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Full Length Research Paper

Liaison of tuberculosis and human immunodeficiency virus (HIV) co-infection in the progression to AIDS: Prognostic value of cluster differentiation 4 (CD4+) cell as marker of disease progression

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This biphasic study investigated the prevalence and co-infection of the human immunodeficiency virus (HIV) and tuberculosis (TB) along major transport routes of Enugu State; their consortium in active HIV disease and the role of cluster differentiation 4 (CD4) cells and other haematologic parameters as markers of HIV progression. Prevalence and co-infection with HIV and TB were studied in the preliminary phase (Phase 1: 1999 to 2001), among 12,000 individuals who were screened for HIV using determine rapid test kits and TB by the Ziehl-Neelsen staining technique. Effect of TB on HIV progression using CD4 cell (estimated by cytometry using Partec Cyflow SL-3 counter) as surrogate marker was studied in Phase 2 (2005 to 2008) among 50 of those subjects presenting with AIDS-related clinical symptoms. Other investigated parameters included white blood cells (WBC) and haemoglobin/packed cell volume (PCV). The rate of co-infection with HIV and TB was 49.6%. The effect of TB on the progression of HIV to acquired immune deficiency syndrome (AIDS) was highly significant at α = 0.05. The study confirmed the role of TB in furthering the progression of HIV to AIDS as well as the importance of the surrogate markers as indicators of immune system deterioration and subsequent decline.

Key words: Prevalence, co-infection, cluster differentiation 4 (CD4) cells, tuberculosis (TB), human immunodeficiency virus (HIV), surrogate markers.

INTRODUCTION

It is not easy to decide which of the deadly duo (human immunodeficiency virus (HIV) and tuberculosis (TB)) precedes the other in the precipitation of acquired immune deficiency syndrome (AIDS) condition. TB has already been recognized as the most common cause of death from a single pathogen worldwide and most of these deaths occur in the developing countries of Africa, Asia and South America (WHO, 1977; UNAIDS, 1977; Sunderman et al., 1986). The relationship between TB and HIV is thought to be synergistic, thus, the combined effect of both is worse than their separate effects added together (Sunderman et al., 1986; Murray et al., 1990). HIV multiplies the problems of TB for individuals and entire communities; TB complicates the management and course of HIV infection (Nunn et al., 1990; Bonecici et al., 1998). Hence, the HIV-TB link has a dramatic impact, particularly in developing countries where 95% of people with TB and HIV live.

One of every four HIV-1-infected persons in the world is diagnosed with active TB, making TB the most frequent

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life-threatening co-infection in HIV-1-infected patients (Harris, 1995). TB is associated with increased HIV-1 viral load, a fall in cluster differentiation 4 (CD4) lymphocyte (LYM) counts, and increased mortality. In a prospective epidemiologic study in Uganda on the impact of pulmonary TB on survival of HIV-infected individuals matched for CD4 cell count, the course of HIV-1 infection was found to be accelerated after TB diagnosis (Whalen et al., 2000). Pulmonary TB occurs before the onset of severe immunodeficiency at a relatively higher CD4+ counts (336 to 441 ml^{-1}) than in other opportunistic infections such as *Pneumocystis carinii* or *Toxoplasma gondii* (Mildvan and Muthur, 1987).

With the staggering worldwide growth of HIV pandemic, the Center for Disease Control and Prevention (CDC) has defined set of guidelines and recommendations for HIV-infected adolescents and adults on the basis of clinical conditions associated with the HIV infection and CD4+ T-lymphocyte counts (CDC, 1997). These CDC guidelines are based on studies done in developed countries, but are under trial in developing countries (Kam and Wong, 1998). Low CD4 T-cell count is considered to be a marker of the progression of HIV, and is associated with a variety of conditions, including many opportunistic infections, burns, trauma, etc. The low CD4 counts caused by some of these conditions often fall below 200 mm^{-3}, which is the level needed to diagnose AIDS in someone who was previously positive for antibodies to HIV (CDC, 1999). The daily increasing incidence of HIV and subsequent diagnostic and management failure especially in the remote and resource poor communities of Nigeria, resulting from lack of trained personnel and infrastructure necessitates this study which investigated the prevalence HIV and TB co-infection in high risk communities of Nigeria, in the geographical area along the major North-South trucking/stop over transport routes of Enugu State, and to evaluate the association of the deadly duo in AIDS progression. This study attempts to assess the prognostic value of CD4 cells and other haematologic surrogate markers in disease progression with the view to establishing a globally accepted, cost effective and efficient tool for effective diagnosis and management of people living with HIV/AIDS in the rural communities of Nigeria.

MATERIALS AND METHODS

Study design

This biphasic study investigated the prevalence and co-infection of HIV and TB along major transport routes of Enugu State; their consortium in active HIV disease (Phase 1, 1999 to 2001), and the role of CD4 cells and other haematologic parameters as markers of HIV progression (Phase 2, 2005 to 2008).

Selection of study areas

This work was a survey of sub-groups in specific locations in part of South-Eastern Nigeria, namely, Enugu North and Enugu Urban, located between Longitude 56° North and Latitude 17° South. Earlier studies by the Federal Ministry of Health had recorded certain locations in this zone as HIV/AIDS high prevalence area on the basis of the following characteristics: (i) geographical location as truck routes/stop-over of commuters from the Northern to the Southern parts of Nigeria; (ii) proliferation of commercial activities (occasioned by the Federal Ministry of Health) including commercial sex working in the specified locations; (iii) high incidence of AIDS cases, as well as the report which indicated that HIV cases are more predominant in transport routes and urban centers (Federal Ministry of Health, 1986; Uwakwe, 1994; Essex, 1992).

Sample size

The sample population was self-selected because of the peculiar characteristics that are relevant or tend to predispose to HIV/AIDS such as sexual promiscuity in forms of concubinage, woman to woman marriage, child-marriage, etc. HIV studies in these areas are therefore very sensitive issues and require a high level of confidentiality and informed consent. Study participants therefore consisted of only those who gave their consent, opting for inclusion following explanation of the study and agreed to have HIV testing. However, a sample size of 4,000 individuals was taken (for consistency and reliability) for each of the 3 years comprising HIV-positive and negative persons. Thus, a total sample size of 12,000 subjects was used in Phase 1 of the study.

Ethical consideration

The consent of the various hospitals and clinics used in the study was sought for and obtained prior to the investigation. Informed consent was obtained from all study participants prior to enrollment. Subject confidentiality was ensured by strict adherence to the University of Nigeria Ethical Guidance for Human Subjects including ensuring privacy of participants by using codes in place of person's names to avoid observation, intrusion or attention of others; storing samples or data in locked cabinets and not disclosing available information.

Laboratory analysis

HIV Screening

A 10-ml blood sample was collected from each participant by venepuncture into a Vacutainer K_3 ethylenediaminetetraacetic acid (EDTA) bottle using automated BD Vacutainer eclipse needle (Vacutainer CPT; Beckton Dickinson, Basel, Switzerland) after pre-test counseling and informed consent. Plasma was separated within 2 h and either processed immediately or frozen at -80°C until use. Screening for HIV-1/2 antibodies was carried out according to manufacturers' instructions using a rapid *in vitro* test kit; the Determine (Abbot Laboratories Japan).

Screening for Mycobacterium TB

Collection of sputa and other nasal secretion: Early morning sputa and nasal secretions from participants were collected in sterile wide-necked leak proof disposable containers soon on waking before mouth wash was done. Sputum production by some patients was enhanced by inhalation of mist. Three different samples were collected from each patient fortnightly and analyzed within 24 h of collection. These were first checked macroscopically by the investigator, and reported as purulent, muco-purulent, mucoid or muco-salivary, or bloody.

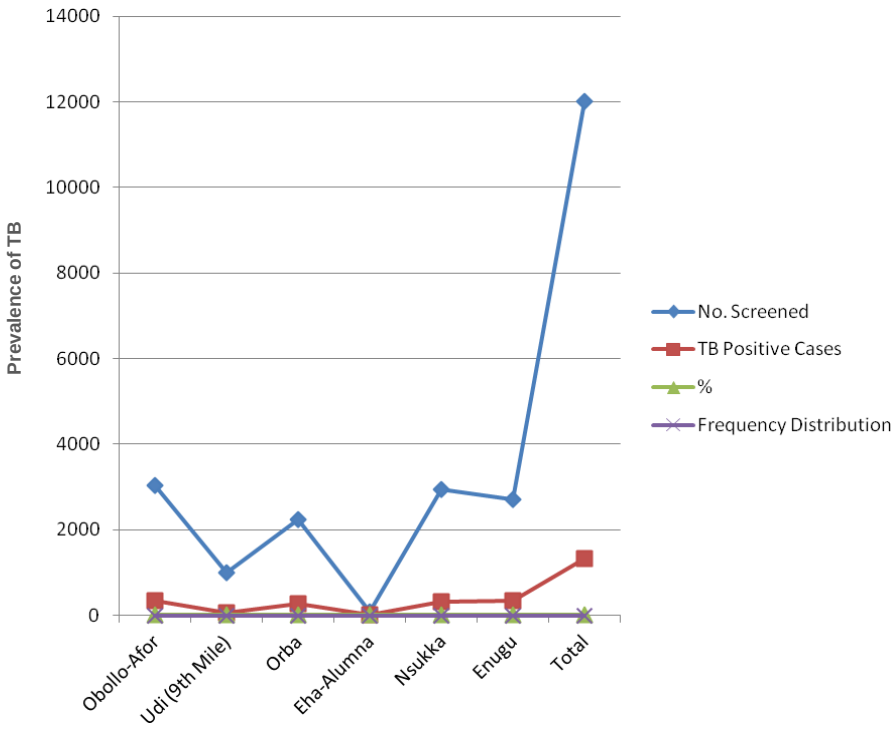

Figure 1. Prevalence of TB cases in the surveyed locations (1999-2001).

Preparation and microscopic examination of sputum smear: Screening for *Mycobacterium tuberculosis* was done using the Ziehl-Neelsen (ZN) method for acid-fast-bacilli (AFB). One drop (50 µl) of purulent, muco-purulent or cheese-like specimens was digested with two drops (100 µl) of 4% potassium hydroxide solution and held at room temperature for 1 h until the viscous samples became fluid (Stokes, 1970). The digest was then smeared on a clean greaseless microscope slide, air-dried and stained by ZN method (Cheesbrough, 1991). The stained preparation was examined microscopically for AFB.

Phase 2, a follow-up exercise was conducted between June, 2005 to July, 2008, using sera and sputa samples from 75 asymptomatic HIV positive people from the cohort. These people were re-tested to determine their HIV and TB status and followed up with regular clinical observation and laboratory screening to determine the effect of TB on HIV progression. Fifty (50) of these subjects came up with AIDS-defining conditions: recurrent fever, weight loss >10% body weight, oral candidiasis, etc. These subjects were subsequently used as a sub-cohort for determining the effect of TB on HIV progression. Patients were also classified into a CDC matrix on the basis of clinical symptoms and CD4 counts. Haematological markers of HIV progression including CD4 were fitted as time-dependent covariates, adjusting for age, sex, transmission category, and risk, using Cox proportional hazards models. Other haematological indicators of disease including white blood cell (WBC), haemoglobin and erythrocyte sedimentation rate (ESR) were used to ascertain the level of HIV disease progression.

HIV screening: Participants were re-screened for HIV1/2 and TB as earlier indicated for HIV and TB screening.

CD4 cell count: CD4 count was done by flow cytometry using an automatic portable single solid-state laser machine (the Partec Cyflow SL-3 counter; wavelength: 30nW @ 532 (green), with flow cell size, (250 × 350 µm)) synthetic quartz flow cuvette for luminar transport with sheet fluid sample and a built-in thermoprinter. 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 milliliter. CD4 cell counts were monitored at 6 monthly intervals. AIDS was defined as clinical stage C of the 1993 classification system (CDC, 1993).

Haematological parameters

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 WBCs, RBCs and lymphocytes (all expressed as number of cells per liter). ESR was done using the Western Green technique.

Statistics

Data were analyzed using the Statistical Package for the Social Scientist (SPSS, Version 18): the analysis of variance (ANOVA) where appropriate. The mean and standard deviation(SD) values were calculated for the CD4 ratios.

RESULTS

Prevalence of TB

The overall prevalence of TB among the studied population was 11.1%. This ranged from 5.3% among screened subjects in Eha-Alumona and the 9th Mile Corner to 12%

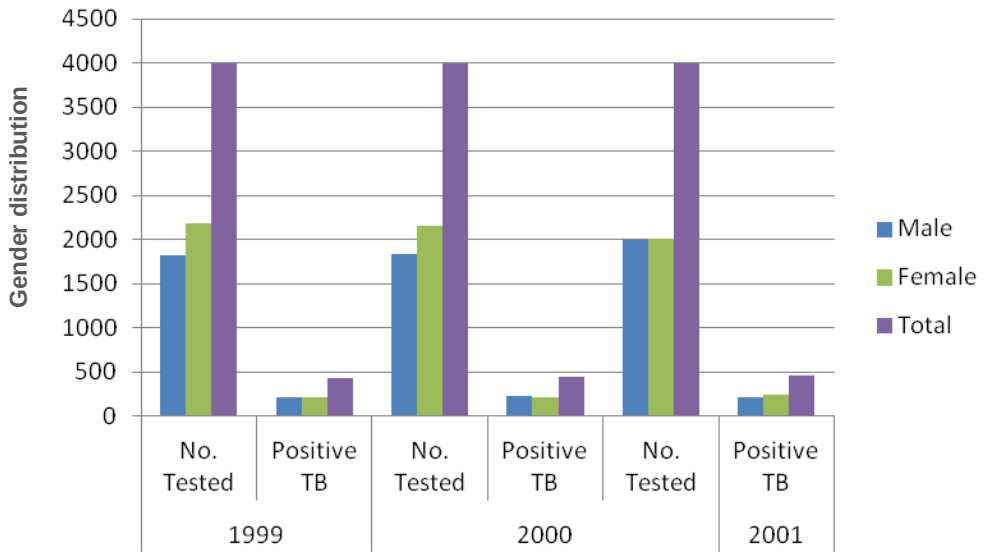

Figure 2. Gender specific distribution of TB infection (1999-2001).

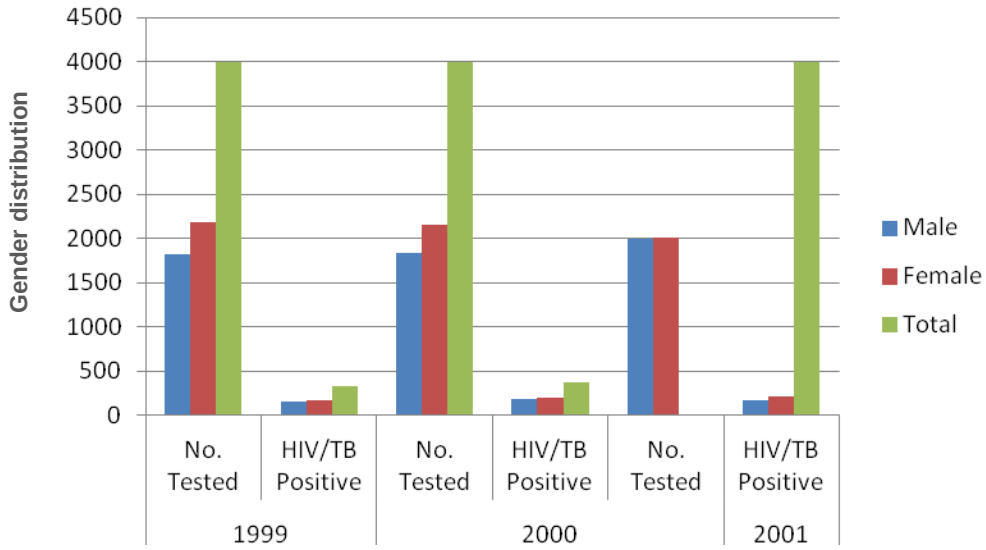

Figure 3. Gender distribution of multiple HIV/TB infections (1999-2001).

in Enugu and Orba, respectively (Figure 1). The pattern of TB infectivity among males and females in the study area for the 1999 to 2001 study period indicated that 667 (12%) of the 5662 males and 668 (11%) of the 6338 females screened were positive for TB (Figure 2). Co-infections with HIV and TB were apparent in the study. Out of the overall 2199 HIV positive subjects, 1,092 (49.6%) were infected by both HIV and TB (Figure 3). Single and pairwise infections are presented in Table 1.

Categorization of the 50 patients followed up after the preliminary screening based on their initial (baseline) and final CD4 count at immunodeficiency is shown Table 2. There was a significant decrease in the final values of all surrogate markers at immunodeficiency. However, there was no association between the decrease and age or gender ($P>0.05$). A significant increase was nevertheless observed in the AFB status of patients (from + to ++ or +++) at immunodeficiency ($P<0.05$), indicative of progression to active TB.

The five (5) surrogate markers used in this study were CD4 count, WBC, haemoglobin (Hgb), packed cell volume (PCV) and lymphocyte counts. These markers were assessed on the sub-cohort 50 HIV patients who were followed up after the preliminary study. Descriptive

Table 1. Prevalence of dual HIV/TB singly and pair-wise in surveyed area, 1999-2001.

Location	No. Screened	HIV positive	TB Positive	HIV Singly	%	TB Singly	%	HIV/TB	%
				1999					
Enugu	901	161	102	73	8.1	14	1.5	88	9.7
Nsukka	984	148	106	60	0.6	26	2.6	80	8.1
(UDI) 9th Mile	330	61	23	45	13.6	7	2.1	16	4.8
Obollo-Afor	1010	170	109	91	9.0	30	2.9	79	7.8
Orba	750	125	89	58	7.0	22	2.9	67	8.9
Eha-Alumona	25	2	1	1	4.0	1	4.0	1	4.0
				2000					
Enugu	901	182	105	89	9.0	12	1.3	93	10.3
Nsukka	984	187	108	90	9.1	11	1.1	97	9.8
(UDI) 9th Mile	330	64	28	44	13.3	8	2.4	20	6.0
Obollo-Afor	1010	199	115	109	10.7	25	2.4	90	8.9
Orba	750	119	92	45	6.0	18	2.4	74	9.8
Eha-Alumona	25	5	2	1	4.0	1	4.0	1	4.0
				2001					
Enugu	901	190	106	99	10.9	15	1.6	91	10.0
Nsukka	984	154	104	66	6.7	16	1.6	88	8.9
(UDI) 9th Mile	330	65	31	43	13.0	9	2.7	22	6.6
Obollo-Afor	1010	223	119	115	11.3	11	1.0	108	10.6
Orba	750	139	94	63	8.4	18	0.2	76	10.1
Eha-Alumona	25	5	2	3	12.0	-	-	2	8.0

Table 2. Baseline and final CD_4 count, AFB status and blood profile of follow-up group.

Code	Age	Sex	Initial/Baseline CD4 count (µl)			CD4 Count (µl) at immunodeficiency syndrome	Other blood profile								AFB status	
							Baseline				At immunodeficiency					
			<200 cells/µl	200-300 cells/µl	≥300 cells/µl	<200 cells/µl	Total WBC cells/µl	HB (g/dl)	PCV (%)	LYM (%)	Total WBC cells/µl	HB (g/dl)	PCV (%)	LYM (%)	AFB status initial/Baseline	AFB at immunodeficiency
1	41	M	-	-	305	102	12,800	14.0	42.2	60	3,200	4.9	20.2	28	+	+++
2	46	F			402	113	13,900	9.8	30.6	47	2,400	6.7	19.6	26	+	+++
3	31	F	-	290	-	101	11,000	13.1	39.9	31	2,601	7.2	18.0	30	-	++
4	25	F	-	252	-	153	15,002	11.1	36.0	36	3,500	5.6	19.5	31	+	++
5	44	M	-	-	335	96	11,900	9.4	36.7	54	2,600	6.0	17.2	29	++	+++

Table 2. Contd.

6	37	F	-	-	301	77	14,400	12.3	35.7	35	2,500	4.9	18.9	30	+	+++
7	31	F	-	-	375	106	14,800	11.7	37.6	27	2,450	5.2	17.0	26	-	+
8	28	F	-	-	384	194	13,700	12.8	36.7	61	3,000	6.3	19.0	32	-	-
9	27	F	-	-	355	131	14,600	11.4	32.8	57	4,000	5.6	20.2	27	++	+++
10	33	M	-	239	-	89	15,700	13.9	40.3	41	2,800	6.5	18.9	30	-	-
11	32	M	-	-	386	102	16,400	13.2	42.5	39	3,700	6.3	19.8	30	+	++
12	42	F	-	-	312	94	17,700	13.5	38.5	35	2,800	5.3	18.1	28	+	+++
13	31	F	-	277	-	189	16,000	11.4	32.4	33	2,520	7.1	17.7	35	-	+
14	30	F	-	296	-	162	15,600	8.1	35.6	55	3,100	5.3	19.0	29	++	+++
15	33	F	-	225	-	82	15,500	11.6	36.0	33	2,500	5.0	19.2	30	+	+++
16	55	M	-	-	395	100	16,800	12.3	36.7	30	2,650	6.0	18.5	26	-	+++
17	40	F	-	-	377	74	14,400	10.6	33.4	26	4,100	6.0	27.0	22	++	+++
18	31	F	-	202	-	68	16,800	10.0	33.5	34	2,700	5.6	20.4	31	+	+++
19	36	F	-	-	333	101	14,500	11.7	36.1	38	2, 500	5.3	18.1	27	+	++
20	38	M	191	-	-	77	14,100	14.3	42.5	35	2,700	4.9	19.8	33	++	+++
21	31	F	-	-	300	95	14,600	9.2	34.0	18	4,000	5.7	17.2	20	++	+++
22	39	F	-	-	261	74	14,200	9.8	30.2	61	3,250	5.0	18.7	46	-	++
23	32	M	-	-	333	88	17,000	11.0	32.2	33	2,600	7.3	17.9	32	-	+
24	51	M	-	-	311	103	13,008	11.7	36.5	53	3,450	7.6	17.0	27	+	++
25	34	F		290	-	160	14,900	6.6	35.5	35	4,000	5.7	19.1	23	++	+++
26	30	F	-	-	326	118	14,900	11.1	36.1	50	3,500	7.2	20.4	29	-	-
27	38	F	-	290	-	152	15,100	12.2	33.7	63	4,150	7.4	19.6	52	-	+
28	49	F	-	266	-	11O	14,000	8.8	37.8	43	3,450	6.3	20.3	40	+	++
29	48	M	-	-	310	150	15,900	12.0	33.8	33	3,500	5.3	19.5	35	+	++
30	27	F	-	-	325	166	11,200	11.9	35.7	44	2,900	5.0	18.9	40	-	+++
31	57	M	-	-	315	81	14,300	10.6	32.6	24	3,820	6.6	17.4	29	-	++
32	58	M	199	-	-	72	16,000	8.0	25.0	06	3,300	7.5	18.2	19	-	-
33	28	F	-	281	-	88	18,200	6.5	36.0	08	2,900	6.0	20.0	25	+++	+++
34	42	F	-	-	307	109	16,600	9.8	37.3	27	2,780	5.8	18.5	22	+	+++
35	39	F	-	282	-	159	14,900	6.1	33.9	43	4,200	7.0	18.9	20	-	+
36	27	F	-	-	309	127	17,200	8.2	35.3	19	2,600	4.9	17.9	22	+	+++
37	34	F	-	-	317	111	16,800	10.5	33.6	39	3,900	5.0	19.8	25	+	++
38	39	F	-	-	302	96	14,700	11.2	36.8	31	4,200	7.6	20.4	30	-	++
39	45	F	-	265	-	108	12,300	9.3	28.1	22	3,610	5.7	18.6	27	+	++
40	35	F	-	285	-	130	16,800	10.1	34.2	47	3,000	5.0	20.0	31	+++	+++
41	55	M	-	275	-	174	11,100	9.3	39.2	32	4,100	7.7	18.8	26	-	-
42	65	F	-	225	-	104	14,500	7.3	33.5	41	2,750	5.3	19.2	20	++	+++

Table 2. Contd.

43	45	M	-	-	388	100	15,000	14.7	38.4	33	2,800	6.3	18.6	32	+	+++
44	25	F	-	-	345	73	12,900	11.5	40.5	41	3,300	6.0	19.5	29	-	++
45	48	M	-	-	306	113	15,100	10.0	30.8	31	2,850	5.6	17.9	30	++	+++
46	34	F			342	120	16,800	10.8	38.7	51	3,000	4.9	18.	27	++	+++
47	48	F	-	260	-	126	14,900	10.0	39.5	48	3,950	5.0	20.	24	+	++
48	35	F	-	215	-	72	10,900	10.9	30.4	64	4,380	6.0	19.	31	-	-
49	34	F	189			68	19,200	5.8	38.1	12	2,810	5.5	19.	28	+	+++
50	64	M		-	385	101	17,000	7.4	36.4	41	2,550	5.0	18.	25	++	+++

Table 3. Descriptives of the surrogate markers on the degree of infection of HIV.

Parameter	CD4		WBC		HB		PCV		LYM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HIV	300.7	55.21	14912	1875.7	10.57	2.159	35.59	3.575	38.00	13.78
AIDS	111.2	32.57	3196	594.6	5.93	0.876	19.07	1.488	28.92	6.15
Total	206.0	105.4	9054	6048	8.25	2.849	27.33	8.738	33.46	11.55

statistics of the surrogate markers on the degree of infection of HIV as well as the means and standard deviations of the patients, computed at both HIVand AIDS levels on the 5 surrogate markers are shown in Table 3. The descriptive analysis indicated a higher mean value for the HIV than that for the AIDS level using the markers as variables. The standard deviations of the variables at AIDS level were all lower than those at HIV levels ($P<0.00$) (Table 4).

The correlation between AFB results (categorized as negative, +, ++ and +++) and HIV progression to AIDS, determined by using the surrogate markers described, showed a steady decline in the mean value of CD4 count from 256.13 to 119.37 across the four categories of AFB from negative (at baseline) to +++ (at immunodeficiency), respectively (Table 5). However, there were differences in SD across the categories; values with most uniformity had least SD of 54.21, and were indicated by +++. The mean WBC level increased from negative to +, but decreased steadily from + to +++, indicating an inverse relationship. Both the mean haemoglobin level and their SD were observed to increase from negative (-) to positive (+), but decreased steadily from + to +++. Similarly, category +++ showed values of haemoglobin with most uniformity, with SD of 1.03. The mean PCV count showed similarity to mean haemoglobin. However, their SD varied at random with least value of 4.59 occurring at +++. The mean lymphocyte values as well as their standard deviations decreased steadily across the four categories.

A test of significance (ANOVA) to determine the effect (influence) of TB on the progression of HIV to AIDS, using the surrogate markers as test variables is shown in Table 6. CD4, WBC, haemoglobin and PCV tests showed P-value as 0.00 ($P<0.05$); the lymphocyte count had P-value as 0.012 (Table 6). The effect of TB on the progression of HIV to AIDS is very highly significant at $\alpha = 0.05$.

DISCUSSION

This biphasic study investigated the prevalence of co-infection with HIV and TB in the geographical area along major transport routes of Enugu State. Their consortium in active HIV disease and the

Table 4. ANOVA test on the difference between baseline and syndrome levels.

Parameter		Sum of squares	df	Mean square	F	Sig.
CD4	Between groups	898135.290	1	898135.290	437.145	0.000
	Within groups	201345.460	98	2054.546		
	Total	1099480.750	99	-		
WBC	Between groups	3431487525.210	1	3431487525.210	1772.476	0.000
	Within groups	189726586.180	98	1935985.573		
	Total	3621214111.390	99	-		
HB	Between groups	537.776	1	537.776	198.222	0.000
	Within groups	265.874	98	2.713		
	Total	803.650	99	-		
PCV	Between groups	6824.412	1	6824.412	910.004	0.000
	Within groups	734.934	98	7.499		
	Total	7559.346	99	-		
LYM	Between groups	2061.160	1	2061.160	18.107	0.000
	Within groups	11155.680	98	113.833		
	Total	13216.840	99	-		
AFB	Between groups	37.210	1	37.210	40.726	0.000
	Within groups	89.540	98	0.914		
	Total	126.750	99	-		

Table 5. Descriptives of the surrogate markers on the effect of AFB on progression from HIV to AIDS.

Parameter	CD4		WBC		HB		PCV		LYM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative	256.13	96.28	11511.7	5026.5	10.0	2.46	31.01	7.89	36.75	14.55
+	263.92	85.14	12826.3	5310.9	10.1	2.61	32.53	8.13	35.46	11.42
++	195.64	111.25	8485.2	5848.3	7.6	2.39	26.26	8.59	34.96	10.57
+++	119.37	54.21	4044.1	3913.8	5.7	1.03	20.43	4.57	27.37	6.95
Total	205.95	105.38	9054.3	6048.0	8.3	2.85	27.33	8.74	33.46	11.55

role of CD4 cells and other haematologic parameters as markers of HIV progression was similarly evaluated. The steady increase in TB and HIV prevalence rates observed has serious public health implications. HIV tends to complicate the problems of TB patients and of course endangers the entire community by increasing the rate of multi-drug resistant strains of the bacterium as well as further compromising the immune status of infected persons as earlier reported (Nunn et al., 1990; Bonecici et al., 1998). Co-infection with HIV and TB investigated in this study has a dramatic impact on the quick progression of HIV/TB patients to full-blown AIDS as well as the possible activation of latent TB and subsequent progression to active TB among the multiply infected persons. Immune suppression or degeneration resulting from the multiple HIV/TB infections will automatically increase the risk for opportunistic infections and AIDS-related malignancies in the population. The reality that HIV/TB co-infection has caused more deaths than the individual infections is confirmed in this study. This view is supported by the reports on the synergistic effect of the co-infections (Murray et al., 1990). The reported occurrence of multiple HIV/TB infection further suggests that an epidemic of TB is going on alongside HIV/AIDS disease among the people within the area being studied, including the transport (truck/stopover) communities of Enugu State. This evidence is supported by the reports on the presence of co-infection in immune suppressed individuals (Lucas, 1992). Co-infection with these pathogens is therefore particularly devastating in

Table 6. ANOVA test on the effect of AFB on progression from HIV to AIDS.

Parameter		Sum of squares	df	Mean square	F	Sig.
CD4	Between Groups	346114.235	3	115371.412		
	Within Groups	753366.515	96	7847.568	14.702	0.000
	Total	1099480.750	99	-		
WB	Between Groups	1172249741.145	3	390749913.715		
	Within Groups	2448964370.245	96	25510045.523	15.317	0.000
	Total	3621214111.390	99	-		
HB	Between Groups	342.869	3	114.290		
	Within Groups	460.781	96	4.800	23.811	0.000
	Total	803.650	99	-		
PCV	Between Groups	2287.833	3	762.611		
	Within Groups	5271.513	96	54.912	13.888	0.000
	Total	7559.346	99	-		
LYM	Between Groups	1413.125	3	471.042		
	Within Groups	11803.715	96	122.955	3.831	0.012
	Total	13216.840	99	-		

the rural communities under study where the burden of disease is high. Though the paradigm of the dual HIV/TB infections and distribution does not seem to show any significant difference, there are however associated socio-economic and socio-cultural implications. On the socio-economic level, the constant rise in HIV/TB infection in the area indicates a mutual interaction between the two infections and also portrays the level of socio-economic status of the area such as poverty. Several people are living below subsistence level which further sustains risk behaviours predisposing to HIV, including sex working (commercial and clandestine) and multiple sex partnering (Dibua, 2010). In addition is overcrowding, a consequence of harsh economy which facilitates transmission of TB and promotes HIV infection thereby further weakening the vulnerability and susceptibility of the entire community.

The surrogate markers used in this study have proven prognostic value in evaluating the influence of TB on HIV progression and the development of AIDS-related conditions. This view is confirmed by previously published reports on the importance of surrogate markers in evaluation of HIV infection (Deyton, 1996). The absolute CD4 T-lymphocyte count is one of the best surrogate markers for assessing the risk of progression to AIDS among HIV-infected individuals. The general tendency for the $CD4^+$ count to decline with time among HIV-infected patients despite the variability among individuals was demonstrated in the study; the decrease in CD4 count is thus indicative of the HIV progression to AIDS, authenticating the categorization of the AFB as indicated. The idea that $CD4^+$ T-lymphocyte numbers are predictive of AIDS-defining illnesses was yet strengthened by the observation of significant differences in $CD4^+$ levels associated with increased AFB (+++). This observation is confirmed by the reports of World Health Organization, in defining its staging system for HIV disease, in which it proposed the utilization of total lymphocyte count as predictor of HIV disease (WHO International Collaborating Group for the Study of the WHO Staging System, 1993). The results of this study confirm the value of using the surrogate markers in limited resource settings, for the diagnosis and management and/or follow-up of infected subjects.

Among the other cellular measures used in the study that could reflect immune defense processes in the HIV disease progression, no one appears to be consistently irrelevant; the reported low haemoglobin and PCV were predictive of steeper decline of $CD4^+$ counts; though did not seem predictive of the onset of AIDS: a typical normal number of RBCs found in a blood specimen can range from four to 6 million cells/µl (one thousandth of a milliliter) of blood depending upon gender and the altitude at which the person lives. Hemoglobin varies with altitude (male: 13.8 to 17.2 gm/dl; female: 12.1 to 15.1 gm/dl). The impact of a decline in the haemoglobin/PCV levels as observed in the study is severe anaemia. This is in conformity with the reports on the association of changes in haemoglobin levels in infants with low birth weight and fetal anaemia (Cessie, 2002).

The statistical analysis (descriptive) of the surrogate markers indicated a higher mean value for the HIV than

that for the AIDS level using the markers as variables. However, the SD of the variables at AIDS level was all lower than those at HIV levels. A test of significance using the analysis of variance to determine the difference between baseline and syndrome levels (the mean HIV and AIDS level) using each of the surrogate markers as test variables, indicated a P-value of 0.00, implying a significant difference between the mean values at HIV and AIDS levels: the more the progression from HIV to AIDS, the more the values of the surrogate markers tend towards decline. This confirms the influence of TB in HIV disease progression. The AFB value, +++, was therefore observed to be the most uniform value of all investigated parameters at immunodeficiency state. The analysis of variance ($P<0.05$), confirmed the view that TB is a significant factor in furthering the progression of HIV to AID; this is very significant at $\alpha=0.05$. The analysis further indicates the role of the surrogate markers as indicators of immune system deterioration and subsequent decline.

Conclusion

Co-infection of HIV and TB has intrinsically been linked to acceleration of the course of HIV and subsequent progression to AIDS, particularly among the local population in Nigeria. The study confirmed the individual role of TB in furthering the progression of HIV to AIDS, and in addition, emphasized the importance of CD4 counts as prognostic marker, as well as the role of other surrogate markers as indicators of immune system deterioration and subsequent decline. The use of these markers in HIV disease monitoring and diagnosis was proposed especially in the local communities where diagnostic resources are limited and the burden of the disease is high.

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