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Comparative Evaluation of HIV Infected Foreign Students and Indian with AIDS in Chandigarh, India

S. Sehgal

SUMMARY **Out of a total of 1,600 foreign students who came to India between June 1989 and October 1990, 22 were seropositive for HIV-1. Ten showed antibodies to all the gene products. Antibodies to gp160 and p24 were present in all the seropositives while antibodies to p53, p15/17 were significantly higher in healthy seropositives than in patients with full blown AIDS. Absence of antibodies to p15/17 and p53 thus appeared to be a more sensitive criterion of end stage disease than absence of anti- p24 antibodies.**

When seropositive samples from African students were checked for HIV-2 antibodies by ELISA, 13/22 were found to be positive. Further, 2/10 Indians with full blown AIDS were also strongly positive for HIV-2. These data could be of relevance for formulating future strategies for population-based screening for HIV-2.

In India the first seropositive case of human immunodeficiency virus was detected in April, 1986 and by May the first full blown case of AIDS was diagnosed in a foreigner. [1] By March, 1990, 2,167 seropositives were detected with a positivity rate of 4.7 per thousand and 6 months later, the number increased to 4,082 with a positivity rate of 10.2 per thousand. [2] On the eastern border, in Manipur more than 1,500 seropositives have been detected among intravenous drug users. [3] The infection has thus taken a slow and steady hold in this sub-continent. Foreign students form another high risk group, particularly those coming from Africa. By October, 1990 a total of 85 foreign students were reported to be positive all over India and one fourth were detected in a surveillance center attached to Nehru Hospital, Chandigarh. The present study was designed to assess the antibody reactivity to different viral gene products in subjects detected to be positive at this centre. The patterns were correlated with ELISA values and compared with those of patients with full blown disease seen in India.

MATERIALS AND METHODS

A total of 1,600 students were screened for HIV-1 antibodies between June 1989 to October 1990 by the conventional enzyme-linked immunoassay using a Wellcozyme Competitive kit. Any subject showing a value below the cut off was subjected to Western blot assay using a Dupont kit. The tests were performed strictly in accordance with the instructions provided with the kits. Briefly, the strips containing the peptides were blocked with 2 ml of blocking buffer for 1 hour and 20 μl of test serum added to the blocking buffer. Incubation was carried out at 37° C in a shaker water bath. The strips were washed extensively with 3 changes of wash buffer and sequentially reacted with appropriately diluted biotinylated antihuman IgG and avidin labelled peroxidase. The color was developed using alpha chloronaphthol and the positive bands evaluated carefully. The intensity of bands was arbitrarily graded from + to + + + + and a scoring system was devised combining the intensity and the number of bands. Presence of strongly positive antibody to all the major nine bands plus extra bands were designated as + + + +. All the sera were checked for VDRL reactivity and IgA levels. Platelet counts were also done in all cases. All positive cases were checked for

From the Department of Immunopathology Postgraduate Institute of Medical Education and Reseach, Chandigarh-160012, India.

Table 1. Western blot patterns in patients and seropositives

Gene products	E ELISA positive		
	Africans (22)	Indians (20)	Indians with AIDS (10)
gp 160	100 percent	100 percent	100 percent
gp 120	60 "	85 "	80 "
p 66	91 "	80 "	70 "
p 55	73 "	80 "	30 "
p 53	82 "	80 "	40 "
gp 41	91 "	100 "	90 "
p 32	86 "	90 "	70 "
p 24	100 "	95 "	70 "
p 15/17	82 "	95 "	40 "

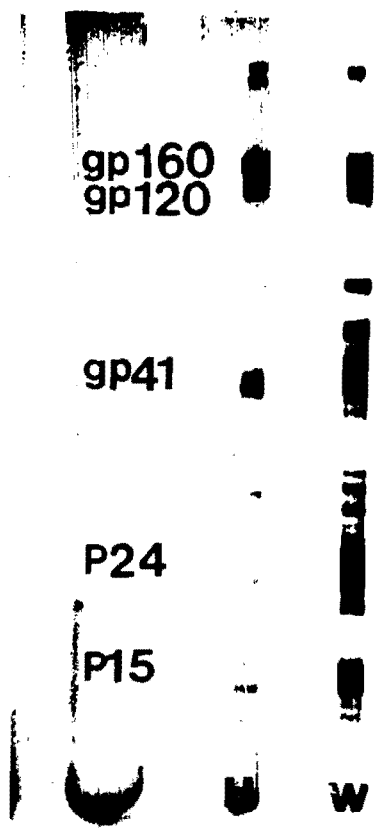

Fig. 1 Paired Western blot pattern from an indian with full blown AIDS (H) with healthy seropositive spouse (W).

HIV-2 infection also by ELISA. The presence of HIV-2 antibodies was confirmed by pepti LAV assay (Pasteur Diagnostics, Paris). Diagnosis of full blown AIDS was substantiated by enumerating the absolute number of CD4 positive cells.

RESULTS

Out of a total of 1,600 healthy students, twenty two were found to be seropositive and the reaction was strongly positive in all the cases. 89% of the students were from Kenya. Their ages ranged from 18 to 30 years with a mean age (± SD) of 23 (±3.5) years. Out of these, 19 were males and 3 females. All the positive students belonged to Kenya. The mean (± SD) ELISA value (OD at 450 nm) was 0.11 (± 0.13) with a range of 0.14 to 0.20, while the cut-off mean was 0.45 (± 0.12) and that of the control sera was 1.68 (± 0.28).

No subject exhibited an indeterminate Western blot pattern. The number of reactive bands varied from 7 to 18. There was a direct correlation between ELISA and Western blot scores ($r = 0.89$). The net results of Western blot pattern are indicated in Table 1. Antibodies to gp160 and p24 were present in all the 22, i.e. 100% were seropositive while anti-p66 and gp41 antibodies were positive in 91%. Antibodies to gp120 and p55 were less frequent, being positive in 60 and 73 percent, respectively. At least 2 students showed distinct bands between 31-36 kDa and five patients depicted positive reaction to 12-15 kDa peptides.

During an initial period of 2.6 years, ten full blown cases of AIDS were also diagnosed in subjects originating from Punjab. Each of them had a history of contact either in an African or a Gulf country. When categorized according to the WHO staging, all were in stage 4 of the disease. When the Western blot patterns of healthy asymptomatic foreign students were compared with those of full blown AIDS cases from India, the most striking differences were the antibodies to p15/17 which were present in only 40% of patients with full blown AIDS as compared to 82% of healthy students. Similarly, antibodies to p55 were present in 73% of healthy students and only 30% of patients with AIDS. On the other hand, anti- p24 antibody was still present in 70% patients 1-2 months before death. Ten students had antibodies to all the structural gene products. All the students were asymptomatic except for a minor sexually transmitted ailment. The VDRL test was positive in 6/22, i.e. 27.2% of the cases. None had thrombocytopenia. The mean IgA was statistically higher than the Indians controls ($p < 0.001$). The total lymphocyte count was normal in all and eosinophilia was observed in 5/22 cases.

Although some false positive ELISA reactions were obtained in early 1989 with Wellcozyme kits, no false positive reaction was obtained with new generation Wellcozyme kits based on monoclonal antibody capturing recombinant antigen onto the microtiter plates. A clear example of fading anti- p15 and p55 antibodies could be appreciated in a couple

where the husband had full blown disease and the wife was a healthy seropositive (Fig. 1). Out of 22 cases, 13 were also positive for HIV-2 by ELISA. While 2/10 Indians with full blown AIDS were also positive for HIV-2.

DISCUSSION

The present study aimed to delineate the Western blot patterns in healthy seropositive foreign students and to compare them with those of full blown cases of AIDS. The criteria of Western blot positivity included the presence of antibodies to at least one glycoprotein peptide each from all the three structural gene products. Recently WHO has redefined criteria for Western blot positivity in which antibodies to envelope gene products (gp160, gp120, p41 kDa) are claimed to be the most reliable.[4]

It has earlier been observed that antibodies to some gene products may disappear during progression of the disease[5] and therefore a critical analysis of the Western blot pattern has important prognostic implications. Our limited data indicated that antibodies to gp120 were less frequent in asymptomatic healthy seropositives (60%) as compared to patients with full blown AIDS (80%). All Indian patients with AIDS were diagnosed preterminally and died within 1-3 months of diagnosis. Antibodies to gp160 were positive in 100 percent of AIDS patients and seropositives. Clearly, gp160 evokes a stronger humoral immune response. Whether it is more immunogenic or more readily accessible to the host to mount an immune response is an intriguing question and needs further analysis. Antibodies to p53, p15/17 were significantly 91% of seropositives. Antibodies to gp53, p15/17 were significantly higher in healthy seropositives than in patients with full blown AIDS. Absence of antibodies to p15/17 and p53 thus appeared to be a more sensitive criterion for end-stage disease than absence of antibodies to p24. In the present study 13/22 students were also positive for HIV-2 by ELISA; similarly two Indians with full blown AIDS were also positive for HIV-2. They were confirmed by a line immunoassay which tested antibodies against gp41 and gp36 detected as discrete bands. Conventional Western blot for HIV-2 was, however, not done. Possibility of cross reaction in some of these cases cannot be ruled out, yet it does indicate that HIV-2 is present in India. Although interaction between HIV-1 and HIV-2 has not been studied in detail, there are indications of increasing incidence of HIV-2 infection in Guinea Bissau and Côte d' Ivoire, respectively.[6,7] Several tests are available for HIV-2 assay.[8-10] In fact Constantine, Callahan and Watts[11] felt the need to evolve a test which could simultaneously check for both the viruses and today several test systems offer this facility. A double dot ELISA dip stick as developed in France could be useful for populations where transmission can be traced to an African country. It has been observed at our Center that several Indians travelled to African countries initially and then migrated to the Gulf region after a number of years. Such a situation was encountered in two of ten patients who died of AIDS. These factors might become responsible for changing the epidemiology of HIV-1 and HIV-2 infections in this region. The government of India has rightly taken initial steps of introducing an HIV-I plus HIV-2 ELISA for screening of blood donors.

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