Multiple Infections of Malaria and Typhoid Fever Among People Living With HIV/AIDS: Effect of Herbal Concoctions on CD4 Lymphocyte Count and Other Haematological Indices

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Abstract
We investigated malaria, typhoid and HIV co-infections among 600 persons in Uzo-Uwani Local Government Area of Enugu State, Southeast Nigeria and the effect of some herbal concoctions administered in the locality on the CD4 counts of those infected with malaria, typhoid and HIV. Of 600 patients screened, 286 (47%), were infected with HIV, 452 (75%) with malaria, and 401 (66%) with typhoid). Only 54 (9%) was uninfected. HIV infection was higher in those aged 26-35 years followed by those between 36-45 years. The prevalence of malaria was higher in males compared to females. The reverse was the case for typhoid although these gender differences were not statistically significant (p > 0.05). Of the 600 screened, 546 persons had malaria, typhoid and HIV co-infection. The greatest number (121) of these triply co-infected persons had CD4 counts that ranged from 201-300 cells/µl. Average counts of other hematologic parameters were: 514±21 for CD4 cell count; 15.01 ± 4.1 for WBC; 84.56 ± 81 for lymphocyte count, and 69.18± 3.0 for red blood cell (PCV). On the gender level, out of the 546 triply infected persons observed, 232 were males, and 314, females, with preponderance of female to male ratios. The effect of conventional antiretroviral therapy (ART), and herbal concoction separately on the CD4 counts of the 50 triply infected volunteers (with baseline CD_4 counts below 200cells/µl) were evaluated in one monthly intervals of treatment. Prior to this treatment, the 50 volunteer patients had mean CD_4 count of 118.60±60.17 cells/µl. Following administration of the antiretroviral and the herbal concoctions, a total of 39 patients responded positively to the treatment, with significantly increased mean CD_4 count of 202.31±86.72 cells/µl. Using the different antiretroviral drugs and effectors, it was observed that the different drug regimen significantly boosted the CD4 count at the end of treatment (p < 0.05). No significant difference (p > 0.05) was found in the CD4 cell count of male and female volunteers before (110.25 ±55.51 vs. 122.78 ±62.81 cells/µl) and after (177.69±82.64 vs. 214.63±87.34 cells/µl) treatment. Similarly, levels of TWBC, HB and PCV were significantly improved (p < 0.05) following the treatment. Furthermore, when the TWBC, HB and PCV of volunteers who had negative and positive response after treatment were compared, only the HB of those who responded positively to treatment showed a significant increase (p < 0.05).

Key words: Malaria, Typhoid fever, AIDS, HIV, CD4, Haematology

Introduction

Malaria remains Africa's leading cause of under-five mortality (20%), and constitutes 10% of the continent's overall disease burden. Malaria infection in Nigeria is caused by *Plasmodium falciparum,* the most severe and life-threatening form of the disease. Typhoid fevers which include both typhoid and paratyphoid fevers are multi-systemic diseases with fluctuating clinical presentations including primary gastrointestinal lesions. *Salmonella typhi* is the dominant stereotype in Nigeria, with percentage incidence of 60-68% (Bello and Tanyigna, 1996). Though with distinct aetiology and transmission modes, malaria and typhoid share some similarities; one mimicking the other in their presenting clinical signs and symptoms especially in the early stages of the disease; and syndromes produced by both diseases often present with diagnostic difficulties and confusions.

Co-infection with malaria and intestinal parasites are indicated to exacerbate the outcome of HIV infection by polarizing the immune response towards Th2. HIV infection is interrupts the acquired immune response to malaria; the risk of parasitemia, high parasite density and malaria fever increase viral local of HIV patients. *Plasmodium falciparum* has been shown to stimulate HIV replication through the production of cytokines (including interleukin 6 and tumor necrosing factor a (FNfa) by activated lymphocytes, thereby increasing the incidence and severity of malaria; and therefore causing an upsurge in the risk of HIV transmission (Froebel *et al*., 2004). These interactions therefore pose a serious public health threat especially in the malarious and heavily HIV-infected populations of Nigeria, thus calling for the strengthening of preventive measures in these locations. Until now, the world has relied on plants for the best malaria drugs: chloroquine from Cinchona tree; and Artemisinin from Chinese salad plant, *Artemisuaannua.* Following the devastating effect of HIV infection, there is the daily upsurge in *P. falciparum* resistance to the World Health Organisation (WHO) celebrated drug, Artemisin combination. Nigerian folkloric medicine employs the use of *Cleistopholis patens* (Benth), *Terminalia avicennioides, Momordicabalsamina, Combretuimpaniculatum* and *Tremaguineensi against* multi-drug resistant parasitic pathogens especially *S. typhi* (MDR) strains (Dalziel, 1937). This study investigated the effect of herbal

concoctions on CD4 cell counts and other haematological indices (surrogate markers) of HIV disease progression among people living with HIV/AIDS in the area of study.

Materials and Methods

HIV screening: A 5-ml blood sample was collected with blood collection tubes (Vacutainer CPT; Becton Dickinson, Basel, Switzerland). Plasma was separated within 2 hours and either processed immediately or frozen at −80°C until use. Samples were screened for HIV antibodies using the *Genie HIV*-1/2 *kit* (Sanofi Diagnostics Pasteur, Montreal, Quebec), a synthetic- peptide solid-phase enzyme immunoassay according to Manufacturer's instructions. The lower limit of detection of this assay was 400 copies per milliliter. Positive results were confirmed using the 'Determine' and 'Stat-pak' kits manufactured by Abbot and Chembio Laboratories respectively.

Malaria parasite estimation using giemsa stain: Five (5) ml blood sample was collected from each patient by venipuncture for malaria parasite assay. Duplicate thin and thick blood films were prepared immediately following blood collection on separate slides. A small volume of blood (3.0-4.0µl) was used for the preparation of a thick film on a grease-free glass slide. Thick smear of the blood was made by gentle mixing for 20-30 seconds using a slide spreader to de-fibrinate the blood and to obtain a round smear of about 1cm in diameter. The film was allowed to air-dry at a horizontal position. Thin film was then made by spreading 2 µl of blood from the edge of a glass slide with a swift sweep using a thin glass slide. The films were then fixed in absolute methanol for 1-2 sec and air dried. Each slide was then labeled with appropriate code. A 10% solution of 3% Giemsa stain (pH 7.). was diluted by dispensing 5mls of the stain to 45ml normal saline. Slides were placed on a staining rack and to each was poured 5mls of the diluted Giemsa. Contact was maintained for 10 minutes, after which stain was washed off with water, and the slides allowed to drain before air-drying in a vertical position away from dust and possible contaminant. All the blood films were stained after 24-48 hr. The slides were then observed under a 100x objective magnification. The WHO approved method for parasite enumeration was adopted(WHO,1985). Quality control was maintained using standard positive and negative films as well as standard operation procedures.

Malaria Parasite Determination:

Dipstick antigen-capture assays for *P. falciparum* histidine-rich protein: The test device is a rapid chromatographic immunoassay for the qualitative detection of circulating *Plasmodium falciparum* in whole blood. The test utilizes colloid gold conjugate to selectively detect *P. falciparum* antigen in whole blood. The membrane is pre-coated with *P. falciparum* antibody. During testing, the whole blood specimen reacts with the dye conjugate, which has been pre-coated on the test strip. Through capillary action the mixture migrates upward on the membrane and reacts with Pf antibody on the test line. The Dipstick, an antigen capture assay is based on immunological detection of *P. falciparum* histidine-rich protein. The procedure takes a total of 15–20 minutes, and the results are read visually and interpreted depending on the presence or absence of colored stripes on the dipstick. The test device was obtained from the foil pouch and placed on a leveled surface. A micro pipette was used to obtain some blood (10µl) from the EDTA bottle and applied to the specimen well of the test device. Two drops of the buffer solution was also added to the specimen well to disrupt the red blood cells, release the antigens and enhance capillary movement of the blood through the test cassette. After 15 minutes, the devices were examined for presence of coloured bands. The results of each assay were recorded as positive or negative on the basis of the observation of the precipitated band. Two distinct coloured bands were confirmatory for the presence of malaria parasite.

Screening for *S. typhi* Using Widal Test:

Qualitative slide agglutination test: The rapid agglutination test by slide titration method using commercial antigen suspension (cal - Test Diagnostic inc. Chino, U.S.A) for somatic (O) and flagella (H) antigens was adopted for the Widal test. One drop (0.5 ml) each of undiluted patient serum samples for the four antigens was placed on each circled card and one drop of each of the four *Salmonella* antigens were added separately, and gently rotated for one minute. Positive and negative controls were also maintained. Appearance of agglutination gave qualitative results. The titre for each of the antigens, was determined by a repeat test with dilutions of serum. This was carefully carried out for precision.

Quantitative slide agglutination test: Approximately 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of patient's serum each for the four antigens were dispensed on the circled card. To each series of serum specimen, was added one drop of specific antigen, mixed and rotated for one minute. Agglutination in each of these was then observed: 80 µl corresponded to 1 in 20 dilution, 40 µl to 1 in 40, 20 µl to 1 in 80, 10 µl to 1 in 160 and 5 µl corresponds to 1 in 320 titre. Serum samples with a titre of 1:160 for *S. typhi* antigens were considered positive results.

Quantitative Tube Agglutination Test

Procedure for tube agglutination test: Approximately 0.5ml of patient serum in sterile test tubes was doubly diluted by mixing and transferring from 1:10 to 1:640 in three-five rows. To the tubes in the first row was added somatic *S. typhi* O antigen, while to all the remaining rows, were added different flagella H antigens. A test tube with only saline was kept in each row as control. All the tubes (including control) in a row were mixed with 0.5ml of antigen suspension. The first row was treated with

S. typhi O antigen, the second row with *S. typhi* H antigen, the third row with *S. paratyphi* AH antigen. The fourth row with *S. paratyphi* BH antigen, and the fifth row with *S. paratyphi* CH. Following addition of the antigens, the tubes contained in a rack were placed in a thermostatically controlled water bath maintained at 37^{o}C for overnight incubation, after which the tubes were examined for agglutination, using the control tubes with no agglutination as reference point. Agglutination test of O antigen appeared as a "matt" or "carpet" at the bottom of each tube; while that of H antigens maintained loose, wooly or cottony appearance. The highest dilution of serum that produced a positive agglutination was taken as the titre.

Other Haematological Parameters:

CD4 cell count: HIV positive samples were assayed for CD4 by the Cyflow technique using the Cyflow counters (Partec), for both counting and analyses of particles and cells. Blood samples were stained with fluorescent dye, and allowed to stand for about 5 mins for the dye molecules to be imbibed by the cells (this was illuminated at defined wavelength). The color intensity for each labeled cell was measured by a ploidy analyzer; the intensity of emitted light was proportional to its CD4. The concentration or volume of fluorescent cell was measured at 0.2 ml by the volume detector, while the ploidy analyzer determined the number of cells per ml. CD4 cell counts were monitored at 6 month intervals. Overall treatment efficacy was defined as an increase of the CD4 cell count by at least 50% of the baseline value, while AIDS was defined as clinical stage C of the 1993 classification system (CDC, 1993).

Haematological tests: An automated Coulter counter T540 machine, standardized against a 4C plus blood control was used for haematological parameter estimation. The machine automatically diluted 29.6 µl whole blood samples, lysed, counted and printed out the result of absolute numbers of White Blood Cells, Red Blood Cells and lymphocyte.

Antiretroviral therapy (ART): Within the context of this study, the highly active antiretroviral therapy (HAART) a combination therapy which targets multiple steps in HIV replication: triple combination treatment with 2 RTIs and 1 protease inhibitor; or 1 RTI and 2 protease inhibitors was used following the WHO recommended guideline as follows:

Zidovudine (AZT): 300 mg twice daily

Lamivudine (3TC): 150 mg twice daily

Nevirapine (NVP): 200 mg twice daily for first 14 days,

Efavirenz (EFV): 600 mg once daily (at bedtime)

Stavudine (D4T): 40 mg every 12 hours for adults of > 60kg body weight and 30mg every 12 hours for adults < 60kg body weight

Didanosine (DdI): 400 mg once daily for adults > 60kg body weight and 250mg daily for adults < 60kg body weight.

Time was measured from the date of starting treatment to the date of the last follow-up visit (June 2, 2009 to June 30, 2010). Patients were maintained in the original regimen for the period of study following initial trial regimen to determine level of tolerance according to body weight. Compliance and/or adherence was maintained by close monitoring and advise. Exclusion criterion included pregnant women as a result of difference in their HAART regimen requiring that women not already taking treatment, but requiring HAART, should defer commencement until second trimester after the period of organogenesis and when symptoms of morning sickness are likely to have settled. Gestational period was not determined among the pregnant women, hence their illegibility for the study. CD4 cell counts were monitored at 6 monthly intervals.

Herbal treatment for typhoid and malaria: Treatment cocktail for malaria and typhoid was carried out using several local medicinal herbs commonly used in folkloric medicine for the dual treatment of malaria and typhoid infections, namely;

1. *Alstonei boonei*
2. *P. nitida*
3. *Cleistropholis patens* (Benth)
4. *Occimum gratissimum*

Herbal preparation and administration: Fresh leaves of the plants were collected from around the local communities of Nsukka and authenticated by a professional taxonomist, Mr A. Ozioko at the Department of Botany, University of Nigeria, Nsukka. Leaves were mixed in equal proportions and thoroughly washed in clean water and boiled for 20-30 min until leaves turned brown. The concoction was then drained into large clean pot and allowed to cool, after which it was dispensed into bottles and preserved by refrigeration. A glass (25cl) of the concoction was administered to each volunteer patient twice daily for two weeks. Fresh preparations were however made weekly to ensure sterility and viability of content, avoid contamination and degradation of product following overstay.

Prophylaxis, Using *Moringa oleifera* Leaves and Seed:

Moringa Processing: *Moringa* leaves and seeds, with their rich sources of macro and micro-nutrients: Vitamins A and C, Calcium, protein and potassium were also incorporated into the cocktail for energy boosting and immune restoration as well as system detoxification. Fresh leaves of *Moringa oleifera* were collected from Ifite-Ogwari in Ayamelum Local Government Area of Anambra State. Leaves were thoroughly washed and drained in a clean local basket (Nkata or Ekete in Igbo), and then allowed to dry indoors for about 2 weeks with regular turning to avoid decay and mold contamination and to ensure even drying. Dry leaves were then pulverized using electric blender (Model -). Ground leaves were further dried in a microwave for 5 mins to ensure proper drying, and

allowed to cool before packaging in dry air-tight plastic containers. Mature *Moringa oleifera* seeds were carefully removed from pods, and stored in tight plastic containers without removing the husk to avoid contamination with mold.

Administration of *Moringa* tea: Each volunteer patient was given a cup of Moringa tea, consisting of 1 tablespoon of dry Moringa leaves mixed in warm water before meal twice daily for 2 weeks at 1 week interval following the herbal treatment for malaria and typhoid fevers. Subsequently, 3 seeds of *Moringa* were given to each patient to chew nightly before going to bed.

Ethical consideration: The ethical consent of the Community leaders of the study population as well as the consenting volunteers was obtained prior to commencement of this study. Oral discussion on the nature and efficacy of the project was carried out before administration of questionnaires and commencement of and laboratory studies. Due to the social stigmatization and rejection associated with HIV seropositivity, the approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control be ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. Administration of the herbal concoction was carefully carried out strictly on consenting participants who were already aware of the local names and uses of the herbs tested (there was therefore assurance of safety of the herbs before administration). This study was therefore carried out in compliance with the international guidelines for human experimentation in clinical research according to the Declaration of Helsinki (World Medical Association and Council for International Organizations of Medical Sciences (CIOMS).

Study end points: In general, treatment efficacy was defined as an increase of the CD4 cell count by at least 50% of the baseline value. *AIDS* was defined as clinical stage C of the 1993 classification system for HIV infection (CDC, 1993).

Results and Discussion

Prevalence studies

Co-infectivity with HIV, Malaria and typhoid were apparent in the study. Out of the 600 persons screened for the triple infections, HIV, Malaria and Typhoid, 286 (47%), were infected with HIV, 452 (75%) with malaria, and 401 (66%) with typhoid). The uninfected persons were 54 (9%). Co-infectivity was therefore observed, an individual was dually infected with either of the triple infections, indicating a case of dual or multiple infectivity in the population (Fig. 1).

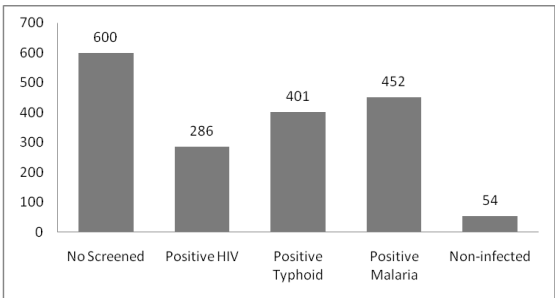


Fig. 1: Paradigm of Malaria and Typhoid co-infection among HIV infected persons

The paradigm of infection is represented below:

Malaria and typhoid co-infection In HIV	
No. screened	600
No positive for HIV	286
No positive for typhoid	401
No positive for malaria	452
Un-infected	54

Prevalence of HIV Infection:

Profile of HIV infected persons in the population : preponderance of infection: A total of 286 HIV infected persons were observed, 106 males (37%), and 180 females (62.9%), indicating a preponderance of female to male HIV infectivity (OR= 4.226, 95% CI 3.00-5.945, P > 0.0001), with those within 26-35years in higher proportion, followed by those in the 36-45years (n=72), 16-25 years (n=40), 46-55 years (n=36) and >15years (n=15) (Fig. 2; Table 1).

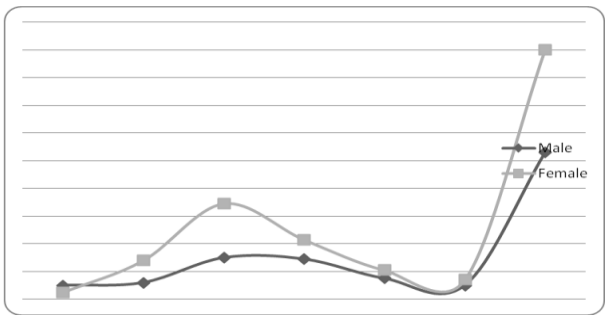
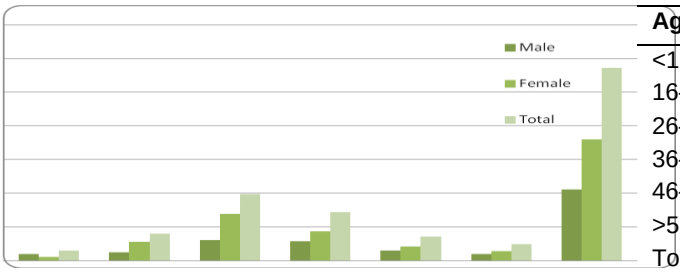


Fig. 2: Sex prevalence of HIV infection in the study population

Table1: HIV positive cases: age and sex distribution (n= 286)

Age Range	Male	Female	Total
<15	10	5	15
16–25	12	28	40
26–35	30	69	99
36–45	29	43	72
46–55	15	21	36
>56	10	14	24
Total	106	180	286



Age Range	Male	Female	Total
<15	13	8	21
16–25	64	48	112
26–35	44	50	94
36–45	32	39	71
46–55	37	35	62
>56	18	13	31
Total	208	193	401

Fig. 2 (alternative): Age and Sex prevalence of HIV infection in the study population

Preponderance of malaria infection

In consideration of the pattern of malaria infection in the studied population (n = 452), males (77.0%) had higher malaria infection prevalence than females (73.3%) although no significant difference (P > 0.05) was found in the prevalence of malaria infection. Malaria infection was most prevalent among those 26-35 years (n=125). Next in prevalence were those in the age group16-25years. The least in the infectivity profile was the >56 age group (n=30) (Fig. 3; Table 2).

Table 2: Malaria positive cases: Age and sex distribution (n= 452)

Age Range	Male	Female	Total
<15	52	34	86
16–25	51	46	97
26–35	76	49	125
36–45	33	28	61
46–55	23	30	53
>56	19	11	30
Total	254	198	452

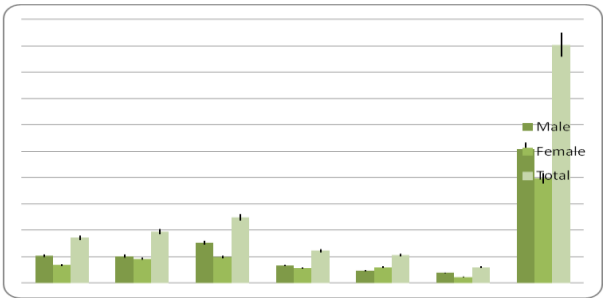


Fig. 3: profile of age and sex distribution of malaria positive cases

Profile of typhoid fever infection

Typhoid fever was more in females than in males (71.5% vs. 63.5%, OR=1.470, 95% CI= 1.04-2.08, P < 0.05). A preponderance of the infection was observed among the 16-25 age group (n=112). Next in the range of infectivity were those between the ages 26-35 (n= 94), 36-45 (n=71), 46-55 (n=62) and lastly, < 15 (n=21) (Figur 4; Table 3).

Table 3: Typhoid positive cases: Age and sex distribution (n= 401)

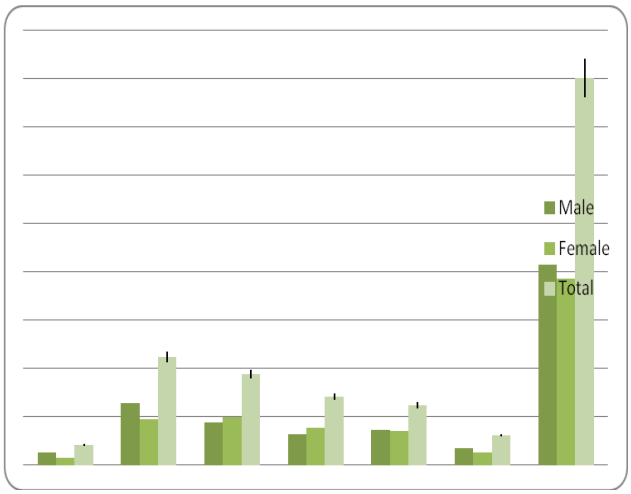


Fig. 4: Age and Sex Distribution of Typhoid Positive Cases

Paradigm of age and sex malaria and typhoid co-infectivity among HIV infected persons

Investigation of the paradigm of sex distribution of the multiple infections in the population indicated as follows: out of the 286 (47%) HIV infected persons, 121 were males, while 165 were females in the ratio of ---; 452 had malaria parasitemia: 219 , males, 233 females (ratio: ---); 401 were had typhoid fever infection; 202, males, and 199 females (ratio ---) (Fig. 5; Table 4).

Table 4: Sex distribution of the multiple infections HIV, Malaria and typhoid in the population studied (n= 600)

Investigated Conditions	No. Positive	Positive HIV	
		Male	Female
Positive HIV	286	121	165
Positive malaria	452	219	233
Positive typhoid	401	202	199

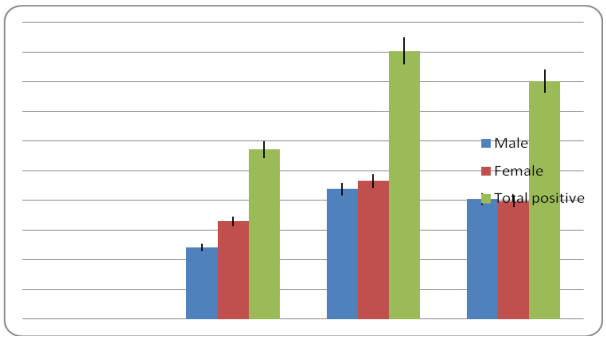


Fig. 5: Pattern of gender and age occurrence of single and multiple co-infectivity with malaria, typhoid and HIV (n = 600)

Pattern of single and Multiple Infectivity of the Triple Infections in the Population:

HIV: single and co-infection

Out of the 286 HIV positive persons, 2 were singly infected, 148 had dual HIV and malaria infections while 136 had dual HIV and typhoid infections

Malaria: single and co-infection

Of the 452 malaria positives cases, 42 had single or mono- malaria infection, 148 were dually infected with both malaria and HIV, while 262 had dual malaria and typhoid infections

Typhoid: single and co-infection

From a total of 401 typhoid cases, 3 had mono or single infection with typhoid, 136 had dual HIV and typhoid, while 262 had both typhoid and malaria (Table 5; Fig. 6)

Table 5: Association of multiple infection with typhoid and malaria in HIV infection

HIV singly	Malaria singly	Typhoid singly	HIV + Malaria	HIV + Typhoid	Malaria + Typhoid	HIV + Malaria + Typhoid	Non-infected
2	42	3	148	136	262	546	54

Table 7: Baseline mean cd4 lymphocyte count of singly and multiply infected

CD4 Range	No of subjects	CD4	WBC	Lymph	PCV
0–100	39	101±24	7.22 ± 0.9	55.71 ± 22	42.11±2.3
101–200	182	409 ± 28	10.13 ± 0.9	71.20 ± 20	56.20 ± 2.2
201–300	121	514 ± 21	15.01 ± 4.1	84.56 ± 81	69.18 ± 3.0
301–400	105	581 ± 22	16.50 ± 2.0	78.35 ± 43	62.20 ± 1.5
401–500	46	699 ± 31	18.35 ± 4.2	61.88 ± 33	58.13 ± 51
501–600	29	714 ± 26	9.55 ± 1.6	66.42 ± 22	55.46 ± 2.1
601–700	14	802 ± 33	9.01 ± 8.1	55.11 ±16	50.10 ± 2.2
701–800	6	919 ± 44	10.23 ± 2.0	40.21 ± 26	52.0 ± 3.9
>800	4	2194 ±65	9.66 ± 4.7	33.19 ± 20	64.12 ±1.1

Individuals in relation to sex

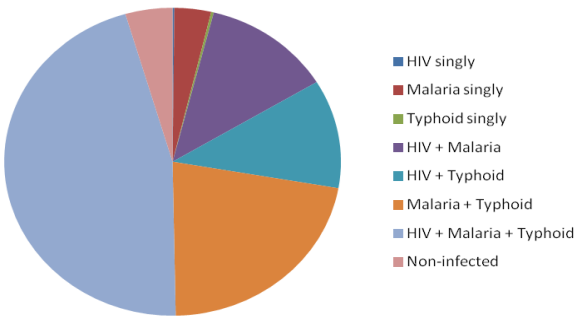


Fig. 6: Paradigm of multiple infection with typhoid and malaria in HIV infection

Investigation of haematological parameters:

The baseline haematological parameters: CD4 count, total white blood count and differential (lymphocyte count), and red blood cell count (measured as Packed cell volume- PCV) of the triply infected participants in their different CD4 categories, and the pattern of the gender distribution are elucidated in Tables 6 and 7. The highest number of triply infected persons (n= 121) were within the CD4 range of 201-300. Average counts of other hematologic parameters were: 514±21 for CD4 cell count; 15.01 ± 4.1 for WBC; 84.56 ± 81 for lymphocyte count, and 69.18± 3.0 for red blood cell (PCV). This was followed by those within the 101-200 CD4 category (n=182), with average CD4 cell count of 409 ± 28; WBC of 10.13 ± 0.9; lymphocyte count of 71.20 ± 20 and PCV of 56.20 ± 2.2. The least in the category were those in the >800 CD4 range (n= 4), with average CD4 count of 2194 ±65; WBC of 9.66 ± 4.7; lymphocyte count of 33.19 ± 20 and PCV of 64.12 ±1.1, respectively. On the gender level, out of the 546 triply infected persons observed, 232 were males, and 314, females, with preponderance of female to male ratios.

CD4 lymphocyte (µl)	Mean CD4 Values	Male	Female	Total
0–100	101± 31	11	21	39
101–200	409 ± 28	43	56	182
201–300	514 ± 21	72	93	121
301–400	581 ± 22	36	47	105
401–500	2194 ±65	27	35	46
501–600	714 ± 26	21	29	29
601–700	802 ± 33	14	16	14
701–800	919 ± 44	6	12	6
>800	2194 ±65	2	5	4
Total		**232**	**314**	**546**

Antiretroviral therapy and herbal concoctions for malaria and typhoid fever

The effect of antiretroviral therapy using conventional ART, and herbal concoction on the CD4 counts of the 50 triply infected volunteers (with baseline CD_4 counts below 200cells/µl) following one monthly intervals of treatment is here presented. Administered ART included:- AZT: Zidovudine, 300mg/12hours; 3TC: Lamivudine, 150mg/12hours; NVP: Nevirapine, 200mg/day; EFV: Efavirenz, 600mg/day; d4T: Stavudine, 40mg/12hours; ddI: Didanosine, 400mg/day; TDF: Tenofovir, 300mg/ day. The volunteers were then followed up **(**at one monthly interval between each treatment) with herbal cocktail: *Alstonei boonei* concoction for malaria, and concoctions of *P. nitida, Cleistropholis patens* (Benth) and *Occimum gratissimum,* in equal proportions for typhoid fever.

The results of the CD4 cell counts, before and after the treatment are here elucidated. Available results indicated that the 50 volunteer patients had mean CD_4 count of 118.60±60.17 cells/µl before treatment. However, following administration of the antiretroviral and the herbal concoctions, a total of 39 patients responded positively to the treatment, with significantly increased mean CD_4 count of 202.31±86.72 cells/µl. Using the different antiretroviral drugs and effectors, it was observed that the different drug regimen significantly boosted ($P < 0.05$) the CD4 count at the end of treatment (Tables 8, Fig. 7). Generally, a significant statistical difference ($P < 0.05$) was found between the CD4 count of volunteers had positive and negative response to drugs after treatment (220.85±85.13 vs. 122.00±28.67 cells/µl, $P < 0.05$). Nevertheless, no significant difference ($P > 0.05$) was found in the CD4 cell count of male and female volunteers before (110.25 ±55.51 vs. 122.78 ±62.81 cells/µl) and after (177.69±82.64 vs. 214.63±87.34 cells/µl) treatment.

Table 8: The CD4 counts of HIV patients before and after treatment with ART and herbal concoctions

Antiretroviral drugs administered	Baseline CD4 count before ART	Final CD4 count after ART
Overall drug effect	118.60±60.17	202.31±86.72*
AZT, 3TC, NVP + Effector	118.43±55.21	183.29±70.86*
D4T, 3TC, NVP + Effector	112.90 ± 58.67	190.80 ±103.51*
DDL, TDF, LPV + Effector	123.70 ± 60.98	201.90 ± 64.06*
D4T, 3TC, EFV + Effector	149.60 ± 65.99	267.00 ± 52.86*
D4T, AZT, NVP + Effector	87.17± 28.72	180.50±41.95*
AZT, 3TC, EFV + Effector	132.67± 78.24	266.67 ±56.08*

*significant difference ($P > 0.05$)

Similarly, levels of TWBC, HB and PCV were significantly improved ($P < 0.05$) following the treatment (Table 9, Fig.s 7 and 8). Furthermore, comparing the TWBC, HB and PCV of volunteers who had negative and positive response after treatment, significant increase ($P < 0.05$) was only observed in the HB of those who responded positively to treatment (Table 10).

Table 9: Haematological Tests of HIV Positive Patients Before and After ART

Haematological indices	Before therapy	After therapy
TWBC	4.57± 1.71	6.14±1.34*
HB	10.51±2.17	30.49±16.65*
PCV	31.97±5.95	41.95±11.97*

*significant difference ($P > 0.05$)

Table 10: Haematological Tests of HIV Positive Patients After ART

Haematological indices	Negative response	Positive response
TWBC	5.88±1.12	$6.18±1.31^{ns}$
HB	15.57±8.97	32.53±16.47*
PCV	37.57±13.06	$42.56±11.34^{ns}$

*significant difference ($P > 0.05$), ^{ns} no significant difference

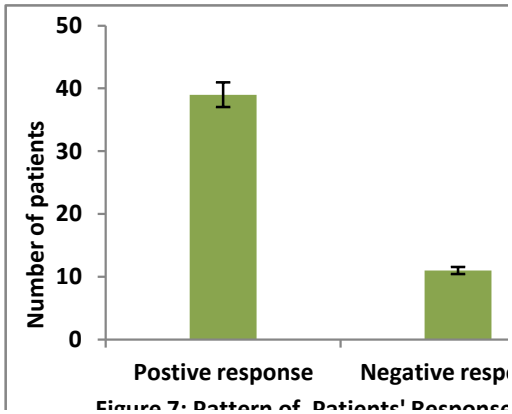
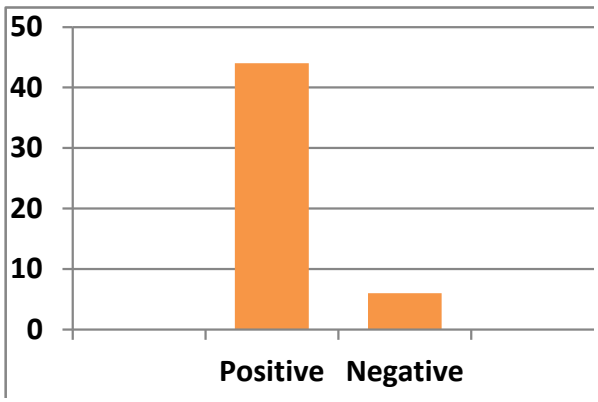


Figure 7: Pattern of Patients' Response



In Nigeria and many developing countries, several healthcare challenges still exist especially among HIV/AIDS infected individuals. The lack of good healthcare facilities and modern diagnostic equipment, lack of adequate and experienced manpower, poor disease surveillance strategies, stigmatization and other socioeconomic factors are some of these challenges. Although very concerted efforts have been made in the last decade to reduce the ravaging effect of HIV/AIDS pandemic all over the world, the impact of HIV/AIDS is still alarming

especially in many developing countries including Nigeria. Opportunistic infections and other kinds of infections such as malaria and typhoid are thought to complicate HIV/AIDS infections in sub-Saharan Africa and have recently drawn more research attention (Chandramohan and Greenwo, 1998). This is the motivation for our study.

It is disturbing to learn that over 80% of malaria deaths in the world today are known to occur in Africa. On the other hand, typhoid infection is also a very well-known healthcare problem in Africa. Unfortunately, the true magnitude of typhoid infection is difficult to estimate due to the confusion arising from the similarity in the clinical picture of typhoid and other febrile illnesses. Malaria, typhoid and HIV co-infection and the subsequent interactions between them may therefore have major implications for the treatment, care and prevention of these infections especially HIV/AIDS.

As expected, co-infections with HIV, malaria and typhoid fever were observed in the study. A very disturbing situation is the finding that a total of 47% of the 600 persons screened had HIV/AIDS. Of the positive HIV confirmed cases, 106 (37%) were males while 180(62.9%) were females. More females were significantly more infected with HIV than males ($P< 0.05$),with those in the age bracket of 26-35 years being the most affected, followed closely by those in the age range of 36-45 years. This is consistent with previous findings. The reason for the higher prevalence in female compared to male population is not known. The unfortunate scenario is that the bulk of those infected with HIV are individuals within the labour force (26-45 years). This portends a very great danger to the economy of Nigeria and other countries with similar situations since any developed or developing country depends largely on its labour force to grow the economy.

The sex and age distribution of malaria and typhoid positive patients appear to follow the same trend: infection was more in males than females although not significantly different ($P>0.05$) while incidence of both infections increased with decreasing age for malaria and typhoid infections respectively. It is difficult to predict the reason why the male population had more incidences of both infections. It might however be due to geographical factors. A possibility also might be due to the fact that the male population in Uzo-Uwani are predominantly farmers who spend most of their time in farms located in remote malaria and typhoid endemic villages where they are more disposed to these infections.

The high incidence of both infections in the younger age groups is consistent with the findings from previous investigators (Agwu *et al*., 2009; Tatfeng *et al*., 2007). Conversely, when malaria, typhoid and HIV cases investigated in the study were pulled together, the number of cases in the female population was higher than what was observed in the male population (597:542). Of this number, 75 and 66% respectively have malaria and typhoid fever, indicating a case of dual or multiple infections among those screened. This observation brings to bear, once more, the risk of malaria and typhoid fever infections among HIV/AIDS patients living in malaria/typhoid endemic areas. Previous studies in both Ekpoma and Benin city in Edo state, Nigeria reported similar findings with respect to malaria among HIV/AIDS patients. Whereas the prevalence of malaria in HIV/AIDS was 74.6% in Ekpoma, that in Benin was 88.8% (Agwu *et al*., 2009; Tatfeng *et al*., 2007). Both values therefore compare closely with 75% found in our study. There are reports which indicate that clinical presentation of malaria does not vary according to HIV status. This report also argued that it is difficult to distinguish the clinical presentation of malaria from other causes of febrile illness in persons with HIV infection (Mbuh *et al*., 2004).

Our findings are not in agreement with the former statement. Nevertheless, another group had previously reported that there are indications that malaria and typhoid infections might have adverse effects in HIV positive individuals by impairing cytotoxic T cell function, stimulating T cell turnover and damaging the placenta in a manner that will aid HIV transmission in the uterus (Chandramohan *et al*., 1998) In a recent review article, (Gordon, 2008) showed that single-center studies previously carried out in Africa and Asia failed to demonstrate any specific interaction of HIV with typhoid fever (Gordon, 2008) In fact, Crump and co-workers (Crump *et al*., 2011) carried out a study in Tanzania where the high incidence of typhoid in an HIV-prevalent area allowed the authors to document an apparent protective effect of HIV against presentation with typhoid fever. They clearly showed that HIV was protective against *S. typhi* bloodstream infection in their febrile adult cohort, in contrast to the strong positive association that has repeatedly been observed between HIV and invasive typhoid and non-typhoid *Salmonella* disease in this and other studies (Gordon *et al*., 2008) In a different study involving the same author (Gordon, 2002) it was demonstrated that this apparent negative association could be due to a lack of cell-mediated immune response to *S. typhi* in HIV-infected individuals, such that although infected, those patients do not show symptoms of fever or other inflammatory features. They rather, have intracellular, quiescent, sub-clinical disease. This looked like an attractive hypothesis. However, if this was the case, then the expectation might be that the availability and high uptake of free ART in Africa might result in clinically obvious *S. typhi*-related disease manifesting during immune reconstitution as an IRIS syndrome, but this has not been reported in Nigeria or other African ART cohorts. This apparently lends credence to our finding in this study that there is a likely interaction between typhoid fever and HIV infections. The high prevalence of typhoid is not surprising either. Since it is transmitted via contaminated food and water, infection is high in areas where sanitary condition is poor and use of untreated water is common. These conditions are peculiar in the environment under study. The situation is not helped by the development of antibiotic resistant strains of

Salmonella typhi, the causative agent of typhoid. Moreover, as more Nigerians relocate/migrate to urban areas, living in crowded, squalid conditions, these new environments may likely lead to a different epidemiology for typhoid and malaria to thrive.

Table 5 shows that HIV patients were more at risk of acquiring malaria or typhoid. The reason is perhaps as a result of the lower immune status of the patient. This trend is expected and has been reported for other infections associated with HIV patients. When pulled together, those who had all the three infections were 546 out of the 600 persons investigated. The finding in our study that 262 persons screened had co-infection of typhoid and malaria emphasizes the severity of these infections in Nigeria. Our data agrees with previous findings in Zaria, Nigeria by (Mbu *et al.,* 2004) . They also reported that there is a high rate of malaria and typhoid co-infection with the widal test. This is because there are certain limitations associated with the use of widal test as a diagnostic tool in patients with malaria. This is one of the limitations of our study. *Salmonella typhi* antibodies cross-react with malaria and non-typhoid *Salmonella* antigens, meaning that the use of widal test as a diagnostic procedure in patents with malaria might lead to misleading results. (Koelman, 1992) also reported cross-reactions due to latent and post-infectious diseases such as chronic hepatitis, rheumatoid arthritis, amoebiasis, tuberculosis and pneumonia (Koeleman, 1992). Mbu and co- workers (2003) also observed that there are more typhoid fever cases in areas with drug resistant malaria and concluded that a cross reaction between malaria parasites and *Salmonella* antigens may lead to false positive widal agglutination test. By using the aforementioned method, we cannot therefore do a 100% accurate assessment that these patients are suffering from a true co-infection of malaria and typhoid. We chose to use the widal test since it is the most widely used procedure in the diagnosis of typhoid fever in Nigeria. It is known generally that blood culture is usually the gold standard for the diagnosis of typhoid fever. Unfortunately, it is not usually requested for by many physicians because of its cost and the length of time (≥ 3 days) required before results are obtained. In future studies, we plan to compare *Salmonella* isolation data from blood samples of those positive for widal tests. Changes in haematological and biochemical parameters have been reported in malaria (Bidaki, 2003) and typhoid infections (Mishra, 2003). These parameters are useful in the diagnosis of many diseases since it helps to detect complications associated with many disease conditions. Ultimately, they are used by clinicians to care for the patient and prevent death which may result due to such complications. Our results indicate that the co-infection of malaria, typhoid and HIV caused some alterations in the haematological parameters of the patients screened in this study. Although their study did not screen for HIV in the samples tested, (Kayode *et al*., 2011) also made similar observations while investigating changes in some haematological indices in patients co-infected with malaria and typhoid fever in Ota, Ogun State Nigeria (Kayode *et al*., 2011). The packed cell volume generally decreased in those that were co-infected. This is expected perhaps due to the breakdown of red blood cells that are common in malaria and typhoid patients, thus suggesting that anemia might be involved. Similar observations were made by other investigators in both malaria (Ogbodo *et al*., 2010; Goselle *et al*., 2009) and typhoid fever patients (Dangana *et al*., 2010) . We also observed a general increase in white blood cell and lymphocyte counts among those with triple infections compared to normal counts in healthy individuals. The increase in white blood cell counts in these subjects might be due to the invasion of the body by the pathogens and consequential secretion of leukocytes to fight against the invading pathogens particularly at onset of the infection. (Kayode *et al*., 2011) and (Sumbele *et al*., 2010) both separately reported increases in leukocytes among malaria patients in Nigeria and Cameroon respectively (Kayode *et al*., 2011; Sumbele *et al*., 2010). However, there were both increased and decreased leukocyte counts in typhoid fever patients screened in the United Arab Emirates (Ali *et al*., 2009).

Of note however is the observation that a significantly higher number of patients (89.4%) with triple infections screened in the study had CD4 counts in the range of 0-400 compared to only 10.6% with CD4 counts of 400 and above. This suggests that either these HIV patients are not responding well to ART, are not using ART (naïve) or are not responding well as a result of the triple infections. This finding motivated us to administer the concoctions used by these villagers and compare their outcomes with a panel of ARTs currently in use in the locality.

The effect of antiretroviral therapy using conventional ART, and herbal concoction on the CD4 counts of the 50 triply infected volunteers (with baseline CD_4 counts below 200cells/µl) following one monthly intervals of treatment showed that all 50 volunteer patients had mean CD_4 count of 118.60 ± 60.17 cells/µl before treatment. However, following administration of the antiretroviral and the herbal concoctions, a total of 39 patients responded positively to the treatment, with significantly increased mean CD_4 count of 202.31 ± 86.72 cells/µl. Using the different antiretroviral drugs and effectors, it was observed that the different drug regimen significantly boosted ($P < 0.05$) the CD4 count at the end of treatment. This is good news. A likely reason for this observation might be due to the efficacy of the concoctions which effectively controlled malaria and typhoid infections and consequently boosted the CD4 counts of the 39 HIV patients. This is not unusual though since it is generally known that controlling opportunistic and other infections in HIV patients usually lead to improvements in CD4 counts. Our findings generally justify why the herbs *Alstonei boonei, P. nitida, Cleistropholis patens* and *Occimum*

gratissimum are used in folkoric medicine to treat malaria and typhoid in southeastern Nigeria. This is also supported by the information in literature.

Overall, this study has shown that malaria and typhoid co-infections are highly common among HIV/AIDS patients in Uzo-Uwani local government area of Enugu State, Southeast Nigeria. Mbu et al noted that this could significantly increase the risk of HIV transmission through blood transfusion (Mbuh *et al*., 2004). Many factors seem to predispose HIV/AIDS patients to malaria and typhoid infections in the area under study: poor sanitation and health facilities, lack of good disease surveillance strategies, occupation and poor control and prevention of malaria among others. Some of the herbal concoctions in the localities might be useful in the management of those with triple infections. Future research ought to focus more attention on these herbal remedies and their possible use in managing malaria and typhoid single infection or in co-infections in this or similar situations.

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