, manufacturing know-how, and clinical development capabilities that will significantly increase the chances of success.

The not-so-good news is that the investigators in developing countries where the disease is ravaging are not with strong clinical and experimental development skills. More importantly, there are clearly insufficient financial and personnel resources identified to date to support the complete development of the most promising candidate vaccines. Indeed, the clinical development of one such candidate that underwent promising field studies in Papua New Guinea was recently abandoned largely due to lack of sufficient financial and industrial resources (35). The way forward will require that researchers will continue to refine target product profiles to be very clear on where and how the vaccines will be used and that there be greater public-private partnership involvement in these discussions.

Malaria vaccine development is at an exciting and very promising stage; it is essential that the momentum achieved to date be sustained in the years ahead.

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# 'MIASMA' THEORY AND THE POSSIBILITY OF MALARIA ERADICATION

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**Malaria as a disease entity caused by plasmodium species only became recognized towards the end of the 19th Century. Prior to that time, Mal'aria or 'bad air' was believed to be the cause of fevers or paludal. This article traces the history of 'Miasma' theory which had been accepted for Centuries before the 'Germ' theory became established. Comparing the 'Miasma' theory with current understanding of Africans about disease causation, it was concluded that there are great similarities. It is therefore recommended that concurrent application of both the 'Germ' theory and the 'Miasma' theory could lead to a more effective control or even global eradication of malaria.**

**Key words: Miasma, malaria, eradication**

## BACKGROUND

The role of the environment in the causation of malaria became clearer with the demonstration of the plasmodium under the microscope by Laveran in 1880 (1). Since that time the germ theory has become established in the causation of diseases, particularly communicable diseases. The further discovery by Ronald Ross in 1897 of the mosquito as the vector of malaria finally nailed the previous theories about causation of diseases (1).

For many decades, quinine and later chloroquine seemed to be the solution to the treatment of malaria. Unfortunately the development of resistance to chloroquine by plasmodium that started in the 1960's became a concern to the medical profession. Various reasons and research findings have been advanced for the probable causes of this resistance. These have included mutation, plasmid transfer, genetic alteration, cell membrane alteration and selection exhibited by the parasite. These various methods have provided answers but not absolute solutions to the problem of malaria (2). On the other hand, the effect of altering the environment was also recognized as being effective in the control of malaria. Hence, measures such as draining of swamps, prevention of stagnant water around houses and other buildings and filling up of ponds and podles were recognized means of reducing mosquito population. In addition, spraying of the surrounding of premises and fields with insecticides such as DDT and the inside of living quarters with combined knockdown and residual insecticide products have been used over the years (3, 4).

However, just like the protozoon plasmodium, the Anopheles mosquito has increasingly developed resistance to these insecticides which had previously proved effective. These developments have therefore resulted in new drugs being developed in the quest to effectively control the disease. Also new techniques of reducing man/mosquito contact were developed with the hope that malaria transmission would eventually be effectively reduced to the barest minimum. The latest technique advocated being the

use of insecticide treated nets. In addition to the above stated knowledge of malaria causation, other environmental conditions such as temperature, vegetation, humidity and topography have been known to be important factors.

Yet despite this vast knowledge about the epidemiology of malaria, the battle against the disease is very far from being over. It is in the light of this development that this proposal is being made in order to stimulate interest in a theory which for centuries had been given consideration in disease causation but was suddenly abandoned for the germ theory. The intention is to establish the fact that this theory and the later embraced germ theory do not necessarily have to be mutually exclusive. Indeed the full understanding of this theory will greatly enable us to understand better the germ theory.

**THE 'MIASMA' THEORY: HISTORICAL ASPECT**

As early as 95BC, Lucretius hypothesized that swamp fever might result from a living organism. However up till the late 19th Century, it was generally believed that 'fevers' (from Italian mal'aria) was caused by a poisonous vapour or "miasma" released from swamps. Climate, season and geographical location were all thought to influence the outbreak of fevers that were thus often referred to as 'paludal'.

However it was known that not all swampy areas were malarious and fevers could be acquired in other geographical zones (1). It was not until mid 1800s that many scientists began to rally behind the animalcular theory (or organismic theory), the precursor of the germ theory which was to come later.

The major breakthrough to confirm these scientific hypotheses occurred in 1880 when the French Physician, Charles Louis Alphonse Laveran discovered the malaria parasite. In spite of Laveran's discovery of the *Plasmodium falciparum*, a large part of the scientific community continued to remain skeptical. It was only in 1887 that William Osler, the great American Physician, first Professor of Medicine and eventual Chair of the Johns Hopkins University, finally gave credit to Laveran and acknowledged the pathogenic nature of the parasite and its aetiological association with the disease (2-4).

In 1897, Ronald Ross, encouraged by Patrick Manson to investigate his hypothesis, saw under the microscope, female pigmented crescents of the parasite in the wall of the stomach of the mosquito *Anopheles stephensi*. In 1898, Ross found the sporozoites of the plasmodium in its digestive tract. This led him to the realization that the germinal rod might be extruded during excretion of the mosquito's saliva and that it might be a way of spreading the disease (2). Ross was of course guided by Patrick Manson's scientific input and intellectual impetus and it was coincidental also in 1898 that Manson founded the London School of Tropical Medicine.

In later years, experiments by other scientists including Giovanni Batista Grassi, Patrick Manson, George Carmichael Low, Louis Sambon and A. Terri were later to establish that the malaria parasite, plasmodium was transmitted to humans by the mosquito (2, 3, 4). With this new knowledge therefore, the 'Miasma' theory

was to receive a final blow within a few years (2).

**'MIASMA' THEORY REVISITED**

It is hereby being advocated that the 'miasma' theory be revisited and seen in the light of knowledge now available in modern science. At the beginning of time earlier referred to, fevers were believed to be caused by contagion and miasma. Contagion of course referred to infections acquired directly from person to person through the air either by sneezing or breathing or by other means by the affected person. On the other hand, 'miasma' referred specifically to fevers acquired from swamps or other water bodies or even waste dumps from which a poisonous vapour (miasma) arises which could result in fever and ague, sore throat, lung diseases and other fevers.

In those days, definitely such knowledge was useful in controlling the environmental problems of European cities. For example, the improvement in general environmental sanitation in Europe including draining of the swamps around the city of Rome, enforcing strict refuse and sewage disposal regulations and prevention of air pollution were instrumental in controlling many communicable diseases. Hence such epidemics as ague, plague, typhoid and malaria were effectively controlled (3, 5). This was during the time the 'miasma' theory was widely accepted in Europe and before the full establishment of the germ theory. If the 'miasma' theory helped Europe so greatly in its development of Public Health why did it become so suddenly relegated to the background at the expense of the germ theory?

**THE AFRICAN EXPERIENCE**

At present, Africa and Asia are in a worse situation as far as Public Health situation is concerned than even Europe before the turn of the 19th Century. Environmental sanitation continues to deteriorate in Africa with attendant health implications (6). Communicable diseases are rampant including vector-borne infections like malaria, yellow fever among others. Open drains, open refuse dumps, stagnant water bodies, indiscriminate faecal disposal and overgrown bushes exist in abundance in the various communities encouraging breeding of mosquitoes and other vectors as well as vermins such as rats and snakes (6), a situation similar to what existed in Europe before environmental sanitation laws were put in place.

Casual observation of the Nigerian community of which this author has had considerable experience has shown that the present African environment definitely needs the same understanding that the European environment had before the turn of the 19th Century. For example, the understanding of the African view about disease causation is not different from what it was during the 'miasma' theory period prior to the mid 19th Century. This may sound retrogressive but unfortunately it is true. It is therefore necessary for the Public Health Practitioners in Africa to understand this historical perspective before setting out to solve the environmental sanitation problems in Africa.

Among most Nigerian ethnic groups including Yoruba, Nupe and Hausa, the beliefs about disease causation especially fevers are similar to the 'miasma' theory. The Yoruba people, for example, believe that fevers (Iba) could be caused by inhaling

'invisible substances in the air' into the lungs. Unfortunately because this is an African belief, it has been labeled up to this moment as superstition without the modern scientists considering that such beliefs were rampant in the developed world, at least, at one time (2).

**'MIASMA THEORY': POSSIBLE CLUE TO MALARIA PARASITE RESISTANCE**

Most Physicians working in the tropical and subtropical areas of the world have been confronted by the issue of treating supposed malaria fever in the same patient repeatedly. Often this failure in treatment has been attributed to the development of resistance by the plasmodium in the blood (7, 8). While this has been proved to be correct in certain situations, it has also been observed that not all such cases could be explained on the basis of resistance. For example, in Nigeria, such observations have been explained by the fact that many of the drugs in circulation are fake, usually not containing the active ingredient in the expected pharmacological concentration in the tablets or capsules.

On the other hand, certain attacks of malaria could only be explained on the basis of recrudescence in which case, plasmodium species somehow re-enter the blood from a quiescent stage in the liver especially in the case of *Plasmodium malariae*.

Malaria recrudescence has also been used to explain a situation when a victim from a malaria endemic zone suddenly develops a bout of malaria more than a year after returning from such an area. The 'miasma' theory could explain such attacks of malaria and much more, if it is first realized that 'miasma' (vapour) inhaled from swamps and effluvium of dirty environment such as refuse dumps as previously understood could cause fevers (not malaria fever alone), hence the unexplained 'Pyrexia of Undetermined Origin' (PUO).

According to the 'miasma' theory, fevers are caused by inhaling miasma (9). What this means, in the light of modern knowledge, is that the demonstration of malaria parasites in the blood would not necessarily account for the associated fever. This fact has been demonstrated in some parasite rate studies in the African. Malaria fever is an entity, no doubt and should not explain away the fevers caused by miasma. In other words, malaria fever and the 'miasma' theory should not be mutually exclusive. It is to be realized that just as 'contagion' is still recognized in modern science and medicine, 'miasma' also should be reinstated to its former status albeit with modern scientific understanding. It could be the only explanation for the cause of many fevers either directly or indirectly by reducing resistance to the various 'animalcule' that were later discovered.

Many doctors in Nigeria have had to treat fevers which responded to anti-malarial drugs but with no demonstrable plasmodium organisms in the blood. On the other hand, two or more members of the same family have sometimes reported in clinics having fevers at the same time yet living in environments which are well netted.

While agreeing that such situations could arise occasionally, the frequent occurrences however, points to a common factor other than mosquito bites, especially when there is no demonstrable malaria

parasite in their blood. The trend in the country's medical practice currently is to label such cases as typhoid fever usually after only one Widal test, rather than a rising titre, had been performed as a diagnostic proof.

**'MIASMA' THEORY: PROBABLE ANSWER TO ERADICATION OF MALARIA**

Rather than totally discard the 'miasma' theory, it is being proposed that it should be reconsidered. Indeed it may be found in the near future to compliment the germ theory rather than being antagonistic. It may even be the 'gap' in our present level of understanding of causes of diseases especially febrile ones. In fact, given the present knowledge of the African concerning disease causation, the 'miasma' theory would appear more appealing than the 'germ' theory and could even elicit his cooperation in utilizing local knowledge and technologies in finding a lasting solution to malaria control (10).

It may even be the first step towards eventual eradication – a feat which is not impossible if the African people and their beliefs and traditions are taken into consideration (10), for it is only through the full participation of the African people themselves (i.e. community participation) which is one of the principles of Primary Health Care that malaria eradication will be possible. In this regard, many African scientists with full orientation in Public Health are needed in this final onslaught against malaria.

**THE WHO ROLL BACK MALARIA PROGRAMME: A STEP TOWARDS ERADICATION**

The roll back malaria programme of the World Health Organization (WHO) is a right step in the right direction. The programme has further received a boost from the commitment of the African Regional Office of the WHO recognizing the fact that an African solution is necessary to the problem through the use of local knowledge and taking into consideration the social and cultural peculiarities of the people (11). Studies on the African environment, which could explain such phenomenon as 'invisible substances' in the air or 'miasma' are needed, preferably by Environmental Health Physicians, Environmental Health Hygienists and other Public Health Practitioners.

Currently the Department of Epidemiology and Community Health of the College of Medicine, University of Ilorin (a WHO Collaborating Centre in Community based Education and Service) runs a one year Master in Public Health (MPH) programme which is inter-disciplinary. This means that candidates from disciplines that are medical or health related are admitted into the programme. In this unique programme, post-graduate students from these diverse backgrounds, including Doctors, Senior Nurses, Pharmacists, Health Educators, and Public Health Engineers among others interact with one another. Through this interaction they are able to see the solution to the problems of the African Environment from both individual and collective perspectives. It is believed that it is from such a Training Centre as this, that scientists will go out in the nearest future to solve the malaria problem together, using the knowledge and local techniques available in Africa once and for all.

**CONCLUSION**

The eventual eradication of malaria will depend on using the local knowledge

and technologies available in Africa and other developing areas of the world. The present impasse in malaria eradication is due to the fact that such local resources are still not accepted in the scientific world. The 'Miasma' theory is in line with the African belief about disease causation. The concurrent application of the 'Germ' theory and the 'Miasma' theory will greatly contribute to solving the malaria problem as they do not necessarily have to be mutually exclusive.

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# EPIDEMIOLOGICAL STUDY OF ASYMPTOMATIC BACTERIURIA AMONG NURSERY SCHOOL CHILDREN IN AHVAZ, IRAN

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**This study was undertaken to determine the prevalence of asymptomatic bateriuria in preschool children of different age and sex groups and to isolate the organisms responsible for asymptomatic bacteriuria and determine their antimicrobial susceptibility pattern. A total of 475 children from 17 nurseries in Ahvaz city, Iran were screened by collecting mid-stream urine samples for the presence of causative organisms. Asymptomatic bacteriuria was observed in 16 cases (3.36%). The isolated pathogens included *Escherichia coli* (50 %), *Proteus mirabilis* (25 %) and coagulase negative staphylococcus (25 %). The antibiograms indicated that Gentamicin (100 %), Nalidixic acid (100 %), Amikacin (75%), Kanamycin (75 %), Nitrofurantoin ( 62.5 %), Tobramycin ( 62.5 %), Tetracycline ( 50 %) and Chloramphenicol (37.5%), were in that order the most effective of the antibiotics tested against *E. coli* isolates. *Proteus mirabilis* showed 100% sensitivity to Amikacin, Gentamicin, Kanamycin, Nalidixic acid and Tobramycin followed by Nitrofurantoin (75%), Cephalotin (50%) and Chloramphenicol (50%). Similarly, antibiogram of coagulase negative staphylococcus showed 75% sensitivity to both Nalidixic acid and Kanamycin. This result indicated a significant rise in the frequency of *Escherichia coli* in asymptomatic bacteriuria.**

**Key words: Asymptomatic bacteriuria, children, urinary tract infections**

## INTRODUCTION

The presence of significant number of bacteria in the urine of asymptomatic patient has been the subject of several long-term studies in the preschool aged children (1-4). Many patients with asymptomatic bacteriuria will declare symptoms of urinary tract infection when questioned closely; many will have intermittent episodes of symptomatic bacteriuria (5-6).

Screening for asymptomatic bacteriuria was undertaken with the belief that early detection of infection and identification of structural abnormalities coupled with appropriate management might lead to prevention of pyelonephritis and renal damage. It is not known how frequently these infections lead to kidney damage (7) or whether their eradication can prevent this outcome, it would be necessary to screen the preschool children of a particular age group. This would be a tedious task unless a simple and economical screening procedure can be used which will be acceptable to the children, parents, school health authorities and clinical microbiologists.

The present study was performed by analyzing urine samples collected from preschool children (age groups, 3-7 years) to determine the incidence of asymptomatic bacteriuria in this group, isolate the responsible organisms and determine their antimicrobial susceptibility pattern.

## MATERIALS AND METHOD

### *Culture media*

Blood and MacConkey agar were used for isolation of pathogens. Diagnostic media used for further characterization of pathogens were Triple Sugar Iron (TSI) agar, Oxidation Fermentation (OF) medium, Lysine Iron agar, SIM medium, Simon Citrate agar, Urea agar, Malonet broth and Triple sugar Iron media. All culture media and reagents were purchased from Biomrieux, France.

***Method***

A total of 475 preschool children aged 3 to 7 years from 13 different nursery schools in Ahvaz, Iran, were screened during 2001-2002 for asymptomatic bacteriuria. The study group comprised of 230 boys and 245 girls. Care was taken to notify the Head Masters/Mistress and other staff about the investigation to be carried out. As the collection of urine sample from the children was difficult, the instruction to the parents on the collection of mid-stream urine sample was typed on a paper and copies were distributed to the children along with sterile wide mouth bottles.

The mid-stream urine samples collected from all the children were transported to the laboratories in ice pack within one hour. In the laboratory, the specimens were examined microscopically for the presence of pus cells, red blood cells and casts.

A standard wire loop (0.01ml capacity) was used to place urine on MacConkey and Blood agar media. The plates were examined after overnight incubation aerobically at 37°C, to quantify the organisms present. All the plates with significant colony growth were examined and colonies identified using the procedures described by Baron and Finegold (8).

***Antibiogram***

Susceptibility testing was carried out on the identified microorganisms using the disk diffusion procedure as described by Jacques and Goldstein (9).

**RESULTS**

Amongst the 475 urine samples, 16 (3.36%) were positive for asymptomatic significant bacteriuria. The frequency of *E. coli* was found to be (50 %) which was followed by *Proteus mirabilis* and coagulase negative staphylococci (25%) (Table 1).

**Table 1: Percent of bacterial isolates among asymptomatic bateriuria in preschool children**

| Organisms isolated | Percent (%) |
|---|---|
| *Escherichia coli* | 50 |
| *Proteus mirabilis* | 25 |
| Coagulase-negative staphylococci | 25 |

Table 2 revealed antibiotic sensitivity pattern of various isolates. Gentamicin was the most effective against *E. coli* isolates and Amikacin was a close second. These isolates were 62.5 % sensitive to Nitrofurantoin and almost less than 50% were sensitive to other antibiotics tested. The *P. mirabilis* isolates were 100 % sensitive to Amikacin, Gentamicin, Kanamycin, Nalidixic acid and Tobramycin. These isolates were 50 and 75% sensitive to Cefalotin, Chloramphenicol and Nitrofurantoin respectively. Similarly, coagulase negative staphylococci were 25% sensitive to Ampicillin, Amoxicillin, Cefalotin and Penicillin G. However, these isolates were 100 % sensitive to Vancomycin.

**Table 2: Percentage antibiotic resistance of isolates**

| Antibiotic | *E. coli* | *P. mirabilis* | Coagulase-negative staphylococci |
|---|---|---|---|
| Gentamicin | 25 | 0.0 | - |
| Amikacin | 25 | 0.0 | - |
| Ampicillin | 100 | 100 | 75 |
| Cephalotin | 75 | 50 | 75 |
| Ampicillin | 100 | 100 | 75 |
| Nalidixic acid | 0.0 | 0.0 | 25 |
| Nitrofurantoin | 37.5 | 25 | - |
| Tobramycin | 37.5 | 0.0 | - |
| Streptomycin | 45 | - | 100 |
| Chloramphenicol | 62.5 | 50 | 50 |
| Tetracycline | 50 | 100 | 50 |
| Kanamycin | 25 | 0.0 | 25 |
| Amoxicillin | 75 | - | 75 |
| Penicillin G | 75 | - | 75 |

**DISCUSSION**

Although, symptomatic and asymptomatic bacteriuria during infancy

is generally characterized by a benign outcome, this phenomenon amongst preschool children rarely leads to end stage renal failure, however, it can not be ignored. It may be the first clue to the important underlying anatomical abnormalities in some patients and can pose a major risk to a child's well being. However, in some children episodes of renal damage have been reported (10).

Although a variety of pathogens have been identified as causing urinary tract infection (11-12), enterobacteriaceae are usually the cause of initial uncomplicated lower tract infections. The organisms most frequently isolated in asymptomatic bacteriuria and urinary tract infection includes species of enterobacteriaceae especially *E. coli* and other Gram-negative bacteria (13). Microbiological culture examination of urine samples from preschool children resulted in the isolation of eight *E. coli* (50 %), four *P. mirabilis* (25 %) and four coagulase negative staphylococci (25 %) bacterial species. Our finding is consistent with earlier reports (2, 12). Several well-documented clinical studies on asymptomatic bacteriuria among school children have reported the prevalence of significant bacteriuria of approximately 0.7 to 3.2% (11, 13).

Our results show the rate for asymptomatic significant bacteriuria to be 3.36%. Therefore, screening infants, toddlers and preschool children for significant bacteriuria might be beneficial in preventing renal damage and other abnormalities.

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**ORIGINAL ARTICLE**

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# CHARACTERISTICS OF NOSOCOMIAL MRSA IN ASSIR CENTRAL HOSPITAL, ABHA, KINGDOM OF SAUDI ARABIA

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**The objective of this study is to determine the characteristics of nosocomial methicillin-resistant and sensitive *Staphylococcus aureus* (MRSA & MSSA) and their minimum inhibitory concentration (MIC) to vancomycin and oxacillin. Over a six-month period a study of *Staphylococcus aureus* isolates from clinical specimens of patients with nosocomial infections in Assir Central Hospital (ACH), Abha, Saudi Arabia, between September 2003 and February 2004, was carried out. Isolation and identification of *Staphylococcus aureus* was performed using standard microbiological methods. MIC to vancomycin and oxacillin was carried out using the E-test strips. Eighty-five *Staphylococcus aureus* isolates were identified. These were made up of 39 (45.9%) MRSA and 46 (54.1%) MSSA. The MIC to oxacillin showed that 37/39 (94.9%) MRSA had MIC >256 µg/ml and only 2/39 (5.1%) had MIC of 4 and 32 µg/ml. Thirty of forty six (65.2%) of the MSSA had MIC < 0.50 µg/ml and 16/46 (34.8%) had MIC of between 0.50 -2 µg/ml. All the 85 isolates were fully sensitive to vancomycin (MIC breakpoint < 4 µg/ml). There is even distribution of sensitivity pattern to vancomycin among MRSA and MSSA isolates. 31/39 (79.5%) of MRSA had MIC of 2 µg/ml while 34/46(74.0%) of MSSA had MIC of 2 µg/ml. The prevalence of MRSA in nosocomial infections in ACH is 45.9%. Thirty-seven out of thirty-nine (94.9%) of the MRSA strains show high resistance to oxacillin (MIC > 256 µg/ml). The use of oxacillin-related drugs to treat nosocomial Staphylococcal infections in ACH should be reviewed and infection control practices should be intensified so as to stem any future increase in MRSA prevalence in the hospital.**

**Key words: MRSA; Characteristics; MICs; Vancomycin; Oxacillin.**

## INTRODUCTION

*Staphylococcus aureus* is one of the most common causes of both hospital and community acquired infections worldwide [1]. Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus epidermidis* (MRSE) levels vary from 5-75% world-wide [2]. Strains of *Staphylococcus aureus* which are oxacillin and methicillin resistant (historically termed methicillin resistant *S. aureus* or MRSA), are traditionally resistant to all beta-lactam agents including cephalosporins and carbapenems as well as commonly used antimicrobial agents used as treatment options on patients infected with such strains [3]. Vancomycin has been used to treat many *Staphylococcus aureus* infections, particularly those caused by MRSA [4].

Accurate detection of methicillin and vancomycin resistance can be difficult using the routine disc-diffusion technique due to the presence of two sub-populations (one susceptible and the other resistant to oxacillin) that may coexist within a culture. Although, all cells in a culture may carry the genetic information for resistance, only a small number can express the resistance *in vitro*. This phenomenon is termed 'heteroresistance' and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin. Heteroresistance frequently presents diagnostic problems to clinical laboratories, because cells expressing resistance may

grow more slowly than the susceptible population. One of the simplest and reliable methods of detecting heteroresistance in staphylococci is by using the Epsilon (E)-test (AB Biodisc, Solna, Sweden).

Conscious of the limitations of our routine disc-diffusion method to detect heteroresistance in *Staphylococcus aureus* isolates in our hospital, we decided to carry out E-test MIC determinations to oxacillin and vancomycin on *S. aureus* from nosocomial infections over a six-month period, September 2003 to February 2004. Our findings are presented and discussed in this communication.

**MATERIALS AND METHOD**

*Staphylococcus aureus* isolates were obtained from clinical specimens of patients with nosocomial infections in Assir Central Hospital (ACH), Abha, between September 2003 and February, 2004. The isolation and identification of *Staphylococcus aureus* were performed using standard microbiological methods [5]. Routine sensitivity testing to Gram positive antibiotics were carried out on Mueller Hinton agar according to NCCLS guidelines [6]. Strains with zone diameter ≤ 12 mm to 1μg oxacillin disc were regarded as methicillin resistant.

***E-test method***

E-test strips for oxacillin and vancomycin (0.016-256 μg/ml), from AB Biodisc, Solna, Sweden, were used for the study. Mueller Hinton agar plus 2% sodium chloride was used as the test medium. The *Staphylococcus aureus* isolates were sub cultured on 5% sheep Blood agar and incubated at 35°C in ambient air for 18-24 hours. Direct colony suspension was made in normal saline to 0.5 McFarland turbidity. The resulting suspension was inoculated on to Mueller Hinton agar containing 2% NaCl, using sterile cotton-tipped swabs. The E-test strips were placed on the inoculated plates aseptically. The plates were then incubated in ambient air at 35°C for 24 hours before reading. Quality control strains were included in every batch of tests.

Isolated colonies in the inhibition ellipse and fine hazes growing beyond the breakpoint are indicative of resistance. *Staphylococcus aureus* were considered oxacillin susceptible when the MIC is ≤ 2 μg/ml and resistant when oxacillin MIC is ≥ 4 μg/ml (Quality control *S. aureus* strain, ATCC 25923, MIC range 0.25-0.50 μg/ml) and vancomycin sensitive when vancomycin MIC is ≤ 4 μg/ml or resistant when MIC is ≥ 8 μg/ml (Quality control *S. aureus* strain, ATCC 25923, MIC range 1.5-3 μg/ml).

**RESULTS**

Out of the 85 *Staphylococcus aureus* isolates, 39 (45.9%) were MRSA while 46 (54.1%) were methicillin sensitive *Staphylococcus aureus* (MSSA). The MSSA strains had very low MIC to oxacillin, 30 (65.2%) had MIC less than 0.50 μg/ml. In contrast, the MRSA were highly resistant to oxacillin; 37 (94.8%) had MIC > 256 μg/ml (Table 1). The MIC to vancomycin was more evenly distributed between the MRSA and MSSA strains; 31/39 (79.5%) and 34/46 (73.9%) respectively, had MIC of 2 μg/ml (Table 1).

Table 1 also shows the antibiogram to some commonly used antibacterial drugs for treating Gram-positive cocci infections. Apart from penicillin G, these relatively cheap drugs are still effective against MSSA strains. On the contrary, only vancomycin was effective against the MRSA strains.

**Table 1: E-Test MIC and Antibiogram of MRSA and MSSA isolates to some routine antibiotics**

| | E-TEST MIC | | | | | | ANTIBIOGRAM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vancomycin MIC (µg/ml) | | | Oxacillin MIC (µg/ml) | | | VA n (%) | OX n (%) | PG n (%) | KF n (%) | CD n (%) | E n (%) | TS n (%) | OT n (%) | GM n (%) |
| | 2 | 3 | ≥4 | ≤2 | 4-32 | >256 | | | | | | | | | |
| MRSA n =39 | 32 (82.1) | 7 (17.9) | 0 (0.0 | 0 (0.0 | 2 (5.1) | 37 (94.9) | 39 (100 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (10.3) | 0 (0.0) | 1 (2.6) | 6 (15.4) | 6 (15.4) |
| MSSA n =46 | 39 (84.8) | 7 (15.2) | 0 (0.0) | 46 (100 | 0 (0.0) | 0 (0.0) | 46 (100 | 0 (0.0) | 0 (0.0) | 43 (93.5) | 41 (89.1) | 36 (78.3 | 42 (91.3) | 37 (80.4) | 45 (97.8) |

Percentages susceptible are shown in brackets ( )
Abbreviations: VA=Vancomycin; OX=Oxacillin; PG=Penicillin G; KF= Cephalothin; CD= Clindamycin; E=Erythromycin; TS = Co-trimoxazole; OT = Oxytetracycline; GM=Gentamicin

## DISCUSSION

Methicillin resistant *Staphylococcus aureus* (MRSA) has become a global concern. They are common causes of both hospital and community-acquired infections [7, 8]. Both forms of infections have been reported from the Kingdom of Saudi Arabia [9, 10]. Detection of MRSA has been problematic when using different antimicrobial susceptibility testing methods [11]. Consequently, NCCLS recommended incubating isolates being tested against oxacillin at 35°C for a full 24hours before reading [6]. Similar problem is encountered when testing *S. aureus* for vancomycin susceptibility. One way of getting round this problem is by using the E-test method (AB Biodisc, Solna, Sweden).

The *S. aureus* vancomycin MIC breakpoint is 4µg/ml. Most susceptible strains have MIC between 0.5-2 µg/ml. In contrast, *S. aureus* isolates for which vancomycin MICs are 8-16 µg/ml are classified as vancomycin-intermediate and isolates for which vancomycin MICs are >32 µg/ml are classified as vancomycin resistant. Strains of staphylococci for which vancomycin MICs are 4-8 µg/ml are not reliably detected using the disc-diffusion method even when these tests are incubated a full 24 hours.

If disc diffusion is the primary method used in a laboratory for testing vancomycin, laboratories should also use a supplemental testing method such as vancomycin screen plate or MIC method. In our study, 76.5% of all our isolates (79.5% of MRSA and 74% of MSSA), have vancomycin MIC of 2 µg/ml while 16.5% have MIC of 3 µg/ml. This is a welcome development from the therapeutic perspective, confirming the absence of vancomycin resistant *S. aureus* in our hospital. It however calls for caution and constant vigilance especially when vancomycin-resistant enterococci have been reported from this hospital [12].

The results of the oxacillin MIC however are not as cheering as those for vancomycin. The MIC susceptibility breakpoint for *S. aureus* is 2µg/ml for oxacillin. The majority of the MSSA (65.2%) have MIC <0.5 µg/ml, while about 95% of the MRSA have MIC > 256 µg/ml. An earlier report from this hospital [13] reported 61% MRSA colonization in hospital personnel (not nosocomial infections). Although at that time MIC determinations were not performed, no isolate was resistant to vancomycin by the disc diffusion method.

The results of the antibiogram of our isolates show that MRSA are completely

resistant to all antibiotics except vancomycin. The MSSA on the other hand, show appreciable susceptibility to all the antibiotics tested except penicillin G. The greatest danger posed by MRSA lies in their multiple antibiotic resistance and their ability to enter the blood stream causing bacteraemia, thus, clinicians are left with few treatment options for infections caused by these organisms and the use of relatively toxic agents. While infection control efforts are being made to identify and control those factors that facilitate the acquisition and spread of MRSA at hospital and community levels, there is an urgent need to source for alternative effective drugs besides the glycopeptides, which currently are over-used in many hospitals, in view of the looming threat of the advent of vancomycin resistant *S. aureus*.

Fortunately, some new agents such as linezolid, quinu/dalfo (Synercid), daptomycin and oritavancin are coming into clinical practice, but these are still in the investigational stage. Since the prevalence rate of MRSA is almost 50%, it is only reasonable to suggest that clinicians should use oxacillin and related drugs to treat infections caused by *S. aureus* in our hospital, only after the susceptibility to oxacillin has been determined.

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**REVIEW ARTICLE**

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# *MYCOBACTERIUM AFRICANUM* – A REVIEW

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**Tuberculosis, a curable infectious disease, remains the leading cause of adult death. The HIV/AIDS epidemic has greatly exacerbate the already grave situation in the developing world by creating a deadly synergy each worsening the course of the other. *Mycobacterium africanum* is a subspecies of *Mycobacterium tuberculosis complex* (MTBC) and is isolated from tuberculous patients in certain parts of Africa. Genotypically, members of the MTBC are closely related, exhibiting 99.9% similarity at the nucleotide level and identical 16s RNA gene (rDNA) and 16s-23s rDNA spacer sequences. However, identification and discrimination between members of the MTBC are important for epidemiological purposes. This paper reviewed current knowledge about this subspecies.**

## INTRODUCTION

Tuberculosis (TB), a curable infectious disease, remains the leading cause of adult death. One third of the world's population is estimated to be infected by members of the *Mycobacterium tuberculosis complex* (*M. tuberculosis, M. bovis, M. africanum, M canetti* and *M. microti*) which are collectively responsible for about three million deaths each year, over 95% of which occur in developing countries (1, 2). The HIV/AIDS epidemic has greatly exacerbated the already grave situation in the developing world by creating a deadly synergy each worsening the course of the other (3).

*Mycobacterium africanum* is a subspecies of the *Mycobacterium tuberculosis complex* (MTBC) and is isolated from tuberculosis patients in certain parts of Africa. Castets and colleagues first described *M. africanum* in 1968 when it was isolated from sputum of a tuberculosis patient in Senegal (4). Genotypically, members of the MTBC are closely related, exhibiting 99.9% similarity at the nucleotide level and identical 16S RNA gene (rDNA) and 16S-23S rDNA spacer sequences (5-8). They however differ widely in terms of their phenotypic characteristics (such as colony morphology, growth rate, and biochemical profile), pathogenicity, epidemiology, geographic range, and host preferences (9, 10). For example, *M. tuberculosis*, *M. africanum* and *M. canetti* are exclusively human, while *M. microti* is a rodent pathogen and *M. bovis* can infect a wide range of mammals (Table 1). The close genetic relatedness, overlapping phenotypes and slow growth make species assignment difficult (5, 11).

In recent times, insights from comparative genomics employing differential hybridization techniques have revealed significant differences in the gene content and genome organization of this group of closely related species, particularly as a number of large sequence polymorphisms (LSPs) (12-15). These differences can be used to more accurately identify subspecies within the MTB complex. Identification and discrimination between members of the

MTBC are important for epidemiological reasons. One of the main reasons for the correct identification of *M. bovis* is its natural resistance to pyrazinamide, a widely used antituberculous drug. In addition, transmission usually occurs through contaminated dairy products and *M. bovis* prevalence rates can be used to evaluate the effectiveness of a bovine TB control program. In contrast, the epidemiologic implication of identifying *M. africanum* is uncertain in view of the limited studies and awareness of this subspecies. However, it is important to correctly identify species within the MTBC to monitor transmission especially in developing countries and zoonotic implications of bovine tuberculosis, and the sporadic report of *M. africanum* in European herds.

Although a number of genetic markers have been described in the literature useful in the identification of *M. tuberculosis* (*stricti sensu*) *and M. bovis*, few have been described for *M. africanum*. Furthermore, there is no single maker available commercially to clearly differentiate individual members within the MTBC or between the sub-species of *M. africanum*. This paper reviews current knowledge about this subspecies.

**Table 1: Some properties of members of the *Mycobacterium tuberculosis complex* (MTBC)**

| Species | Host range | Colony form | Niacin | Nitrate reduction | TCH | PZA |
|---|---|---|---|---|---|---|
| *M. africanum* | Humans | Dysgonic | +/- | +/- | R/S | S |
| *M. bovis* | Cattle, goats, lions Elephants, humans etc. | Dysgonic | - | - | S | R |
| *M. bovis* BCG | | Eugonic | - | - | S | R |
| *M. canetti* Humans | | Smooth | + | + | R | S |
| *M. microti* Voles | | Tiny | + | - | S | S |
| *M. tuberculosis* | Humans | Eugonic | + | + | R | S |

Adapted from Cole ST (46)

## BACTERIOLOGY

*M. tuberculosis* strains have functional nicotinamidase, produce niacin, reduce nitrate, and have enhanced growth in in the presence of glycerol, and are sensitive to thiophene-2- carboxylic acid hydrazide (TCH). In contrast, *M. bovis* strains lack nicotinamidase function, do not produce niacin or reduce nitrate, are not stimulated by glycerol, and are resistant to TCH. However, strains of *M. africanum* exhibit a high degree of phenotypic heterogeneity having biochemical and morphologic characteristics that are intermediary between strains of *M. tuberculosis* and *M. bovis*, complicating laboratory identification.

All members of the MTBC have a doubling time close to 24h and visible growth on solid media takes 3-4 weeks to form colonies on Petri dishes (16, 17) (Table1)

Difficulties in precisely defining or identifying *M. africanum* increase the potential to misclassification of clinical strains and complicating attempts to accurately determine the true prevalence of tuberculosis caused by *M. africanum* (18). Furthermore, data from pyrolysis mass spectrometry do not support a species status for this group of strains, causing some authors to question the validity of this species (10, 16).

## EPIDEMIOLOGY

*M. africanum* has been reported from several regions of sub-Saharan Africa and it is estimated to account for between

5% of patients with pulmonary TB in Ivory Coast and 60% of those in Guinea Bissau with variable proportion of subtypes (19-24). According to their biochemical characteristics, two major subgroups of *M. africanum* have been described, corresponding to their geographic origin. Subtype I or variant I, is a *M. bovis* like organism that is nitrate negative and predominantly clustered in West Africa. Subtype II or variant II is nitrate positive like *M. tuberculosis* and is clustered in East Africa (19, 25-29) though both subtypes have been reported in Guinea –Bissau (30).

In Uganda *M. africanum* subtype II is the main cause (67%) of human tuberculosis in Kampala (18) and transmission probably occurs at low rates on other continents as *M. africanum* has been isolated from patients for whom no link with Africa could be established. *M. africanum* was found to account for 1.25% of bacteriologically confirmed cases of tuberculosis in Southeast England. Over half of the patients infected with subtype I strain in South-East England were of Indian subcontinent origin, whereas patients of African ethnic origin predominated in the subtype II group, and a fifth of patients with either type were of European origin (31).

The prevalence of *M. africanum* in Cameroon dropped from 56% of all TB cases in 1970 to just 9% in 2002. One possible explanation is the widespread use of BCG, which could be effective against less virulent strains of *M. africanum* as observed in experimental models (24, 32). However, whether this phenomenon is the same in other parts of Africa needs to be evaluated and is likely to shed light into the changing pattern of the population structure of the MTBC complex being driven by various selective pressures (Table 2).

In humans, the clinical manifestations of *M. africanum* are similar to those of other members in the group except that *M. africanum* is rarely isolated from patients with genitourinary tuberculosis (31). In experimental models, *M. africanum* appears to have reduced virulence compared to *M. tuberculosis* (33), but this laboratory observation is not supported by clinical evidence. *M. africanum* remains a highly pathogenic and transmissible tubercle bacillus rather than an opportunist or atypical mycobacterium. It can acquire mutations leading to isoniazid and rifampicin resistance similar to those seen in *M. tuberculosis* and *M. bovis* (17).

*M. africanum* can cause extra-pulmonary TB, like other members of the MTBC. For example, it was isolated from a patient with cutaneous tuberculosis with

**Table 2: Reported studies of *M. africanum***

| Country (N) | Prevalence of *M. africanum* % | Type of Study | Author |
|---|---|---|---|
| Burkina Faso (300) | 18.4 | P | (20) |
| Cameroon | 56 | P | (32) |
| Cameroon (455) | 9 | P | (24) |
| Guinea-Bissau (229) | 61 | P | (30) |
| Ivory Coast (321) | 5.3 | P | (47) |
| SE England (6800) | 1.3 | H | (31) |
| Uganda | 16 | H | (22) |
| Uganda (234) | 67.1 | P | (18) |

P* population based study, H* Hospital based study.

bilateral nodular scleritis, nasal sinus invasion, and nasal septum perforation, who had concurrent pulmonary disease. In HIV positive patients enrolled in a trial substituting rifabutin for rifampicin in short course therapy for pulmonary tuberculosis, 49% had *M. africanum* isolated, indicating a high prevalence of *M. africanum* in human TB in Uganda (21).

*M. africanum* is not confined to the human population; an outbreak of tuberculosis due to this strain has been reported in pigs and cattle in Norway. The lesions in pigs were similar to those caused by *M. tuberculosis*, *M. bovis* and *M. avium*, with caseation in the lymph nodes of the head and jejunum. The source of the infection could not be established (34) and in Northern Bavaria, *M. africanum* was isolated from a mediastinal lymph node of a young bull from a herd of cattle, the source of infection was thought to be a member of the family of the farmer (35). Thorel reported the first isolation of *M. africanum* in monkeys and emphasized the potential public health hazard that animals may present for humans (36).

**MOLECULAR INSIGHT**

Recent advances in comparative genomic technologies include DNA arrays that can identify deletion events (13, 14, 37) and highly sensitive whole-genome sequence comparisons, which detect the full range of polymorphisms from single nucleotide polymorphisms to gene rearrangement. Brosch *et al* (12) have uncovered several variable genomic regions in the members of the *M. tuberculosis* complex. Differential hybridization arrays identified 16 deleted regions (Region of difference (RD 1-16), ranging in size from 2 to 12.7 Kb, that were absent from BCG Pasteur relative to H37Rv. Based on the presence or absence of these regions, a degree of relatedness to the last common ancestor of the *M. tuberculosis* complex was proposed that showed the progressive loss of genes (deletions) and the lineages existing within members of the group (Fig.1). This opened new perspectives in tuberculosis epidemiologic and diagnostic research, as one of these deletions (RD1) is believed to have been the primary attenuation event in the derivation of *M. bovis* BCG from *M. bovis*, since all *M. bovis* BCG isolates bear this deletion (37) and reinsertion of this region into *M. bovis* BCG partially restored its virulence (38).

Relating these findings to previously suggested differences in virulence and other factors is providing better insights into the epidemiology and pathogenesis of tuberculosis in general. These data, which suggested a sequential accumulation of Large Sequence Polymorphism (LSPs), served to construct a phylogenetic scheme for the evolution of the MTBC (Fig.1).

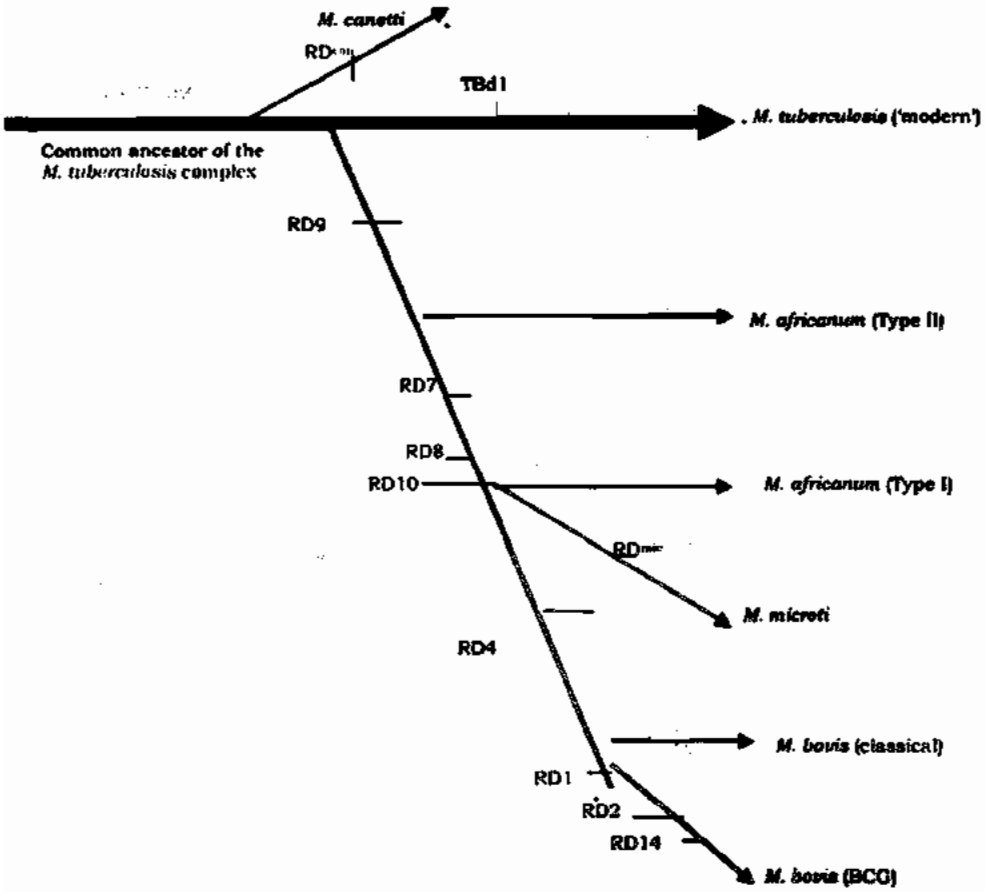


Fig. 1: **Scheme of the proposed evolutionary pathway of the tubercle bacilli illustrating successive loss of DNA in certain lineages (RD). The scheme is based on the presence or absence of conserved deleted regions genes. Note that *M. africanum* has an intermediate position between *M. tuberculosis* and *M. bovis*. Adapted from Brosch *et al* (12)**

In addition, these regions of difference (RD) can be used to identify sub-species within the MTBC. From the evolutionary pathway proposed (Fig 1), isolates characterized as *M. africanum* form two distinct genetic groups and are close to the common ancestor of the *M. tuberculosis* complex in that they are not lacking the TbD1 specific deletion common in 'modern' *M. tuberculosis*. What differentiate the genetic groups of *M. africanum* presently are RD7, RD8, RD9, and RD10. In the first group RD9 alone is deleted while RD7, RD8 and RD10 are present, while in the second group RD7, RD8, RD9 and RD10 are all deleted (12, 39-41).

There have been reported isolates of *M. africanum* which express genetic profile similar to those of 'modern' *M. tuberculosis* in that the TbD1 is missing while other regions of difference are present, however, some have suggested that these isolates should be called *M. tuberculosis* (41). 16S rRNA gene and internal transcribed spacer sequences, specific repetitive elements like IS610 and the direct repeat locus, in combination with phenotypic testing have shown to accurately differentiate several MTBC groupings and were used to evaluate large collections of clinical isolates (40, 42, 43). They are useful in identifying members of the MTBC as a group from other species

of mycobacteria but less efficiently for inter or intra species identifications. Table II shows some of the markers that have been described.

Spacer oligonucleotide typing (Spoligotyping) is the only DNA- based methodology for which most MTBC members are believed to have signature features (44, 45). Based on results with three independent markers (IS*6110* RFLP analysis, spoligotyping and VNTR), Viana-Niero *et al.* have suggested that *M. africanum* has a specific spoligotyping signature with the absence of spacers 8,9,and 39 (18, 23) while another group (18) proposed a different set of criteria to define *M. africanum*. A combination of the geographic origins of the strains, susceptibility to TCH, hybridization to at least two of the *M. bovis* derived spoligotype spacers 33 to 36, and a specific *gyr*B DNA sequence define subtype I. Resistance to TCH and lack of hybridization to spacer 33 to 36 define subtype II (18).

Yet, Parsons and colleagues (40) proposed another classification of subspecies of the *Mycobacterium tuberculosis complex* based on sequences that are highly conserved with respect to RD 1, RD 9, and RD 10. They suggested these three regions could be used to differentiate *M. tuberculosis* strains from the other members of the MTBC by screening isolates that lacked RD 9 for the presence of RD 10. If both are deleted, they subsequently tested these isolates for the presence of RD 3, RD 5, and RD 11. Isolates lacking only RD 9 were identified as *M. africanum*. Hence, RD9 is a common deletion found in *M. bovis*, *M. bovis* BCG, and *M. africanum* but not in *M. tuberculosis*. This finding supports the accepted view that *M. africanum* is evolutionarily intermediate between *M. tuberculosis* and *M. bovis* (12) but does not distinguish between subtypes I and II.

A large population based study in an area where *M. africanum* is endemic could compare these proposed classification methods and potentially achieve a consensus definition of the subspecies. This in turn will facilitate the identification of phenotypic associations and host preferences of the organism.

**Table 2: Genetic differences among members of the TBC**

| Component evaluated | Difference | Reference(s) |
|---|---|---|
| **Variable alleles** | | |
| *oxyR* nucleotide 285 | A in *M. bovis*, G in other members of the TBC | (48) |
| *pncA* nucleotide 169 | G in *M. bovis* and *M. bovis* BCG, C in other members of the TBC | (49) |
| *katG* codon 463 | CTG (Leu) in group 1, CGG (Arg) in group 2, CGG (Arg) in group 3[a] | (8) |
| *gyrA* codon 95 | ACC (Thr) in group 1, ACC (Thr) in group 2, AGC (Ser) in group 3[a] | |
| *GyrB* | Sequence differences among members of the TBC | (29) (42) |
| Variable sequences for spacers between direct repeats[b] | | |
| Spacers 33 to 36 (derived from BCG) | *M. tuberculosis* does not hybridize to the spacers | (45) |
| Spacers 39 to 43 (derived from *M. tuberculosis*) | *M. bovis* and BCG do not hybridize to the spacers | (45) |
| Spacers 37 and 38 | *M. microti* has a very short direct repeat region; many strains only hybridize to spacers 37 and 38 | (50) |
| Spacers 8, 9 and 39 | *M. africanum* does not hybridize to the spacers | (23) |

*M. africanum* subtype I can be determined by spoligotyping, note also other genetic makers used in the differentiation of the MTBC. Table II. Adapted from Parsons *et al.* (40)

## CONCLUSION

*M. africanum* accounts for a sizeable proportion of tuberculosis in Sub-Sahara Africa,[1] although its identification has been hampered by lack of clear criteria. Comparison of several proposed identifications methods will hopefully lead to a consensus on the identification of this subspecies, which in turn allows for a better understanding of its clinical characteristics and host predilections.

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