BETA-2-MICROGLOBULIN LEVELS IN HUMAN-IMMUNODEFICIENCY VIRUS INFECTED SUBJECTS

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ABSTRACT

We studied beta-2 microglobulin (β -2M) levels in 44 HIV infected subjects belonging to 3 clinical stages as well as in 25 healthy controls. The method used was a competition enzyme immunoassay. In this study, levels of β -2M were measured in two groups of HIV infected individuals, the asymptomatic and those with progressive and advanced disease, in order to affirm its role as a surrogate prognostic marker. It was found that mean β -2M levels were 1.28 mg/L in the controls (normal range 0-2.4 mg/L), 11.41 mg/L in the HIV infected subjects, 2.69 mg/L in the asymptomatic HIV infected, 12.14 mg/L in those with persistent generalized lymphadenopathy (PGL) and 39.29 mg/L in the patients with acquired immunodeficiency syndrome. It was concluded that β -2M levels were significantly higher in the HIV infected as compared to the controls. Further, the levels were much higher in the HIV infected with progressive disease/PGL and highest in those who had developed AIDS. β -2M is an important surrogate serological marker useful in prognostication of disease process in the HIV infected. Advantages of measuring β -2M levels over p24 antigen detection and CD4 counts were highlighted.

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KEYWORDS: HIV infected, Beta-2 Microglobulin, Progressive HIV disease, AIDS.

Introduction

Beta-2 microglobulin (β -2M) is a globular protein of molecular weight 11.8 kilo Daltons. It is present on the surface of nearly all nucleated cells in the body linked non-covalently to the class-I major histocompatibility (MHC) antigens. It forms the light chain of MHC class-I. While its precise function is unknown, its association with the MHC class-I antigens, its homology with the constant region of the immunoglobulin molecule, and its release by lymphocytes in response to mitogens suggests a possible role in the immune response [1].

It is normally found in circulation and about one half of the endogenous serum levels (normal mean levels of up to 1.2 mg/L with a range of 0-2.4 mg/L) of β -2M are contributed to by the turnover of lymphocytes. Serum concentrations depend upon renal function as it is normally ex-

creted through the kidneys, hence elevated levels occur in renal impairment. Serum β -2M levels are increased in HIV infection because HIV targets T lymphocytes and macrophages and enhances their turnover and destruction [2]. *In vitro* studies [3] have shown that secreted products of HIV-specific cytotoxic T lymphocytes may enhance MHC-class I expression and release of β -2M in the infected tissue.

In this study, levels of β -2M were measured in two groups of HIV infected individuals, the asymptomatic and in those with progressive and advanced disease, in order to affirm its role as a surrogate prognostic marker.

Materials and Methods

During the period April to December 1995, 44 HIV infected individuals admitted to Command Hospital (SC) were enrolled in the study for

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estimation of serum β -2M levels. Their HIV status was confirmed by the application of ELISA and rapid test, followed by confirmation by western blot. Their blood was collected aseptically, and the sera were stored at -20°C till tested. The controls for this study were 25 age-matched HIV negative healthy male subjects.

The β -2M levels were determined by enzyme immunoassay (EIA) using the *in vitro* diagnostic test kit (Immunotech, France). The EIA test used was a competition assay. Samples and standards were incubated in the wells of a microtitre plate, coated with monoclonal antibody, in the presence of a tracer. The tracer consisted of β -2M conjugated to alkaline phosphatase. Following incubation, the liquid contents of the wells were aspirated and the wells were rinsed to remove excess tracer. Bound enzymatic activity was then measured by addition of the chromogenic substrate. The intensity of the colour developed and the measured optical density (OD) was inversely proportional to the concentration of β -2M in the samples. The concentration of β -2M in the test samples was obtained from a standard curve established using standards supplied with the kit employing a semilogarithmic paper and 2-scale plotting of results. The 6 standards provided in the kit were 0, 0.23, 0.45, 1.3, 4.5 and 13.5 mg/L. Concentration of β -2M in samples whose OD value could not be extrapolated to the standard curve were determined by log transformation of the OD and subjecting transformed log values to a linear regression analysis, using log concentration as the dependent variable and log OD as the independent predictor.

Further statistical analyses included calculation of 'p' value by chi square test, estimation of odds ratio and relative risk. 'P' values less than 0.05

were taken as significant.

Results

All HIV infected subjects chosen for this study were males. Their age range was 29-44 years, with a mean age of 32.7 years. The duration after detection of their HIV seropositive status was from 3-18 months. All were biochemically confirmed to have normal renal function. By the CDC staging system [4], 29 amongst the HIV infected, were in stage III and had PGL. Three patients had acquired immunodeficiency syndrome (AIDS) (Stage IV C). Those in stage III and IV C were considered as having progressive and advanced disease respectively. There were 12 subjects in clinical stage II (asymptomatic infection).

The controls were 25 age-matched healthy males, who had been subjected to HIV testing after staying abroad for a duration of more than 30 days and were negative for anti-HIV antibodies.

The range and the mean serum levels of β -2M found in 25 HIV seronegative healthy controls, 12 asymptomatic HIV-seropositives, 29 patients with PGL and 3 AIDS patients are shown in the Table. 'P' values and odds ratio derived for each group on comparison with the controls are also shown in the Table. Evident from Table is the fact that the mean serum β -2M level in all HIV infected, regardless of the stage, is more than the mean value in the control group. Also, the mean serum β -2M level in the groups with progressive/advanced disease (PGL, AIDS) is higher than that seen in asymptomatic HIV infected.

Elevated β -2M levels (>2.4 mg/L) were found in 36 of the 44 HIV infected subjects in this study as compared to only 3 out of 25 controls (chi square=31.62, $p < 0.001$). Thus, the likelihood (odds ratio=33.00) of having higher β -2M levels is

TABLE
Serum beta 2-M level in various groups

Group (n)	Range (mg/L)	Mean (mg/L)	p value	Odds ratio
Controls (25)	0.94 - 3.10	1.62	—	—
All HIV infected (44)	1.15 - 79.83	11.41	< 0.001	33.00
Asymptomatic HIV-infected (12)	1.15 - 3.85	2.68	0.011	7.33
PGL groups (29)	2.15 - 49.30	12.13	< 0.001	63.56
AIDS (3)	15.99 - 79.83	39.29	< 0.001	Undefined

33 times more in the HIV infected as compared to healthy controls.

Similarly, there is statistically significant difference in the number of subjects with high β -2M between the PGL/AIDS group and the controls as also between the asymptomatic HIV infected and the controls. Significantly higher number of patients with progressive/advanced HIV disease (PGL, AIDS) showed elevated β -2M levels as compared to asymptomatic HIV infected (chi square=8.85, $p=0.002$). The odds of having elevated β -2M levels in the presence of progressive/advanced disease is nearly 10 times more as compared to the asymptomatic HIV infected (odd ratio=9.67). In this case-control study the relative risk of developing advanced disease in the presence of higher β -2M levels could not be assessed.

Discussion

In the present study the value of a one-time measurement of serum β -2M levels was ascertained in HIV infected individuals in three clinical states [4] of HIV disease. The aim of the study was also to further establish the correlation between the estimated values of serum β -2M with the range of the disease.

Several markers have been used in recent studies to prognosticate disease progression in the HIV infected [5,6]. These include both cellular markers like CD4/CD8 counts and their ratio and serological markers like serum β -2M, serum neopterin, soluble interleukin-2 receptors, IgA, anti-cardiolipin antibodies, HIV p24 antigen and even HIV-RNA. We studied serum β -2M levels in our control population and found a mean normal value of 1.62 mg/L. The mean normal value among our controls was lower than that found in African population (2.35 mg/L) [7].

Serum β -2M level has been found to be an independent predictor of outcome of HIV infection in various studies [8,9]. Our results show that all HIV infected patients, regardless of the stage of the disease, showed raised β -2M, with a mean value of 11.41 mg/L. Patients of PGL and AIDS showed significantly higher mean values (12.13 mg/L and 39.29 mg/L respectively). This adds an important prognostic information in determining the risk or progression to advanced disease in HIV infection.

β -2M measurement has several advantages over other prognostic markers. In contrast to CD4 cell counts which require special procedures for specimen handling and expensive equipment for processing, β -2M can be measured by a serological test like EIA. The equipment for this is available in most laboratories even in developing countries. Several studies show [9] that β -2M levels rise earlier than the fall in CD4 count. β -2M level monitoring has more to offer in prognostication even in the absence of CD4 cell depletion. Hoffman et al [10] found that higher β -2M concentrations predicted subsequent decline in CD4 counts; their study substantiates that changes in β -2M occur sooner after HIV seroconversion than changes in CD4 count. Further, serum β -2M level is a superior marker than p24 antigen [11] because p24 rises transiently during primary HIV infection and again after years of infection. Isolated studies [9] have shown p24 antigen positivity in only a minority of HIV infected patients. The finding that β -2M concentration is a prognostic marker implies that immune system activation is heightened in HIV infected patients progressing to AIDS. Immune system activation may simply be a consequence of greater HIV replication or may theoretically reflect presence of other co-factors that promote HIV disease progression [9]. Three AIDS patients included in our study had disseminated tuberculosis and highest levels (up to 79.83 mg/L) were recorded in their sera. Other studies [12] have concluded that HIV infected patients with post-primary tuberculosis are likely to be in more advanced stage of HIV caused disease and in them, HIV infection would have a strong independent effect on β -2M levels. Indian studies on β -2M in the HIV infected are few. Thakar et al [13] found β -2M levels significantly higher than healthy controls in 234 HIV-asymptomatic seropositives. In a 2-year follow-up of 54 HIV seropositives they found rising levels correlating well with disease progression.

Other situations in the HIV infected where β -2M measurement could help are: (a) in predicting vertical transmission from HIV infected mothers [14], (b) in predicting HIV- dementia through CSF levels [15], (c) in predicting neurological involvement through serum/CSF ratio of β -2M levels [16], (d) in assessing therapeutic response and

patient survival in those who receive anti-retroviral therapy since β -2M levels fall with therapy [17,18] and (e) in helping counselling of the HIV-infected.

We have underscored the serious prognostic implication of high β -2M levels. The opposite implication of low β -2M levels should not be overlooked while counselling the HIV infected. Subjects with low levels have a good prognosis for many years of AIDS-free life, albeit they cannot be assured of no risk of eventually developing the disease.

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