Impact of prophylaxis with cotrimoxazole on malaria parasitaemia, PCV and CD4+ count in HIV-infected pregnant women in Jos, Nigeria

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Abstract

The effect of prophylaxis with Cotrimoxazole (CTX) against malaria parasitaemia and other pregnancy outcomes among HIV-infected pregnant women in Jos was investigated. Peripheral blood from study women was used to prepare thin and thick blood smears for the identification and estimation of malaria parasitaemia as well as immunological and haematological investigations. Sulfadoxine pyrimethamine (SP) was used as control, being the standard used for prophylaxis against malaria in pregnant women. Result shows that Prevalence of malaria infection was reduced from 34.1-7.2% and from 25-10% among group A and B respectively. Mean parasitaemia was reduced from 1984.76 asp/µl to 1000.00 asp/µl and from 1960.38 asp/µl to 1118.18 asp/µl for CTX an SP respectively. Mean packed cell volume (PCV) was reduced from 33.09-33.02% with CTX and from 33.11-32.90% with SP. Mean CD4+ count increased from 223.55 cells/µl to 300.54 cells/µl with CTX but decreased from 570 cells/µl to 536.4 cells/µl with SP. Prophylaxis with CTX is associated with 89% reduction in parasitaemia, with immunological but no haematological benefits, while prophylaxis with standard Sp is associated with 76% reduction in malaria parasitaemia with neither immunological nor haematological benefits. There was no statistical difference between the two drugs throughout the study. The use of cotrimoxazole for antimalarial prophylaxis among HIV-infected pregnant women is strongly supported but with close monitoring of their PCV so as to detect and treat any possible anaemia.

Introduction

Malaria remains a major global health threat with substantial burden on sub-Saharan Africa where an estimated 90% of all malaria deaths occur particularly among pregnant women and children under 5 years of age [1]. Co-infection of HIV and Malaria makes malaria in pregnancy more severe by impairing the ability of women to control the illness [2,3] owing to the effect of HIV in destroying the natural immunity against malaria.

Intermittent preventive treatment (IPT) with anti-malarial drugs, has been proven to be an effective means of controlling malaria in endemic countries, with Sulfadoxine Pyrimethamine (SP) being the drug of choice among pregnant women [4]. The WHO [5] had however advocated for the use of Cotrimoxazole, a broad-spectrum antibiotic consisting of Sulfamethoxazole and Trimethoprim, commonly used as prophylactic agent against opportunistic infections in HIV-infected individuals, for antimalarial prophylaxis in pregnancy. This is thought to minimize toxicity effect arising from concurrent use of the two drugs which are both sulfur-based. There is paucity of data from the West African region on the effectiveness of Cotrimoxazole in reducing malaria parasitaemia among HIV-infected pregnant women. Such evidence-based information is essential for safe and effective management and prevention of malaria among this population.

Our specific objectives were to; Determine the prevalence and intensity of malaria parasitaemia among HIV-infected pregnant women before and after antimalarial prophylaxis with Cotrimoxazole versus SP in Jos, Plateau state. Determine the Packed Cell Volume (PCV) of HIV-infected pregnant women before and after prophylaxis with Cotrimoxazole as compared with SP in Jos, Nigeria. Determine the Immunological characteristics (maternal CD4+) of HIV-infected pregnant women before and after prophylaxis with Cotrimoxazole versus Sp among the study population.

Methodology

Study sites and population

The study was conducted in three different sites within Jos metropolis; Faith alive Foundation Hospital (FAFH), Bingham University Teaching Hospital (BHUTH) and Plateau State Specialist Hospital (PSSH), all of which have comprehensive HIV care centres. Plateau State Specialist Hospital (PSSH) and BHUTH are Tertiary Health Institutions while Faith Alive Foundation Hospital is a non-Governmental Hospital that cares for people living with HIV, in Plateau state and environs. A total of 235 HIV-infected pregnant women were recruited for the study from these Hospitals after obtaining ethical consents of the study subjects. The use of cotrimoxazole for antimalarial prophylaxis among HIV-infected pregnant women is strongly supported but with close monitoring of their PCV so as to detect and treat any possible anaemia.

Figure 1 shows the flow diagram of the study was conducted. Two hundred and thirty-five (235) eligible HIV-infected women who...
235 women who fulfilled the inclusion criteria and gave consents, enrolled into the study from FAFH, BHUTH and Women randomly allocated groups based on their CD4+ count. (≤350=A, >350=B)

135 women placed on daily CTX for prophylaxis against falciparum malaria
-10 lost to follow up (including 2 AR)
125(92.6%) women analysed after wk. 36
23(17%) lost to follow-up including 3 Still birth.

100 women placed on SP-IPT for prophylaxis against falciparum malaria
- 3 lost to follow-up
97(97%) women analysed after wk. 36.
4(4%) lost to follow-up (home delivery)

102(75.6%) women followed-up to delivery
93(93%) women followed-up to delivery

Figure 1. Flow diagram of activities involved in the study of the effect of prophylaxis with CTZ against falciparum malaria parasitaemia in HIV-infected pregnant women in Jos (CTX: Cotrimoxazole; IPT-SP: Intermittent preventive treatment with Sulfadoxin Pyrimethamine; AR: Adverse drug reaction)

Addresses and GSM numbers of participants were collected for follow-ups and other informal interactions. Participants were then followed-up to delivery.

At week 36, peripheral venous blood was again collected for the determination of CD4+ count, presence and intensity of plasmodium parasites and PCV. Use of insecticide-treated bed nets (ITNs) by participants was also ascertained at this point, which also marked the end of prophylaxis.

Parasitological investigations

Both thin and thick blood smears were prepared for the screening and identification of malaria parasites according to the methods described by WHO [6].

The diagnosis of malaria was based on the identification of asexual stages of *Plasmodium* on the thick blood smears, while thin blood smears were used to identify species of *Plasmodium*. *Plasmodium* parasite density was determined by counting the number of asexual parasites against 200 leucocytes on the thick blood film and converted to parasites per µl using an assumed total white blood cell (WBC) count of 8,000 µl. Blood films were declared negative or insignificant if no parasite was seen after viewing 500 WBC [7].

Parasite density (Parasitaemia)\(=\frac{X\times8000}{F}\)
Where; \(X=\)Number of parasites counted, 8000=standard WBC count
\(Y=\)Number of White blood cells count. (WBC)
All slides read, were confirmed by a microscopist with the roll
back malaria program. Where there were discrepancies, a second
microscopist was involved for the final verdict. Parasite density was
classified according to the methods described by Trape [7] and Boisier,
et al. [8].

Percent (%) reduction in Malaria parasitaemia was determined as
shown in equation 2 below

\[ \text{MpR} = \frac{\%\text{Mpbf} - \%\text{Mpaf}}{\%\text{Mpbf}} \times 100 \]

Where \( \%\text{MpR} = \) Per cent malaria parasite reduction
\( \%\text{Mpbf} = \) Per cent malaria parasitaemia before prophylaxis
\( \%\text{Mpaf} = \) Per cent malaria parasitaemia after prophylaxis

Determination of CD4+ cell count

The CD4 T lymphocytes count was determined according to the
method described by WHO [9] as follows:

Determination of CD4+ count

i) 20 microlitre whole blood (EDTA as anticoagulants) was added
   into a partec test tube properly labelled with the patients' code for
   proper identification

ii) 20 microlitre (μl) of CD4 mAb M antibodies was added and
    mixed properly.

iii) Mixture was incubated for 15 mins at room temperature
    protected from light.

iv) This was followed by addition of 800 microlitres of non-
    lyse buffer and shaken gently without allowing for bubbles to form.

v) Mixture was then analysed on a partec device.

vi) CD4 cells were measured and counted using the flow cytometric
    analyser in a partec flow cytometer (Cylow counter) with an
    excitation light source of 488 nm or 532 nm (blue or green solid-
    state laser). The dilution factor was 42.

Determination of Packed Cell Volume (PCV)

The PCV of study population was determined using the capillary
tube method and read in a Microhaematocrit reader as described by
Bull, et al. [10].

Data analysis

Data generated from the study were analysed using SPSS statistical
software for Windows 11.0 (SPSS Inc. USA). Occupation, educational
status and other categorical variables were compared using chi square
to determine any association between the various outcomes in the
two groups. Bar charts were used for graphical display of categorical
variables. Regression analysis was used to determine the association of
other confounding factors was ascertained using the multivariate analysis
and a 5% level of probability (P<0.05).

Results and discussions

Malaria infection among study population

Mean (Range) parasitaemia at enrollment was 1984.76 (1200-3800)
asp/μl and 1960.38 (1200-5050) asp/μl for groups A and B respectively.
Prevalence of malaria infection was significantly (P<0.05) higher
among women who took CTX [46 (34.1%)] than those who took SP
[25 (25%)] before prophylaxis. However, prophylaxis with CTX
reduced more parasitaemia compared to SP [9 (7.20) vs 10 (10.31)],
but the difference was not significant statistically (P≥0.05) as shown
on Table 1. Our result is similar to earlier reports by Houmsou, et al.
[11], in which pregnant women with low CD4+ counts (201–500 cells/
μL) were significantly and four times more infected than those with
higher CD4+ counts. This is expected since those in group A had lower
immunity compared to those in group B, as also indicated by their low
CD4+ count. Very low immunity is said to allow for easy establishment
and multiplication of infectious agents.

The parasite density was generally low to moderate (500-5050 asp/
μl). This may be partly attributed to the reconstitution of their immune
system, resulting from the use of HAART.

Statistical summary of malaria infection in relation to demographic
and other base-line characteristics of study population is as shown on
Table 2. There is no significant statistical difference in malaria infection
in relation to patients’ demographic characteristics throughout the
study. This shows that malaria infection affects HIV-infected pregnant
women regardless of their demographic or obstetric status. Coinfection
of malaria and HIV is said to impair the woman’s antimalarial immunity
[2], known to develop with age in areas with stable malaria transmission
so that infection occurs regardless of patient’s demography.

The use of insecticide treated bed nets (ITBNs) was significantly
associated with malaria parasitaemia in this study (P=0.02 and 0.012
before and after prophylaxis with CTX respectively). Although malaria infection increases with duration of HIV, such difference
was not significant statistically (P≥0.05). Similarly, malaria infection
did not vary significantly with duration of ARV usage throughout the
study (P≥0.05) as shown in the Figure. Although malaria infection
rates varied among women taking the different ARV regimes, such
differences were not statistically significant (P>0.05).

Multivariate analysis of age, gravidity, duration on ARV & use of
insecticide treated bed nets (ITBNs) revealed that their individual
contributions to the overall reduction in peripheral maternal malaria
parasitaemia after prophylaxis was not statistically significant as all
the regression coefficients were greater than the 0.05 significance level
(Figure 2).

Packed cell volume of study population

The mean PCVs in the two groups are both slightly lower than
the normal ranges (36-46%) for the study population. Mean PCV was
reduced from 33.09 to 33.02% for group A and from 33.11 to 32.9%
among group B, but the difference between the groups is statistically
insignificant (P≥0.05).

PCV similar to 33.1%, previously reported by Panti, et al. [12],
but slightly lower than the 34.3% reported from Coastal Ghana [13].
Differences attributable to the difference in the characteristics of the two
populations, since they worked with non-infected pregnant women.

Severe anaemia (<25%) was higher among those who had
prophylaxis with CTX than those who took SP. This is higher than
that reported by Achidi, et al. [14] from Cameroon, Kapito-Tembo,
et al. [15] from rural Malawi and Stephens, et al. from Coastal Ghana.
However, the low number (6 & 1 for group A & B respectively) of
women with mild-moderate anaemia in the study is expected, given the
high percent reduction in parasitaemia after prophylaxis. Apart from
its significant contribution to maternal mortality and both maternal
and infant morbidity, anaemia in pregnancy is a risk factor for infant
iron deficiency anaemia [16], and it may be associated with adverse
behavioral and cognitive development if left unchecked [17] (Figure 3).
CD4+ count (cells/µl) of study population

Prophylaxis with CTX associated with slightly but significant (P=0.001) increases in CD4+ count. (Mean CD4+ count increased from 223.55 to 300.54 among group A but decreased from 570 to 536.4 among group B. Only 2 (1.6%) had CD4+ count of ≤50 cells/µl, just as the group with 251-350 cells/µl increased to 78 (57.8%). Among group B however, there were slight but statistically significant (P=0.001) decreases in CD4+ count as shown in the figure. It is also noteworthy that the lower classes of ≤ 200 cell/µl as well as 201-350 cell/µl that were not found at enrollment, now had up to 3 (3.0%) and 11 (11.0%) women after prophylaxis with the standard SP.

Malaria parasitaemia was inversely but significantly associated with CD4+ T cell count. T-cell immunity in HIV-infection is impaired, which is of importance for antimalarial responses. This result is consistent with previous studies [11,18,19].

Table 3 shows the statistical summary of the prophylactic activity of the drug. Parasitaemia was significantly reduced by 89.4% and 76.0% (P=0.0000001 and 0.0001 for A and B respectively). PCV was significantly (P=0.015) reduced by 6.87% among group A, but the 3.5% reduction in group B was not significant (P≥0.05). There was a significant (P=0.00006) increase of 24.5% in CD4+ count among the CTX group, whereas a slight but significant (P=0.011) decrease of 8.7% was recorded among those who took SP for prophylaxis. These may be accounted for by the prophylactic activity of the drugs (Figures 4 and 5) [20].

Conclusion and recommendation

Prophylaxis with CTX was associated with 89% reduction in parasitaemia, with immunological but no haematological benefits, while Prophylaxis with standard Sp was associated with 76% reduction in malaria parasitaemia with neither immunological nor haematological benefits. The need to closely monitor women using...
Table 1. Prevalence of malaria parasites before and after prophylaxis (36 weeks) among study population.

| Study group | Before prophylaxis | After prophylaxis | Loss to follow-up |
|-------------|--------------------|-------------------|------------------|
|             | No. examined | No. +ve (%) | Prev | No. examined | No. +ve (%) | Prev | No. examined | No. +ve (%) | Prev |
| Group A (CTX) | 135 | 46 (34.07) | 19.58 | 125 | 9 (7.20) | 3.83 | 10 | 2 (20) | 0.85 |
| Group B (SP) | 100 | 25 (25.00) | 10.64 | 97 | 10 (10.31) | 4.26 | 3 | 1 (33.33) | 0.43 |
| Total | 235 | 71 | 30.21 | 222 | 19 (8.56) | 8.09 | 13 | 3 (23.08) | 1.28 |

CTX: Cotrimoxazole; SP: Sulfadoxine pyrimethamine

Table 2. Statistical summary of Malaria infection in relation to demographic and other characteristics of study population.

| Parameter       | Group A: 135 | Group B: n=100 |
|-----------------|--------------|--------------|
|                 | Before       | After        |                      | Before       | After        |                      |
| Age (yrs)       | 46 (34.1)    | 0.148        | 09 (7.2)             | 0.54         | 25 (25)      | 0.08                    | 10 (10.3) | 0.036 |
| Occupation      | 46 (34.1)    | 0.613        | 9 (7.2)              | 0.67         | 25 (25)      | 0.417                   | 10 (10.3) | 0.1 |
| Educational Status | 46 (34.1) | 0.157        | 09 (7.2)             | 0.34         | 25 (25)      | 0.932                   | 10 (10.3) | 0.577 |
| Marital Status  | 46 (34.1)    | 0.712        | 09 (7.2)             | 0.82         | 25 (25)      | 0.409                   | 10 (10.3) | 0.062 |
| Gravidity       | 46 (34.1)    | 0.104        | 9 (7.2)              | 0.12         | 25 (25)      | 0.138                   | 10 (10.3) | 0.13 |
| Blood Group     | 46 (34.1)    | 0.802        | 9 (7.2)              | 0.79         | 25 (25)      | 0.921                   | 10 (10.3) | 0.045 |
| Genotype        | 46 (34.1)    | 0.31         | 9 (7.2)              | 0.38         | 25 (25)      | 0.054                   | 10 (10.3) | 0.267 |
| Use of ITBN     | 46 (34.1)    | 0.02         | 09 (6.7)             | 0.01         | 25 (25)      | 0.021                   | 10 (10) | 0.038 |
| Duration of HIV | 46 (34.1)    | 0.209        | 9 (7.2)              | 0.97         | 25 (25)      | 0.282                   | 10 (10.3) | 0.291 |
| Duration on ARV | 46 (34.1)    | 0.204        | 9 (7.2)              | 0.86         | 25 (25)      | 0.262                   | 10 (10.3) | 0.271 |

Table 3. Summary statistics of the effect of prophylaxis with CTX and SP on Malaria Parasitaemia among study population.

| Parameter                        | Antimalaria |
|----------------------------------|-------------|
|                                  | CTX         | SP          |
| Mean paired difference in Parasitaemia before and after prophylaxis | 598         | 381.4       |
| %Reduction in Parasitaemia       | 89.4        | 76          |
| P value of the difference        | 0.0000001   | 0.0001      |
| Mean paired difference in PCV before and after prophylaxis | 2.27        | 1.19        |
| % reduction in PCV               | 6.87        | 3.58        |
| P value of difference            | 0.015       | 0.154       |
| Mean paired difference in CD4+ count before and after | 54.8        | 49.9        |
| % difference in CD4+ count       | 24.5        | 8.74        |
| P value of difference            | 0.00001     | 0.011       |

cotrimoxazole for prophylaxis in order to detect and treat any possible anaemic tendencies stressed.

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