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Prognostic Significance of Serum Beta-2 Microglobulin in Patients with HIV Infection and AIDS

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SUMMARY

Evaluation of beta-2 microglobulin [b_2M] levels was undertaken in 34 cases of full blown acquired immunodeficiency syndrome [AIDS], 20 apparently healthy human immunodeficiency virus (HIV) seropositive individuals and 20 normal controls to evaluate its usefulness as a surrogate marker to distinguish between full blown AIDS cases and HIV seropositive individuals. However, it had a poor predictive value in establishing a diagnosis of full blown AIDS in an individual patient. Further, the coefficient of correlation between CD4+ T-lymphocyte count and b_2M was low both in patients with full blown AIDS and HIV seropositive individuals.

INTRODUCTION

Human Immunodeficiency Virus (HIV) epidemic has taken deep roots in India ever since the first case was documented in a sex worker in Tamil Nadu (1). It is estimated that by the year 2000, India will have 5 million infected individuals. Stunning advances have been made in a short time spanning a decade regarding the pathogenesis of acquired immunodeficiency syndrome [AIDS]. It was recognized quite early that CD4+T-lymphocytes cells are the key targets (2) for the HIV virus and during the development of full blown AIDS a substantial reduction in the numbers of CD4+T-lymphocytes is an essential component (3). Enumeration of CD4 + T-lymphocytes has therefore been adjudged the best prognostic marker for the development of full blown AIDS and has been used regularly to monitor therapeutic regimes (4). Subsequently, however, it was revealed that the viral load assay was the most sensitive documentation of viral replication (5). Even when peripheral CD4+T-lymphocyte count is normal, there is a substantial replication of the virus in the tissues and blood (6-8). The draw back of these tests are that they are expensive and not available for routine use. While CD4+T-lymphocyte estimation requires sophisti-

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cated and expensive instrument i.e., flowcytometer and antibodies, the cost of viral assay is prohibitive. Most of the Indian patients belong to poor socio-economic background and they cannot afford these expensive tests. Surrogate markers thus have an important role in assessing prognosis of HIV infected patients. Beta-2 microglobulin [β_2M] is one such marker used in patients with HIV infection and AIDS.

β_2M is a small polypeptide that forms invariant chain with HLA class-I molecule and is present on all nucleated cells (9). Small amounts of β_2M occur in various body fluids (10). Increased [β_2M] levels in serum have been found in patients with malignancies including multiple myeloma where it is the most powerful prognostic and therapeutic marker (11). β_2M also reflects lymphoid activation, destruction and hence is indicative of HIV replication (12).

In the present study, we evaluated the usefulness of serum β_2M estimation in Indian patients with HIV infection and AIDS. We have attempted to address the question whether serum β_2M can be a reliable surrogate marker to diagnose full blown AIDS in patients with HIV infection.

MATERIAL AND METHODS

Cases reporting to the AIDS Surveillance Centre, Department of Postgraduate Institute of Medical Education and Research, Chandigarh were included in the study. The diagnosis of HIV infection was established by two different ELISA tests, i.e., Recombigen (Cambridge Biotech, Ireland), Detect (Biochem Immunosystem Inc., Canada) and a rapid test (Cambridge Biotech, Ireland) or Immunocomb (Orgenics, Israel). If all the three tests were frankly positive, the case was designated as HIV positive. In case of even a minor discord, Western blot test was performed (Newlav Blot-I/II, Sanofi Diagnostics, Pasteur, France). Clinical diagnosis of AIDS was established in 34 patients by the CDC clinical criteria or a CD4+T-lymphocyte count less than 200/mm^3. Twenty apparently healthy HIV-positive individuals and 20 healthy normal controls were also included in the study and subjected to similar investigations.

Serum β_2M estimation

β_2M was estimated using an enzyme immunoassay (United Biotech, Inc. USA) based on a sandwich enzyme-linked immunosorbent assay [ELISA] principle. Briefly, β_2M kit employs microwell coated with anti-β_2M antibodies. Patient sera diluted 1 : 1000 and standards were incubated in the microwell for 30 minutes at room temperature. After washing in phosphate buffer saline, 100 ml of the enzyme conjugated second antibody was added and the plate was incubated for another 30 minutes at room temperature. After several washings, the colour was developed using tetramethyl benzidine. After 10 minutes of incubation, the reaction was stopped by adding 100 ml of 1N H_2SO_4. The intensity of the colour was read at 450nm. This was quantitated by plotting standards vs O.D. on a double log graph paper according to the manufacturer's instructions.

Estimation of CD4+T-lymphocytes

In twenty randomly selected cases absolute CD4+T-lymphocyte count was measured in a Becton Dickinson flowcytometer using anti-CD4 + and anti-CD8+ antibodies and consort-30 programme. Briefly, 100 µl of EDTA blood was incubated with 20 µl of the Simulset reagent for 30 minutes. The mixture was subjected to red cell lysis using a 1 to 10 dilution of the lysing fluid (Becton Dickinson). After incubating for 10 minutes, the cells were washed thrice in phosphate buffer saline pH 7.2 and viewed in the flowcytometer. The percentage of CD4 + T-lymphocytes was enumerated and their absolute number was calculated from absolute number of total lymphocytes.

Statistical analysis : Unpaired 't' test was employed in different groups to evaluate significance of β_2M and CD4 + T-lymphocyte count. Correlation coefficients (r) were computed between CD4 + T-lymphocyte count and serum β_2M levels in patients with full blown AIDS and apparently healthy, HIV-seropositive individuals.

RESULTS

The mean age of full blown AIDS cases (n=34) was 35 [SD 12] years. There were 25 males. The mean age of apparently healthy HIV-seropositive individuals (n=20) was 26.7 [SD 7.5] years. The mean serum β_2M level in patients with full blown AIDS was 6.7 (SD 2.3) mg/ml. Considering a level of 7 µg/ml as the cutoff, 17 of the 34 patients with full blown AIDS (50%) had levels >7 µg/ml. Among the HIV-seropositive individuals the mean serum β_2M level was 4.15 (SD 2.2) µg/ml. Two cases (10%) had levels > 7 µg/ml. Mean serum β_2M levels in 20 healthy controls was 1.7 (SD 3.5) µg/ml. These details are shown in Table 1 and Figure 1.

Considering 4.15 µg/ml, 26 cases [76.5%] of full blown AIDS and 9 HIV-seropositive individuals [45%] had values > 4.15 µg/ml. However, when the cut off was taken as the mean normal value in

Table 1 : *Serum β_2 microglobulin levels in 30 patients with full blown AIDS, 20 HIV-seropositive individuals and 20 normal controls*

Cutoff value (mg/ml)	AIDS n (%)	HIV-seropositive individuals n (%)	Normal controls n (%)
> 7.0	17 (50.0)	2 (10.0)	0 (0)
< 7.0	17 (50.0)	18 (90.0)	20 (100)
> 4.15	26 (76.5)	9 (45.0)	0 (0)
< 4.15	8 (23.5)	11 (55.0)	20 (100)
> 2.4	33 (97.6)	16 (80.0)	0 (0)
< 2.4	1 (2.9)	4 (20.0)	20 (100)

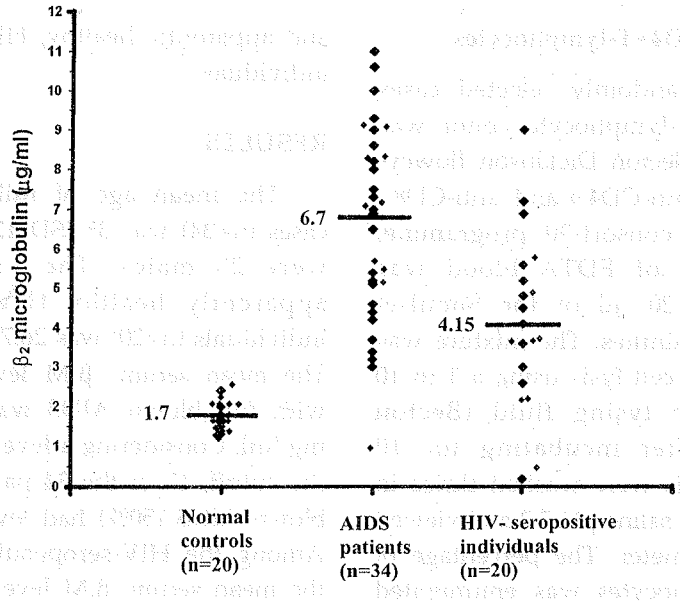

Figure 1. β_2 Microglobulin levels in control, HIV-postive individuals and AIDS patients.

controls ± 2SD i.e., 2.4 μg/ml., then 33 patients with full blown AIDS (97%) and 16 seropositive (80%) had values above 2.4 mg/ml. Thus this cut off was not able to distinguish AIDS patients from HIV seropositive individuals.

The serum β_2M values were significantly higher in patients with full blown AIDS compared to HIV-seropositive individuals ($p<0.04$) and normal controls ($p<0.001$). The serum β_2M values in HIV seropositive individuals was significantly higher than that observed in normal control ($p<0.001$). The sensitivity of β_2M for diagnosis of full blown AIDS was 76.5% considering a CD4+T-lymphocyte count of 200/mm^3 as the gold standard and serum β_2M level > 4.15 μg/ml as a cutoff value.

There was no significant correlation between CD4 + T-lymphocyte count and serum β_2M in patients with full blown AIDS ($r = 0.16$) and HIV seropositive individuals ($r = 0.26$).

DISCUSSION

The time interval between HIV infection and full blown AIDS is variable ranging from 2-10 years, depending upon the route of infection, dose of the virus, institution of the type of therapy, nutritional factors of the host and presence or absence of coreceptors (3,6,7,14). Assessment of the stage of the disease is crucial for initiation of appropriate therapy. Since it is not possible to study the extent of viral replication repeatedly in India, lot of stress has been laid on CD4 + T-lympocyte count and surrogate markers such as serum β_2M, p24 assays, neopterin levels etc (15). Assessing CD4 + T-lymphocyte count may be normal in the face of significant tissue viral replication

(7, 8). Therefore there is a need to have a set of adjunct markers which could be used to assess progression to AIDS as the cost of sequential viral load assay is prohibitive and will never be affordable.

In the present study there was a poor correlation between CD4 + T-lymphocyte count and β_2M serum in both HIV-seropositive individuals and patients with full blown AIDS. Thus serum β_2M levels cannot be used as a single marker to distinguish between full blown AIDS disease HIV-seropositivity.

In fact Fahey et al (16) also reiterated that although β_2M levels followed longitudinally had a strong predictive value, yet CD4+T-lymphocyte counts were the single most important parameters of activity (11). We conclude that although β_2M is a useful surrogate marker to differentiate a group of patients with full blown AIDS from HIV-seropositive individuals without AIDS, yet it cannot be used interchangeably with absolute CD4+T-lymphocyte count and results in a given case should be interpreted with caution.

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