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CD4⁺ and CD8⁺ lymphocytes and clinical features of HIV seropositive Nigerians on presentation

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Summary

Eighty of 200 HIV seropositive patients admitted in the medical wards of Ahmadu Bello University Teaching Hospital, Zaria from year 1995 to 1997 were studied on presentation and compared to 40 age and sex matched controls. The main clinical features observed included weight loss, pyrexia, diarrhoea, lymphadenopathy, anaemia and pruritic dermatosis. Sixty-two of the 80 patients (73.2%) presented at stages 3 and 4 of WHO Clinical and Laboratory staging. Thirty (30) percent of these patients died between a period of one to four months after presentation. The main diseases complicating HIV infection at presentation of the 80 patients were *Mycobacterium* tuberculosis infection (30%), acute bacterial infections (with *Salmonella typhi*, *Streptococcus pneumoniae* and *Staphylococcus aureus*) (24%), candidiasis (14%) and Kaposi sarcoma (2%). Seropositivity for HIV types was found to be HIV-I alone in 43.5% of cases; HIV-II alone in 14% and both HIV-I and II in 42% of cases. Risk factors associated with HIV infection were multiple sexual partners (73%), sexually transmitted disease (70%), and unscreened blood transfusion (1%). HIV positive patients had a mean CD4⁺ T-cells of 0.24 x 10^9 ± 0.17 which was significantly lower than the mean of 0.6 ± 0.17 x 10^9 /L for controls ($P < 0.05$ students t-test). Thirty (35%) of the patients had CD4⁺ counts of less than 0.2 x 10^9 /L (200 cells/µl) at presentation. The mean CD3⁺ lymphocytes count was 0.51 ± 0.24 x 10^9 /L for patients and 1.04 ± 0.71 x 10^9 / L for controls. The mean CD8⁺ lymphocyte count in patients was 0.29 ± 0.19 x 10^9 /L and 0.44 x 10^9 /L for controls. Both CD3⁺ and CD8⁺ lymphocyte populations were statistically lower in patients than controls ($P < 0.05$).

Keywords: *HIV, CD4, CD8, TB, Kaposi, sarcoma, diarrhoea*

Résumé

80 des 200 patents VIH seropositif admis an centre hospitalier universitaire de l'universite Ahmadu Bello, Zaria entre 1995-1997 ont ete etudies sur presentation et compares a 40 individus de controle d'age et sexe mixtes. Les principaux trait characteristiques observes etaient la perte du poids, diarrhes, lymphadenopathie, anaemie, pyrexie et la dermatose puritique; 62 des 80 patients (73.2%) se presentaient an stade 3 et 4 des etapes cliniques de laboratoire de l'OMS. 30% de ces maladies sont morts an cours de la periode allant d'un a 4 mois après s'etre presentes. Les malades principales complicant davantege l'infection an VIH a l'arrivee des 80 malades etaien. L'infection mycobacterium de la tuberculose (30%), infections bacteriennes poussees (avec la Salmonelle typhi, streptoccoque pneumoniae et staphyloccoque aureus) (24%), candidiase (14%) et la sarcoma kaposi (2%). La seropositivite au VIH etait du type VIH-I dans 43.5% des cas, VIH-II, 14% et a la fois VIH-I et II dans 42% des cas. Les facteurs de risques associes a l'infection an VIH etaient les partenaires sexuels multiple (73%), MST (70%) et la transfusion sanguine sans test de dipistage (1%). Les maladies VIH positif avaient une moyenne CD4⁺ des cellules –T de 0.24x10^9 ± 0.17 qui etait significativement bas par rapport a la moyenne de 0.6 ± 0.17 x 10^9/L pour les contoles ($P< 0.05$ test de l'etudiant) (student t-test). 35% des patients avaient un comptage de CD4⁺ moins de -.2 x 10^9/L (200 cellules /il) a l'arrivee. La moyenne de comptage du lymphocyte CD3⁺ etait de 0.51 + 0.24 x 10^9/L pour les patients et 1.04 ± 0.71 x 10^9/L pour les controles. La moyenne des lymphocytes CD8⁺ comptes chez les malades etait de 0.29 ± 0.19 x 10^9/L et 0.44 x 10^9/L pour les controles. Las populations des lymphocytes CD3⁺ et CD8⁺ etaient statistiquement bas chez les patients contrairement aux individus de controle ($P<0.05$).

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Introduction

Human Immunodeficiency Virus (HIV) attaches to CD4⁺ cell surface molecule with the aid of co-receptors like chemoreceptor CXCR4 in lymphocytes and CCRS for macrophages [1]. The resultant clinical sequelae of HIV infection have affected virtually every corner of the globe [2], with Africa recording an approximate total of 300,000 AIDS cases by the end of 1993 [3] (WHO, 1994). By the year 1997, Nigeria had reported 10,803 AIDS cases to the National AIDS and STD Control Programme with a general prevalence rate of 4.5% in 1995. Northern Nigeria has shown a slowly increasing rise in HIV infection in most of the northern cities like Maiduguri, Kano and Kaduna [4,5]. HIV-1 serotype contains sub-groups A – J with A, C and D subtypes predominating in Africa, while group O contains several heterogenous viruses. There are at least five subtypes of HIV-2, which is predominantly found in West Africa [6]. It has been shown by Endress and colleagues that HIV-2 may infect CD⁴ positive cells using CXCR4 as the primary receptor [1].

Some of the common clinical presentation in Africans include persistent cough, prolonged fever, chronic diarrhoea and generalised lymphadenopathy and the mode of transmission is primarily heterosexual [5]. Study of any asymptomatic seroconverters in Stockholm showed that within four years symptomatic seroconverters progressed to CDC stage IV disease [6].

The production of strong cell-mediated immunity together with a dominant type I cytokine profile (IL-2, IL-12, gamma IFN) by type I T helper cells (TH_1) is important in delaying progression and resisting HIV viral infection, while co-factors stimulated by parasites in Africa may interact within the patient to switch to type II cytokine profile (IL-4, IL-5, IL-6, IL-10) produced by Type II helper cells (TH_2) with progression of disease. CD8⁺ suppressor cells normally produce antiviral factor (CAF) which blocks HIV RNA expression and it is notable that type I cytokine profile increases CD8 antiviral response while type II cytokines inhibit CAF production in vitro [7]. The points at which such events occur within patients with HIV including our patients are usually not determinable on presentation of the patients to hospital. The virus causes depletion of CD8⁺ subset of lymphocytes with progression to an immunodeficient state and subsequent decrease in cytotoxic lymphocyte (CD8⁺ cell) responses against all major HIV patient [8].

It had been stated that the risk of developing an opportunistic infection becomes significant when the CD4 lymphocyte count falls beneath 200 cells/mm³ which worsens as the CD4⁺ cell count further declines [9]. While reports on the sero-epidemiology of HIV infection in Nigeria are increasing [10,11,15]; there is paucity of information in Nigerian patients on cell-mediated immunological parameters correlated with clinical features. This study presents the lymphocyte populations and clinical features of HIV seropositive patients on presentation at the medical wards of the Ahmadu Bello University Teaching Hospital, Zaria.

Materials and methods

The study was carried out in the Department of Medicine, Ahmadu Bello University Teaching Hospital, Zaria, between 1991 and 1997 as an ongoing study of HIV seropositive patients in northern Nigeria. A total of eighty patients whose laboratory and clinical investigations were analysed and subsequently followed up as out-patients were studied. Controls for the immunologic assays and laboratory investigations were forty HIV seronegative, age and sex matched volunteers within and around the hospital environment. A detailed history and physical examination to exclude sexually transmitted diseases and any disease that causes immunodeficiency was undertaken for each control.

Clinical assessment: All HIV patients were assessed clinically using a standardized study protocol based on WHO 1990 criteria [12].

Laboratory investigations

HIV antibody detection: Antibody to HIV was measured from 1991 to 1996 using a competitive enzyme immunoassay ("Wellcozyme HIV-1 + 2" Wellcome Diagnostics) and from 1996 to 1997 using an indirect solid phase enzyme immunoassay ("Organics" Immunocomb BISPOT) with a 100% sensitivity and specificity (WHO/GPA, 1995) [13]. All patients and controls had a full blood count and differential count using standard methods [14]. Microbial cultures of urine, blood and stool were carried out routinely where indicated. All subjects had serum total proteins, serum albumin and electrolytes evaluated using the methods described by Tietz, 1990 [15].

Immunological assays

Separation of lymphocytes was carried out using the method of Gupta and Good, 1977 [16]. Ten millilitres of heparinized blood was diluted 1:2 with Eagles Minimum Essential Medium (MEM), layered on 3ml of LYMPHOPREP (SIGMA CO.) and centrifuged at 1,800rpm for 30 minutes at room temperature. The lymphocyte layer at the FICOLL-MEM interphase was harvested, washed and adjusted to a cell concentration of 4.0 x 10^6/ml.

These lymphocytes were then labelled with monoclonal antibodies to CD3 total T cells (anti-Leu-4), Cd4 T helper cells (anti-Leu-3a) and CD8 cytotoxic/suppressor cells (anti-Leu-2a), conjugated to fluorescein. The monoclonal antibodies were produced by Becton Dickinson (USA) [17] and the brochure of the company (1981) was used in laboratory staining. Immunoflourescence was read with fluorescent microscope.

WHO clinical and laboratory staging

The laboratory axis is CD4 cell counts per microlitre at presentation while the 1990 reviewed WHO clinical staging (Stage I-IV) were combined with CD4 cell counts in assessment and staging of patients (WHO, 1994).

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Statistical methods

The mean of groups of parameters were analysed statistically using the students t-test and a p-value of < 0.05 was considered significant.

Results

Figure 1 shows the annual progressive increase in the number of patients presenting with HIV infection from 1991-1997 in Department of Medicine and Immunology, Ahmadu Bello University Teaching Hospital, Zaria from 10 cases in 1991 to 102 cases in 1997).

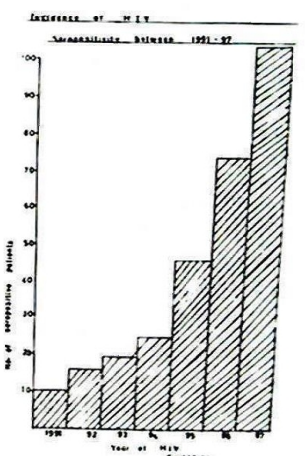

Fig. 1:

Age and sex distribution: Fifty-eight male and 22 female patients (male:female ratio 2.6:1) positive for HIV were studied immunologically while the controls included 27 males and 13 females (M:F = 2.1:1) who were HIV seronegative. The mean ages for male and female patients and controls were 33.1, 32.3 and 33.2 years, respectively. Table 1 shows HIV types with HIV-1 in 35 patients, HIV-II alone in 11 patients and HIV-I and II in 34 patients.

Table 1: HIV type in the patient population at presentation (80 patients)

HIV status	Number (%)
Type 1	35 (43.75)
Type II	11 (13.75)
Type I and II	34 (42.5)
Total	80 (100)

Table 2: Clinical features of HIV patients at presentation

Feature	Number (%)
Weight loss	65 (81%)
Pyrexia	53 (66%)
Diarrhoea	44 (55%)
Lymphadenopathy	42 (52%)
Anaemia	23 (29%)
Persisten cough	23 (29%)
Pruritic dermatosis	22 (27%)

Clinical features: Ninety-eight percent of 80 patients had positive heterosexual behaviour. Fifty-six of the male patients admitted to having had multiple sexual partners and 26 had a past history of sexually transmitted diseases. Two male patients had a positive history of homosexuality. One female ex-convict reported sexual harassment several times by prison warders. Five female patients agreed to having had more than one sexual partner. Three patients including one sickle cell disease (SS + F) patient claimed that unscreened blood transfusion was the likely source of their HIV infection. Seventy-five percent of both male and female patients were married and 15 of the patients had polygamous marriages. Table 2 shows that the common clinical features on presentation were fever, malaise, diarrhoea, persistent cough, oral thrush and significant weight loss. Twenty four patients had active tuberculosis and nine of them died during follow-up. Twenty one patients had acute bacterial infections, 8 with typhoid fever due to *Salmonella typhi*, 2 due to *Salmonella paratyphis*, 6 with lobar pneumonia due to *Streptococcus pneumoniae*, four had septicaemia due to *Staphylococcus aureus* while 2 had septicaemia due to *Klebsiella* spp. *Streptococcus pneumoniae* was also responsible for meningitis in one of the patients. Another common feature in 44 (55%) of the patients examined was diarrhoea. Twelve of the patients examined had diarrhoea. Twelve of the patients with diarrhoea died at follow-up. Three patients were histologically proven to have Kaposi sarcoma (KS). Two of the patients died and one was lost to follow-up. Fifty-three (66%) of the patients had fever and malarial parasitaemia was detected in 12 of them. Other features and complicating diseases are shown in Table 3.

Laboratory features

Haematology: The mean haemoglobin levels were 11.3 ± 5.85g/dl and 12.6 ± 2.92g/dl in patients and controls, respectively (P <0.05). The mean total lymphocyte. The white cell differential counts also showed marked neutrophilia in 50% of the patients.

Biochemistry: Twenty-one (27%) of the patients were hypoalbuminaemic (serum albumin less than 28gm/l). Random blood sugar was however normal (range 3.0 – 7.7, mean 4.9 mMol/L).

Immunology: The distribution of CD3, CD4 and CD8 positive lymphocyte counts is shown in Table 4. Total lymphocyte count, $CD3^+$, $CD4^+$ and $CD8^+$ lymphocyte counts as well as CD4 : CD8 ratio were significantly lower in HIV patients than controls ($P < 0.05$). Fig II shows the distribution of $CD4^+$ and $CD8^+$ cells in patients and controls while Fig. III shows the distribution of absolute total lymphocytes in patients and controls. The mean $CD4^+$ T-cell count among patients with tuberculosis (TB) was 0.48 ± 0.19 x 10^9/L while among those with acute bacterial infection it was 0.24 ± 0.25 x 10^9 /L. pulmonary tuberculosis

Table 3: Diseases complication HIV + VE patients on at presentation.

Disease	Number (%)
Tuberculosis	24 (30%)
Acute bacterial infections	21 (25%)
- Typhoid fever	8
- Lobar pneumonia	6
- Septicaemia	6
- Meningitis	1
Candidiasis	17 (21%)
- Oral thrush	12
- disseminated	5
Kaposi sarcoma	3
Herpes zoster	3
Lepromatous leprosy	2
Facial nerve palsy	1
Seizures/Convulsions	2
Meningeal cryptococcosis	1
Encephalopathy	2

Table 4: T-lympocyte populations in HIV + VE patients and controls at presentation.

	Total Lympho cytes	CD3 Mean + SD	CD4 Mean + SD	CD8 Mean + SD	CD4:CD8
HIV patients	1.18±0.9	0.51±0.24	0.24±0.17	0.29±0.19	0.77
N = 80	x10^9/L	x10_9/L	x10^9/L	x10^9/L	
Controls	1.89±0.53	1.04±0.71	0.60±0.17	0.40±0.78	1.12
N = 40	x10^9/L	x10^9/L	x10^9/L	x10^9/L	
	P <0.05	P < 0.05	P < 0.05	P <0.05	P <0.05

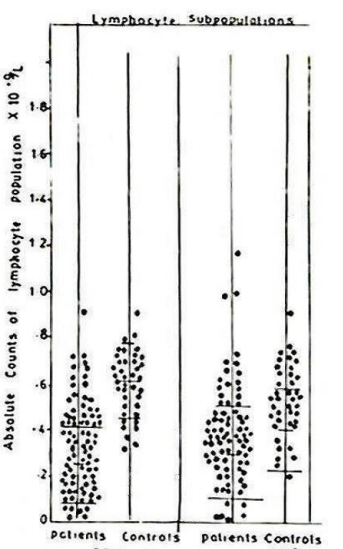

Fig. 2:

occurred in patients with CD4 counts between 400 to less than 200 cells per ml. Mean $CD4^+$ cell count in three KS patients was 0.25 ± 0.16 x 10^9 /L while in diarrhoeal patients it was 0.22 ± 0.17 x 10^9 /L. Thirty of the 80 patients had $CD4^+$ T-cell count of less than 200 cells/ml (<0.2 x 10^9/L).

WHO Clinical and Laboratory Staging: 80 HIV patients with full immunological results were staged according to WHO Clinical and Laboratory Staging method and presented in Table 5.

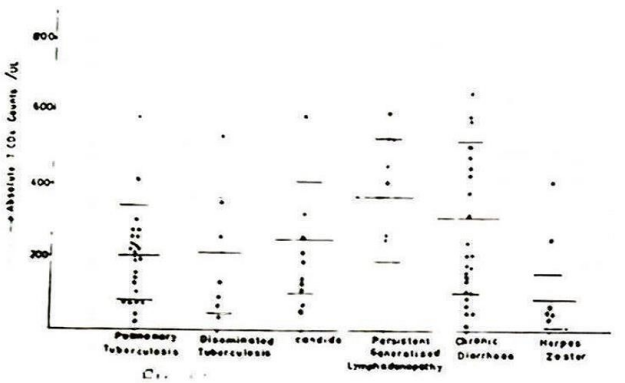

Fig. 3:

Table 5: Staging at presentation in 80 HIV patients. WHO clinical and laboratory staging

Stage	No(%)	Stage	No(%)	Stage	No(%)	Stage	No(%)
1A	4(5%)	2A	1(1.3%)	3A	3(3.8%)	4A	6(11%)
1B	10(15.5%)	2B	2(2.5%)	3B	17(21.3%)	4B	11(13.8%)
		2C	1(1.3%)	3C	6(9.8%)	4C	19(23.9%)

Discussion

This study confirms that HIV infection is on the increase in northern Nigeria (Fig. 1) and that transmission via the heterosexual route is still the main mode of infection. In another study from Nigeria, all but one HIV seropositive patient had concurrent sexually transmitted disease (STD) and transmission was confirmed as via the heterosexual route [11]. While this seems to be a common feature of HIV infection/AIDS in the African sub-region [18,19]; and with the recognition of the role of sexually transmitted disease in the transmission of HIV, it becomes obvious that awareness of these risk factors which promote the spread of HIV such as male patronage of prostitutes, the high rate of STDs, multiple sexual partners and low rate of condom use is mandatory for the successful control of the HIV/AIDS epidemic in Nigeria. Some of these have important attitudinal complications for the culturally operative polygamous marriage pattern predominant in northern Nigeria.

The clinical features consisting of significant weight loss, diarrhoea, tuberculosis, candidiasis and acute severe bacterial infections are similar to other reports from the African subregion [13, 20]. Tuberculosis and diarrhoea coupled with undernutrition may be responsible for the wasting syndrome as evidenced by the low body mass index and lower serum albumin in such patients [21]. Cryptococcal meningitis was found in one patient, while pulmonary tuberculosis (PTB) was found in patients with CD4 cell counts in all ranges (above 500 cells/ml, between 200 and 500 and below 200 cells/ml). This is especially important in an environment of global tuberculosis re-emergence and the spread of multi-drug resistant strains [29, 30].

Using 1990 WHO staging system for HIV infection and disease which is based on clinical and laboratory evaluations, our results showed a mean total lymphocyte count of 1183 cells/μl and mean $CD4^+$ T-cell count of 240 cells/μl in patients and this was significantly lower than in controls. Most of our patients on presentation were in stages 3 and 4 signifying late presentation as well as rapid deterioration of cell-mediated immunity since their symptoms occurred within one to three years. Another study from the United Kingdom [25] showed that AIDS patients (aged between 15 and 24 years) were white unaware of whether they were HIV positive for up to nine months before diagnosis, while our cases who also presented late were in the age bracket of 19 – 65 years. This findings prompt the suggestion that cell-mediated immunity switches early to the elaboration of type II cytokines in blacks and non-whites which deserves confirmatory study. Furthermore, late presentation with HIV seropositivity has been shown to be associated with poor maintenance and high mortality [26]. Indeed 30% of our patients died within a period of four months from presentation. Such high mortality rates have also been recorded by other workers in the West African subregion [22]. High mortality and rapid progression from asymptomatic to full blown AIDS may also be contributed to by lack of awareness of infection, poor utilisation of health services, inadequate health services, financial handicap, prevailing undernutrition and additive effect of other chronic bacterial and parasitic infections in developing countries including Nigeria [7].

Total lymphocyte counts ($CD3^+$ cells) were significantly lower in our patients than controls and while 35% of our patients presented with $CD4^+$ T-cell counts of less than 200 cells/μl, 40 patients (50%) presented with CD4 cell counts between 200 and 500 cells per μl. This is similar to findings from a study in Ivory Coast with a rapid emergence of AIDS [20] and the depletion of CD4+ T-cells by HIV infection. Mechanisms proposed for the depletion of $CD4^+$ T-cells include a direct cytopathic effect, inhibition of cellular protein synthesis, fusion of uninfected $CD4^+$ cells with viral envelope proteins to form syncytia, putative autoimmune mechanisms and virus programmed cell-death or apoptosis [23,24]. Also Type II (T_H2) cytokine profile (IL-4, IL-5, IL-6< IL-10) may be predominant in Nigerians and Ivoriens leading to more rapid progression and loss of cell-mediated immunity as proposed in the renegade HIV immune hypothesis [7].

Increasing evidence also points to the role of $CD8^+$ T-cells in early HIV infection [27]. Levy [1] had shown that CD8 cells produce an antiviral factor (CAF) which blocks HIV RNA expression and this protective CD8 response is lost before CD4 counts begin to fall. Type I dominant cytokines (T_H1 response with IL-1, IL-12 and gamma IFN) increase CD8 antiviral response while type II dominant cytokines, IL-4 and IL-10 inhibit CAF production. Our results showed a significantly lower $CD8^+$ count in our patients when compared to controls which may tend to support the renegade hypothesis. Thus it is possible to have a CD4 : CD8 ratio approaching normal values because of progressive decline of both $CD4^+$ and $CD8^+$ cells in an individual patient. Further studies on the activation and surface expression of new CD molecules on T-$CD8^+$ cells such as CD38, CD57 with disease progression as well as the decrease of CD26, CD28 and CD45RA will contribute to the understanding of the role of $CD8^+$ cells during progression of HIV infection [28].

In conclusion, our study further shows that a few normal Nigerians (controls) may have low normal CD4 and CD8

counts. Such may be as a result of being in an environment of persistent parasitic bacterial and viral confrontation as well as micronutrient deficiency and needs further study.

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