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Tetanus antibody in Nigerians living with HIV/AIDS: A preliminary report

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ABSTRACT

The aim of this study is to investigate the need for anti-tetanus immunisation in Nigerians living with HIV/AIDS by quantifying antibody to tetanus organism in them. Both symptomatic and asymptomatic consenting Nigerians positive for HIV infection and aged 15 years and above were included in the study. Apparently healthy age- and sex-matched subjects were enrolled as controls. Immunisation history was recorded in all participants. The PCV, WBC, platelet and CD4+ cell counts were done on automated counter. Serum levels of antibody to tetanus were quantitated using standard ELISA method. There was no significant difference (t = 0.138, *p* = 0.89) in the mean serum levels of antibody to tetanus in patients with HIV/AIDS (0.5 ± 0.86 IU/mL) when compared with the controls (0.46 ± 0.52 IU/mL). About 85.7% (36/42) of patients with HIV/AIDS had protective tetanus antibody and only six (14.3%) had non-protective antibody levels. In patients with CD4+ T lymphocytes of < 200 cells/µL, the mean anti-tetanus antibody was 0.50 ± 0.98 IU/mL, while in those with CD4+ T lymphocytes of > 200 cells/µL, it was 0.53 ± 0.53 IU/mL. The difference was not statistically significant (t = 0.1, *p* = 0.918). The majority of our patients presented in advanced stage with 69% of them having CD4+ T lymphocytes < 200 cell/µL. This study found a significant number of Nigerians with HIV/AIDS having protective levels of antibody to tetanus in their sera. We therefore suggest continue efforts at improving on the National Programme on Immunisation, as immunisation in patients with HIV infection may not yield adequate responses and may be fraught with the risk of increase in viral replication.

Keywords: infection, immunisation, tetanus antibody, HIV/AIDS, Nigerians

INTRODUCTION

Tetanus is serious but preventable disease that affects the neuro-muscular system of the body. The disease is caused by the toxin of *Clostridium tetani*, a bacterium found in the soil, stool and anything lying on the ground. Among HIV-infected individuals, skin lesions/diseases are known to cause significant morbidity, and could either as a result of the disease (Mohammad *et al.*, 2003; Resneck *et al*., 2004) or due to adverse drug reactions (Namayanja *et al.*, 2005; Manosuthi *et al.*, 2006; Forna *et al.*, 2007). In view of the possible contamination of the skin lesions from the environment or autoinfection from diarrheal stool, the need for vaccination (Kurtzhals *et al.*, 1992) against this disease has been suggested to avert the serious disease in the already immunocompromised individuals. The aim of this study is therefore to investigate the level of antibody to tetanus toxin in Nigerians living with HIV/AIDS, a study that has not been done before.

MATERIALS AND METHODS

Confirmed HIV/AIDS patients seen between July and December 2006 at the Communicable Diseases and Haematology clinics of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria, were consecutively recruited into the study. Ethical clearance was obtained from the hospital's Research and Ethics Committee. Written consent was also obtained from all patients after been assured of confidentiality. Both symptomatic and asymptomatic consenting Nigerians positive for HIV infection and aged 15 years and above were included in the study. Apparently healthy age- and sex-matched subjects were enrolled as controls. Age and gender of subjects and controls were also documented. History of immunisation was also documented in both patients and controls.

Blood samples were taken, in appropriate bottles, for blood counts (packed cell volume, white blood cells and differentials, platelets), CD4+ cell count and serum tetanus antibody level. Haematological parameters were estimated within 6 h of sample collection, while sample for tetanus antibody level were stored at temperature of –20 °C and estimated in batches. The packed cell volume, white cells and platelet counts were done on automated counter (ADVIA-60 Bayer Corporation, New York, United States of America), while CD4+ cell count were done on

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automated counter (Partec Cyflow, GmbH, Otto-Hahn-Str. 32; D-48161 Munster, Germany). Serum levels of antibody to tetanus were quantitated using Tetanus Toxoid IgG Elisa Kit (Demeditec Diagnostic GmbH * Lise-Meitner-Straβe 2 * D-24145 Kiel, Germany) following the manufacturer's instructions (Bio-Rad, 2005). Serum samples for IgG antibody to tetanus quantitation were retrieved from the –20 °C freezer, allowed to thaw to room temperature and analysed in one batch. After passing the samples and standards through ELISA reactions, their concentrations (and optical densities) were then read with a microplate reader (Model 550; Bio-Rad Laboratories, Hercules, USA) at 450 nm, according to the manufacturer's instructions (Bio-Rad, 2005). Tetanus antibody was expressed in International Units per millilitre (IU/mL) and results interpreted according to manufacturer's recommendations which state that value of antibody less than 0.1 IU/mL requires immunization (Demeditec Diagnostics)

Data analysis: Data are presented as means and standard deviations (means ± SD). Student's t-test was used to test the significance of differences between mean values with statistical significance set at p value less than 0.05. Data were analysed using SPSS 11 (SPSS Inc, Chicago, USA, SPSS Inc 1989-2001) statistical software.

RESULTS

Characteristics of the study population: Forty-two patients living with HIV/AIDS and 10 apparently healthy persons were investigated. Of the patients living with HIV/AIDS, 14 were males while the rest 28 were females with a male to female ratio of 1:2; while more females (six females, four males) were also investigated in the control arm, with a male to female ratio of 1:1.5.The mean age in the patient group was 36.69 ± 9.29 years and in control group was 43.00 ± 16.24 years (t = –1.648, p = 0.106) with no significant difference. The majority of participants admitted to have had immunisation during childhood (Table 1).

Haematological characteristics: The mean (± SD) haematocrit in the HIV/AIDS subjects (32.40 ± 16.24%), as expected, was significantly lower compared with the controls (39.40 ± 4.58%) (t = –3.64, p = 0.001). However, the mean WBC though also higher in the controls (4289.29 ± 2016.62/cmm and 5380.00 ± 1250.60/cmm for HIV/AIDS patients and controls, respectively), was not significant (t = –1.63, p = 0.11). Similarly, the mean platelet was expectedly higher in the controls (191857.14 ± 9330.92/cmm and 225200.00 ± 58537.36/cmm for HIV/AIDS patients and controls, respectively), was not significant (t = –1.076, p = 0.29).

Seroprevalence of tetanus antibody: There was no significant difference (t = 0.138, p = 0.89) in the mean tetanus antibody levels in patients living with HIV/AIDS (0.5 ± 0.86 IU/mL) compared with that of the controls (0.46 ± 0.52 IU/mL) (Table 1). By internationally accepted serum level of anti-tetanus antibody (Bonetti *et al*., 2004), 85.7% (36/42) of patients living with HIV/AIDS had protective levels of antibody to tetanus, with only 6 (14.3%) of the 42 having non-protective antibody to tetanus in their sera.

CD4+ cell count in subjects: The mean CD4+ cell count in patients (166.10 ± 102.95 cells/µL) was significantly lower (t = 12.22, p = 0.00) that what was found in the controls (708.00 ± 122.28) (Table 1). Twenty-nine (29/42 or 69%) of the patients had CD4+ cell count of less than 200 cells /µL, while the rest 13 (31%) had CD4+ T lymphocytes of 200 cells or above showing that the majority of how patients presented in advanced stage of the disease.

Tetanus antibody according to CD4+ T lymphocyte counts: Mean antibody levels to tetanus in patients living with HIV/AIDS were not significantly different based on the circulating CD4+ T lymphocytes counts (Table 1). In patients with CD4+ T lymphocytes of < 200 cells/µL, the mean anti-tetanus antibody ± SD was 0.50 ± 0.98 IU/mL, while in those with CD4+ T lymphocytes of > 200 cells/µL, it was 0.533 ± 0.531 IU/mL (t = 0.1, p = 0.918). In regression analysis, there were also no significant differences in subjects' age and serum level of tetanus antibody (t = –1.15, p = 0.25) or subjects' number of circulating CD4+ cells count and serum level of tetanus antibody (t = –0.32, p = 0.75).

DISCUSSION

This preliminary study on the quantification of serum level of antibody to tetanus organism was done to assess the need for prophylactic vaccination of people living with HIV/AIDS as they are likely to present with more severe outcome than the seronegative individuals. The tetanus organism is found in the soil, stool and anything lying on the ground and as such could easily contaminate open wounds, such as dermatological lesions (Garbe *et al*., 1994; Munoz-Perez *et al*., 1998) that are not uncommon occurrence in HIV/AIDS patients either resulting from the disease or secondary to antiretroviral (ARV) or other medications in the management of the patient. In this study, we do not found any significant difference in the serum levels of antibody to tetanus in Nigerian patients living with HIV/AIDS and the seronegative controls. This is an important finding in HIV infection since the infection alters immune functions, immunisation of HIV infected persons may not confer the same protection as may be possible in immunocompetent persons. Also, immunisation of HIV-infected persons can enhance viral replication as a result of immune stimulation (Castello-Branco and Ortigao-de-Sampaio, 1998) leading to a rapidly progressive disease. Although studies have also shown that HIV-infected children are able to mount both cellular and humoral immune responses to commonly administered vaccines in the first two years of life (Borkowsky *et al*., 1992), antibody level tend to be lower and to decrease more rapidly over time compared to immunocompetent individuals (Ryder *et al*., 1993). The implication of this is that attempt at prophylactic vaccination of our patients (if the anti-tetanus antibody are non-protective) is that the desired effect may not be

Table 1: Immuno-haematological and demographic parameters of Nigerians living with HIV/AIDS

	HIV/AIDS	Control	t-value	*p*-value
Age (in years)	36.69 ± 9.29	43.00 ± 16.24	-1.18	0.11
PCV (%)	32.40 ± 5.64	39.40 ± 4.58	-3.64	0.001
WBC (/cmm)	4289.29 ± 2016.62	5380.00 ± 1250.60	-1.63	0.109
Platelet (/cmm)	191857.14 ± 93301.92	225200.00 ± 58537.36	-1.08	0.87
Tab (IU/mL)	0.50 ± 0.86	0.46 ± 0.65	0.14	0.89
CD4+ (/µL)	166.10 ± 102.94	708.00 ± 122.28	12.22	0.00
*CD4+ < 200	0.50 ± 0.98			
*CD4+ > 200	0.49 ± 0.53		0.04	0.97

Tab: serum tetanus antibody; IU/mL = international unit per millilitre; *CD4+: serum anti-tetanus antibody with CD4+ cells of < 200 and CD4+ cells of > 200 cells/µL; PCV: packed cell volume (in percentage); WBC: white blood cell (per cm^3)

achieved. Although the CDC approved the use of inactivated vaccines (such as diphtheria, pertussis and tetanus in HIV patients (Centre for Diseases Control, 1988), studies have shown that even tetanus toxoid can cause increase in viremia and proviral burden in patients with HIV infection when given the Toxoid (Stanley *et al*., 1996)

The majority of our patients had advanced disease with 69% of them having CD4+ T lymphocytes < 200 cells/µL. This is also reflected in their reduced haematocrit, WBC and platelet counts. This is probably a disadvantage in achieving good results if they are to receive immunisation as studies have shown that in patients with advanced HIV infection had fewer antibody production responses than patients with asymptomatic infection (Janoff *et al*., 1988). It has also been documented that even among patients that are asymptomatic, responses of patients with CD4+ T lymphocyte count of > 500 cells/µL were similar to HIV-negative individuals, whereas the responses of patients with CD4+ T lymphocyte count of < 500 cells/µL were significantly lower (Rodriguez-Barradas *et al*., 1992). A similar study also showed that antibody production to inactivated polio vaccine is better with CD4+ T lymphocytes > 200 cells/µL (Vardinon *et al*., 1990). Therefore, the level of CD4+ T lymphocytes in HIV/AIDS patient could be an important factor in patient's response to vaccination. The role of CD4+ T lymphocytes in antibody production have also been documented in animal model (Kennedy *et al*., 2003).

In conclusion, immunising HIV/AIDS patients against infectious diseases will no doubt reduce morbidity, mortality, and improve quality of life in them. However, because HIV infection alters immune functions, immunisation in HIV-infected persons may not confer the expected protection as in the immunocompetent individuals and might even worsen their viral status. This study has shown that the majority of our cohort of patients has protective anti-tetanus antibody level, which is probably a reflection of the effectiveness of the National Programme on Immunisation in country.

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