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Seroprevalence of HIV-1 and HIV-2 infection among children diagnosed with protein-calorie malnutrition in Nigeria

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SUMMARY

Excessive weight loss due to protein calorie malnutrition (PCM) is a significant problem in Nigerian children. This syndrome may be difficult to differentiate from the wasting disease caused by human immunodeficiency virus type 1 (HIV-1) infection. We studied 70 children admitted to the Baptist Medical Center in Ogbomosho, Nigeria in 1990 with PCM for prevalence of antibodies to HIV-1 and HIV-2. The cohort was from low-risk mothers and had a median age of 25 months (range, 4 months–9 years) with a weight deficit of at least 20% of the theoretical weight for age. Two sera were positive for anti-HIV-1 by both ELISA and Western blot (WB). A high prevalence of samples negative for HIV-1 antibody by ELISA were repeatedly reactive (11%, 8/70) or indeterminate (46%, 32/70) by WB. None of the sera was positive for antibody to HIV-2. There was no correlation of ELISA positivity or extent of WB banding with successful recovery from malnutrition. These results indicate a relatively low but significant prevalence of HIV-1 infection in Nigerian children with PCM. The high prevalence of indeterminate reactions in WB assays for HIV-1 suggests that other procedures may be necessary for confirmatory diagnosis of HIV-1 infection in this African population.

INTRODUCTION

Nigeria is a coastal West African country with approximately one-fifth the population of the continent. It has been shown recently that there is a significant and increasing incidence of infection with human immunodeficiency virus type 1 (HIV-1) among high-risk groups in Nigeria, even though the seroprevalence of HIV-1 is relatively low, i.e. $< 1\%$ [1]. Little is known, however, about the seroprevalence of HIV-1 infection in the paediatric population of Nigeria. This is

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of particular interest because in areas of central Africa, HIV-1 infection is recognized as a factor associated with protein-calorie malnutrition (PCM) in children [2].

The purpose of this study was to determine the seroprevalence of HIV-1 infection in Nigerian children diagnosed with PCM. The prevalence of HIV-2 infection was also examined to determine whether this retrovirus had spread to the Nigerian region and was associated with PCM.

MATERIALS AND METHODS

Study population

Clinical histories and serum samples were taken from 70 children (34 males, 36 females) consecutively admitted with a diagnosis of PCM to the Baptist Medical Center in Ogbomosho, located in the Oyo state of the Southwestern region of Nigeria, from 6 June to 2 August 1990. Informed consent was obtained from participants. This study was approved by the Institutional Review Board of the University of Pittsburgh. The population studied was from low risk mothers (traders, farmers, seamstresses) of the Yuruba tribe, residing in or near the cities of Ogbomosho ($n = 54$), Ilorin ($n = 15$) and Lagos ($n = 1$). The children ranged in age from 4 months to 9 years (mean, 31 months; median, 25 months), and had a weight deficit of at least 20% of the theoretical weight for age. All of these patients showed clinical signs of kwashiorkor, marasmus or marasmic-kwashiorkor.

Serological tests

The sera were tested for antibodies to HIV-1 by a commercial enzyme-linked immunosorbent assay (ELISA) that utilizes whole viral lysate as antigen (LAV-EIA, Genetic Systems, Seattle, WA). A serum was interpreted as positive by ELISA if the optical density absorbance value was equal to or greater than the sum of 0·225 plus the average of three negative control absorbance values. We also examined whether high absorbance values were associated with reactive WB assays. For this, serum samples were defined as 'high-negative' in the ELISA if their absorbance values were equal to or greater than the sum of the average of three negative control values plus 0·08. This is based on the absorbance values of sera from homosexual male subjects in the Pittsburgh portion of the Multicenter AIDS Cohort Study that were above the 95th percentile of the ELISA-negative range.

All sera were tested for HIV-1 antibody by Western blot (WB) (Novapath Immunoblot, Bio-Rad, Hercules, CA) regardless of the ELISA result. The WB bands were read visually, and sera which had bands of any intensity were retested at least once using a different lot of WB kit for each test. Results of WB tests were interpreted as follows, using criteria recommended by the Association of State and Territorial Public Health Laboratory Directors and the Centers for Disease Control [3]: negative when no bands were present, reactive ('positive') when antibodies were detected against any two of the following HIV-1 gene products: p24, gp41 or gp120/160, and indeterminate if other combinations were present. Only results of WB bands that were repeatedly present in two or three separate assays were interpreted as indeterminate.

All sera were tested for HIV-2 antibodies by the Central Blood Bank laboratory in Pittsburgh with an ELISA that uses a whole virus lysate as antigen (HIV-2 EIA, Genetic Systems). Specimens were defined as positive if the absorbancy value was equal to or greater than the sum of 0·15 plus the average of three negative control values. Sera that were positive by HIV-2 ELISA were tested for HIV-2 antibody by WB (HIV-2 Western blot, Cambridge Biotech, Worcester, MA) in the New York Blood Center laboratory. A reactive ('positive') result was defined by the manufacturer's criteria as bands corresponding to p26 and either gp34 monomer or trimer.

RESULTS

Two of the 70 samples (3%) were positive for anti-HIV-1 by ELISA (Table 1). These sera were also positive by ELISA when retested. Nine samples (13%) were in the high negative ELISA range for anti-HIV-1, and 59 (84%) were ELISA negative.

Ten (14%) of the 70 sera were reactive for HIV-1 by WB using established United States criteria [3] (Table 1). The two ELISA positive samples were reactive by WB, for an overall prevalence of 3% (2/70). These sera had bands at p24, p55 and gp41 (both samples), and bands at gp160 (one sample), on repetitive testing. Eight (11%) of the 70 sera were either ELISA negative or high negative, and were reactive by WB. There was a high prevalence, 32/70 (46%), of samples that were indeterminate by WB. Only 28 of 70 (40%) were negative for HIV-1 by WB.

Among the high-negative ELISA samples, 44% (4/9) were repeatedly reactive for HIV-1 by WB and 56% (5/9) were WB indeterminate (Table 1). In comparison, only 7% (4/59) of the group that tested negative for anti-HIV-1 by ELISA were reactive by WB ($P = 0{\cdot}008$, Fisher's exact test), and 46% (27/59) were indeterminate by WB ($P = 0{\cdot}04$). The most common bands seen by WB were in the *gag* and *env* regions. One or more bands were *gag*-specific in 86% (36/42) and *env*-specific in 48% (20/42) of the WB positive and indeterminate sera. All of the 28 WB negative sera were also negative by ELISA.

The two children who were ELISA and WB positive were 36 and 51 months of age. The mean age of children with ELISA positive and high negative samples was 46 months (range: 26–74 months), whereas the mean age for ELISA negative samples was 28 months (range: 4 months–9 years; $P \leqslant 0{\cdot}005$; unpaired t test). The association of mean ages with WB results was: 45 months for positive sera, 31 months for indeterminate WB sera, and 27 months for WB negatives.

Only one of the 70 sera was positive for antibody to HIV-2 by ELISA. This was the specimen from the child aged 51 months which was positive for HIV-1 antibody by ELISA and WB assay. The serum was, however, negative for HIV-2 antibody by WB assay.

Our results showed no correlation between HIV-1 ELISA positivity or extent of WB banding with successful recovery from malnutrition. All patients from the HIV-1 ELISA positive and high-negative groups were discharged in good health, having satisfied all the criteria for discharge at the Baptist Medical Center. That is, each patient was clinically healthy with improved nutritional status, having gained at least 2 kg to come within 15% of their theoretical weight for age. Two children who died during hospitalization were HIV-1 ELISA and WB negative.

Table 1. *Analysis of results of ELISA and Western blot methods for detection of antibody to HIV-1 in sera from 70 Nigerian children with protein calorie malnutrition*

HIV-1 Western blot reactive bands*†	Total number of sera	ELISA results‡		
		Positive	High-negative	Negative
Positive				
p24+gp41+p55	7	1	2	4
p24+gp41+p55+gp120/160	3	1	2	0
Indeterminate				
p18	1	0	0	1
p18+p24+p55	1	0	1	0
p18+gp41	1	0	1	0
p18+gp41+p55	1	0	0	1
p18+p55	1	0	0	1
p24	14	0	1	13
p24+p32	2	0	0	2
p24+p55	2	0	1	1
p55+p65+gp120/160	1	0	0	1
p55+gp120/160	1	0	0	1
p32	1	0	0	1
p32+gp41	1	0	1	0
gp41	4	0	0	4
gp41+p55	1	0	0	1
Negative				
No bands	28	0	0	28
Total	70	2	9	59

* Interpretive criteria used by the Association of State and Territorial Public Health Laboratory Directors and the Centers for Disease Control [3].

† HIV-1 gene products: *gag* = p18, p24, p55; pol = p32, p65; *env* = gp41, gp120, gp160.

‡ Optical density absorbancy values were 0·668 and 0·666 (cut-off, 0·253) on first testing, and 0·543 and 0·709 (cut-off, 0·254) on repeat testing, for the two ELISA positive specimens. There was an average absorbancy value of 0·171 ± 0·05 (cut-off, 0·107) for the 9 ELISA high-negative sera, and an average of 0·068 ± 0·02 for the 59 ELISA negative specimens.

DISCUSSION

We found that 3% of 70 children from Southwestern Nigeria in 1990 admitted with a diagnosis of PCM were HIV-1 positive by ELISA and WB testing using standard criteria recommended by the Centers for Disease Control for United States populations [3]. These probably represent true HIV-1 infection, rather than passively transferred maternal antibodies, because the two seropositive children were 36 and 51 months of age. The mothers of the children in our study were at low-risk for infection with HIV-1. This is in agreement with other studies that show a relatively low but significant seroprevalence of HIV-1 infection in low- and high-risk adult populations in Nigeria [1, 4–6]. These surveys indicate that HIV-1 seroprevalence increased from 0 to 0·03–0·3% in low-risk adults in Northeastern, South and Southwestern regions of Nigeria from the early to late 1980s. The seroprevalence is higher (0·5–5·1%) in high-risk, female prostitutes from these areas of Nigeria. There was no serological evidence of HIV-2 infection in these children, even though an increase in HIV-2 infection has been reported in Nigeria [1].

PCM is a common presentation of paediatric AIDS in Africa. The relatively low

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proportion of paediatric patients seropositive for HIV-1 by both ELISA and WB in this study, however, suggests that PCM is not associated with HIV-1 infection in these Nigerian children. Our rate of 3% seropositivity for HIV-1 in 1990 is also several-fold lower than the 12% seroprevalence in children with PCM in the Central African Republic in 1986 [2]. This difference probably reflects the higher prevalence of HIV-1 infection in the female, child-bearing aged population in the Central African Republic.

Our results show that children with PCM of low-risk Nigerian mothers have a very high prevalence of reactivity by HIV-1 when tested by WB alone. Eleven percent (8/70) of the sera which were ELISA negative and interpreted as reactive ('positive') by WB assay, and 46% (32/70) were ELISA negative and indeterminate by WB using criteria recommended by the Centers for Disease Control [3]. Several other studies have shown that sera from African patients exhibit a significantly high number of indeterminate WB patterns [7–9]. The most common bands present in ELISA negative and either WB reactive or indeterminate sera in this investigation were in the *gag* region (p18, p24 and p55). One or more of the *gag* proteins were evident in WB assays of 85% (34/40) of these sera, with p24 being the predominant type (65%, 26/40). This is similar to the predominance of *gag*-specific bands in indeterminate WB results in low-risk blood donors in the United States [10–12].

The significance of the indeterminate WB results in African sera is unclear. It is possible that such results are due to methodological factors in the WB assay. For example, lot-to-lot variability in the WB kit is reported to be a major contributor to indeterminate WB results [12]. In the present study, however, WB bands were only acceptable if they were repeatedly present on retesting using a different lot of WB kit. Our study did indicate that there were significant associations between ELISA determinations in the high negative range and both WB reactive and indeterminate results. This could be related to *gag*-specific reactivity in both of these tests [11]. Persons in the United States and Europe who are at low risk for HIV infection and who have persistently indeterminate HIV-1 WB are rarely, if ever, infected with HIV-1 or HIV-2 [13–16]. Studies have also shown that the majority of high-risk individuals with indeterminate WB results do not develop fully diagnostic serology for HIV-1 [17], and are HIV-1 negative by culture and DNA amplification [16].

The very high prevalence (57%) of reactive and indeterminate WB tests for HIV-1 antibody in ELISA negative specimens from this young population is greater than the 20–32% prevalence reported in low-risk adults in the United States [12, 14]. This suggests that WB assays carry less diagnostic significance in Africa. It should be noted, however, that the ELISA negative sera that were either WB reactive or indeterminate in this study would have been interpreted as HIV-1 negative, and would not have been tested by WB based on the standard procedures used in the United States [3]. Nevertheless, alternative tests for HIV-1 antibody may be needed for accurate diagnosis of HIV-1 infection in the Nigerian population.

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