Effective Control of HIV-1 Disease Progression: Impact on Serum Concentration of β_2-Microglobulin

Adedeji AL[1, 5], Adeleke AB[2, 5], Adekunle AS[1], Adebolu TT[3], Fakande I[4] and Olawoye TL[5]

[1.] Department of Biochemistry, Ladoke Akintola University of Technology, P. M. B. 4000, Ogbomoso, Nigeria
[2.] Department of Chemical Pathology, Federal Medical Centre, P. M. B. 201, Ido-Ekiti, Nigeria
[3.] Department of Microbiology, The Federal University of Technology, P. M. B. 704, Akure, Nigeria
[4.] Living Hope Care, P. O. Box 173, Ilesa, Nigeria
[5.] Department of Biochemistry, The Federal University of Technology, P. M. B. 704, Akure, Nigeria
aladedeji@lautech.edu.ng

Abstract: Progressive defects in cell mediated immune function, are usual features of HIV-1 disease progression. Lack of HIV disease progression can be independently achieved by the host's effective immune responses and highly active antiretroviral therapy. Serum β_2-microglobulin (β_2M) concentration is a known correlate of HIV disease progression. We thus determined whether different patterns of serum concentrations of β_2M characterize immunological and pharmacological controls of HIV-1 disease progression in asymptomatic subjects and compared the potentials of the two conditions in restoring the abnormal concentration of serum β_2M consequent to HIV-1 infection. Although different patterns of β_2M characterized the two conditions, both tend to restore the abnormal serum concentration of β_2M consequent to HIV-1 infection. Increased renal excretion of β_2M was associated with HAART and may be a possible mechanism by which antiretroviral therapy reduces serum concentration of β_2M. No significant association was found between the blood CD4 T-cell count and serum β_2M concentration in subjects with CD4 T-cell >500/µl in asymptomatic HIV-1 infection. We also discovered additive effect of HAART/infection duration and clinical status on serum β_2M concentration. Serum concentration of β_2M may not be a useful marker of immune status in HIV-infected subjects with blood CD4 T-cell >500/µl. We conclude that pharmacological control of HIV-1 disease progression had a higher potential than the host immunological response in restoring the impairments in serum concentration of β_2M consequent to HIV infection.
[Adedeji AL, Adeleke AB, Adekunle AS, Adebolu TT, Fakande I, Olawoye TL. **Effective Control of HIV-1 Disease Progression: Impact on Serum Concentration of β_2-Microglobulin.** *N Y Sci J.* 2012;5(5):18-27]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork

Keywords: HIV-1, Disease Control, CD4 T Cells, β2-Microglobulin

1. Introduction

β_2-microglobulin (β_2M) is a ubiquitously expressed 12-kDa glycoprotein chaperone of the class I major histocompatibility complex (MHC I) molecule. Although, β_2M is a non-MHC gene product, it associates and interacts non-covalently with the α_3 domain thereby performing a vital role in stabilization and presentation of proteasome-generated peptide antigens to cytotoxic T lymphocytes (Salter *et al*., 1990; Abbas and Lichman, 2005). Free β_2M molecules are also detectable in the plasma as products of immune cells turnover. Increased serum concentration of β_2M has also been associated with abnormal renal functions as kidneys are the main site of clearance (Ando *et al*., 2011). As a non-specific marker of immune activation, its serum concentration is a correlate of human immunodeficiency virus type-1 (HIV-1) disease progression to acquired immune deficiency syndrome (AIDS) (Gupta *et al*., 2004; Chitra *et al*., 2011).

A progressive increase in HIV-1 viral load and defects in cell-mediated immune functions are usual features of HIV-1 disease progression. Host effective cell-medicated immune machinery is known to stem the tide of HIV disease (Dyer *et al*., 2008). Furthermore, controls over progression in a subset of HIV-1-infected individuals, who have never been on antiretroviral treatment have been reported elsewhere (Rosenberg *et al*., 1997; Wiliams and Burdo, 2009). This subset can be either long-tern non-progressors or elite controllers and they have been reported to exhibit low viramia and elevated clusters of CD4 T-cell count (Poropatich and Sullivan, 2011). Antiretroviral chemotherapies also decrease AIDS related mortality even though immune restoration following the treatment may not be complete. However, It is usually enough to provide protection from most opportunistic infections (Lederman and Valdez, 2000; Arminio *et al*., 2005; Hanna *et al*., 2009). These reports show that lack of HIV disease progression could be independently achieved under the host's potent immune responses and chemotherapy. In this study, we determined whether different patterns of serum concentration of β_2M characterize immunological and pharmacological controls of disease progression in asymptomatic subjects and compared the potentials of these two conditions in restoring the abnormal

concentration of serum β_2M consequent to HIV-1 infection.

2. Material and Methods

2.1 Selection of Subjects

Sixty-two (62) HIV-1-infected subjects attending Living Hope Care (LIHOC), Ilesa, Nigeria and ten healthy HIV-1 sero-negative volunteers were recruited within the vicinity of the centre for this study as controls. LIHOC is a Non-Governmental Organization, providing care for people living with HIV/AIDS. Information on demographic characteristics and antiretroviral therapy records was collected. Thirty-three of subjects had been receiving HAART [Lamivudine (300 mg/day), Stavudine (60 mg/day) and Nevirapine (400 mg/day)] while the remaining twenty-nine subjects had not received any antiretroviral therapy as at the time of enrolment. Volunteers with conditions that could affect evaluated parameters, such as pregnancy tuberculosis infection were excluded from the study. All subjects had no evidence of renal impairment. Informed consent was obtained from all volunteers before initiation of the study. The LIHOC Ethical Committee of approved the study.

2.2 Specimens Collection and Preservation

Blood sampling was done by venepuncture while urine specimens were collected using appropriate standard methods. Specimens were transported under ice-cold condition to the laboratory within six hours. Urine samples was immediately stored at -20 ^{0}C. Serum was separated from whole blood by centrifugation at 1000 x g for ten minutes and stored in aliquots at -20 ^{0}C until analyzed. Peripheral blood mononuclear cells (PBMCs) were isolated from ACD-anticoagulated whole blood using Ficoll-Hypaque density gradient centrifugation. Briefly, 5.0 ml whole blood layered on 3.0 ml lymphocyte separation medium, (Density 1.077 g/ml, Cambrex Bio Science, USA) and were centrifuged at 500 x g for 15 minutes. PBMCs layer was separated and washed twice in phosphate buffer saline, pH 7.2. The cells were counted, pelleted and re-suspended in appropriate volume of 0.1% triton X-100 [polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether] in phosphate buffer saline to make 10^6 cells/ml. The mixture was incubated for 30 minutes at room temperature and later stored at -20 ^{0}C until when analyzed. Analyses on both test subject and control samples were carried out concurrently.

2.3 Diagnosis of HIV Infection

The diagnosis of HIV-1 infection was performed by enzyme link immunosorbence assay (ELISA) and confirmation was done by HIV Western Blot using Immunetics Qualicode HIV-1/2 Kit (USA). Subjects with indeterminate results were excluded from the study. Control subjects were also confirmed to be negative.

2.4 Enumeration of Blood CD4 T-cell

EDTA-anticougulated blood CD4 T-cell was enumerated using Cyflow Cytometer according to the manufacturer's instructions (Partec, Germany).

2.5 β_2-Microglobulin Determination

The concentration of β_2M in the sera of HIV-1-infected on HAART and naïve to HAART as well as uninfected control subjects were determined by a conventional capture ELISA method (Diametra srl, Italy). β_2M standards provided were by the manufacturer and determination was carried out according to manufacturer's instructions.

2.6 Statistical Analysis

Descriptive analysis, and student t-test was used for the comparisons of data. Spearman correlation was use to test association between variables using GraphPad 5 software (San Diego, CA). p-values <0.05 were considered significant.

3. Results

3.1 Study Subjects

HIV-1-infected subjects were recruited into this study to investigate and compare the impact of immunological and pharmacological control of disease progression on serum concentration of β_2M in HIV-1 infected Nigerians. All HIV-1-infected subjects were asymptomatic and had CD4 T-cell counts >200 cells/µl. There was no history of AIDS diagnosis including a CD4 T-cell count <200 cells/µl, or self-reported occurrence of any AIDS-defining illness, or AIDS diagnosis in HAART naïve subjects. HIV-infected subjects under HAART had a median (quartiles) CD4 T-cell count of 419 (271, 600)/µl which was not significantly different from 441 (319, 511)/µl in HIV-infected subjects naive to HAART (Table 1).

3.2 Comparison of the Serum Concentrations of β_2-Microglobulin in HIV-1-infected Subjects Naïve to HAART (HAART-) and those under HAART (HAART+)

The serum concentrations of β_2M in group in which control of HIV disease progression was achieved in the absence of treatment (HAART-) and the group in which the control was achieved by HAART (HAART+) were compared with uninfected control. The plot representing this relationship is shown in Figure 1. The serum median (quartiles) β_2M

Table 1. Characteristics of Study Subjects

CHARACTERISTICS	HIV-	HAART-	HAART+	p-Value
N	10	31	31	
Age (year)	37 (35, 45)	35 (34, 49)	35 (32, 47)	0.262
Mid Arm Circumference (cm)	27 (24, 29)	27 (25, 30)	26 (24, 31)	0.405
Body Mass Index (Kgm^{-2})	24 (22, 25)	24 (21, 26)	25 (22, 27)	0.505
Sex (M/F)	3/7	7/24	8/23	0.605
Infection Duration (months)	NA	12 (4, 44)	16 (10, 55)	0.236
HAART Duration (months)	NA	0	14 (5, 48)	NA
CD4 count (/µl)	734(634, 825)	441 (319, 511)	410 (300, 600)	0.527

Values are median (25^{th} and 75^{th} percentile). p-values were determined by Student's 't' test and Fisher's exact test, as appropriate to compare HAART- and HAART+, $p < 0.05$ was considered significantly different. NA = Not applicable.

in HAART- and HAART+ groups were respectively 3.60 (2.50, 5.90) µg/ml and 2.90 (2.10, 4.50) µg/ml. This was respectively significantly higher than the concentration of 1.5 (0.80, 3.60) µg/ml in uninfected controls. (p=0.0004 and p=0.005; respectively (Figure 1A). However, the difference between HAART- and HAART+ was not significant (p=0.057). We observed inverse correlation between β_2M and blood CD4 T cell count (Figure 1B, upper panel) and Infection/HAART duration (Figure 1B lower panel).

3.3 Comparison of the Serum Concentrations of β_2-Microglobulin in HAART- and HAART+ at Different HIV Clinical Status

To investigate whether clinical status will affect the pattern of serum concentration of β_2M, we classified the HIV-infected subjects according to HIV clinical status (CD4 T-cell count <500 or >500/µl). In subjects in whom control of HIV disease progression was achieved in the absence of HAART (HAART-), subjects with CD4 T-cell count <500/µl, exhibited a higher serum concentration of β_2M compared to subjects with a CD4 T- cells count >500/µl, although not statistically significant (p=0.259). Similarly, under HAART, subjects with >500 CD4 T-cell/µl, exhibited a lower serum concentration of β_2M compared to subjects with a CD4 T-cells <500/µl, although not statistically significant (p=0.089) [Figure 2A]. We observed a significant inverse correlation between blood CD4 T-cell count and serum concentrations of β_2M in subjects with CD4 T-cell count <500/µl in both HAART- and HAART+ (R=-0.478, p=0.033; R=-0.473, p=0.041; respectively) [Figure 2B, upper panel] while no correlation was found in subjects with CD4 T-cell count >500/µl (Figure 2B lower panel).

3.4 Comparison of Serum β_2-Microglobulin Concentrations in Acute and Chronic Infection/HAART Durations

To investigate the effect of infection and treatment durations on serum β_2M concentration, we classified HIV-infected subjects according to infection/HAART durations. Infection/HAART durations <12 or >36 months were grouped as acute and chronic; respectively. The plot representing this relationship is shown in Figure 3. In asymptomatic subjects naïve to treatment (HAART-), subjects under acute infection duration had statistically similar serum β_2M concentration as those with chronic asymptomatic infection (p=0.185). Similar trend in group undergoing HAART (HAART+) was evident (p=0.099) [Figure 3A]. We observed no significant association between serum β_2M concentration and HAART/infection durations (Figure 3B).

3.5 Dynamics of β_2-Microglobulin under Effective Control of HIV-1 Disease Progression

To propose a possible mechanism underlying the behaviour of β_2-microglobulin under effective control of disease progression, cellular, serum and urinary concentrations of β_2M were compared in urine. The results showed a significant higher cellular expression of β_2M in HIV-1 infected group when compared with HIV uninfected controls ($p < 0.05$) and essentially the same in subject under HAART and those naïve to HAART ($p > 0.05$). However, urinary excretion of β2-microglobulin was found to be significantly higher under HAART ($p < 0.05$). This translates to significantly lower serum concentration of β_2M under effective HAART compared with subjects naïve to HAART (Table 2).

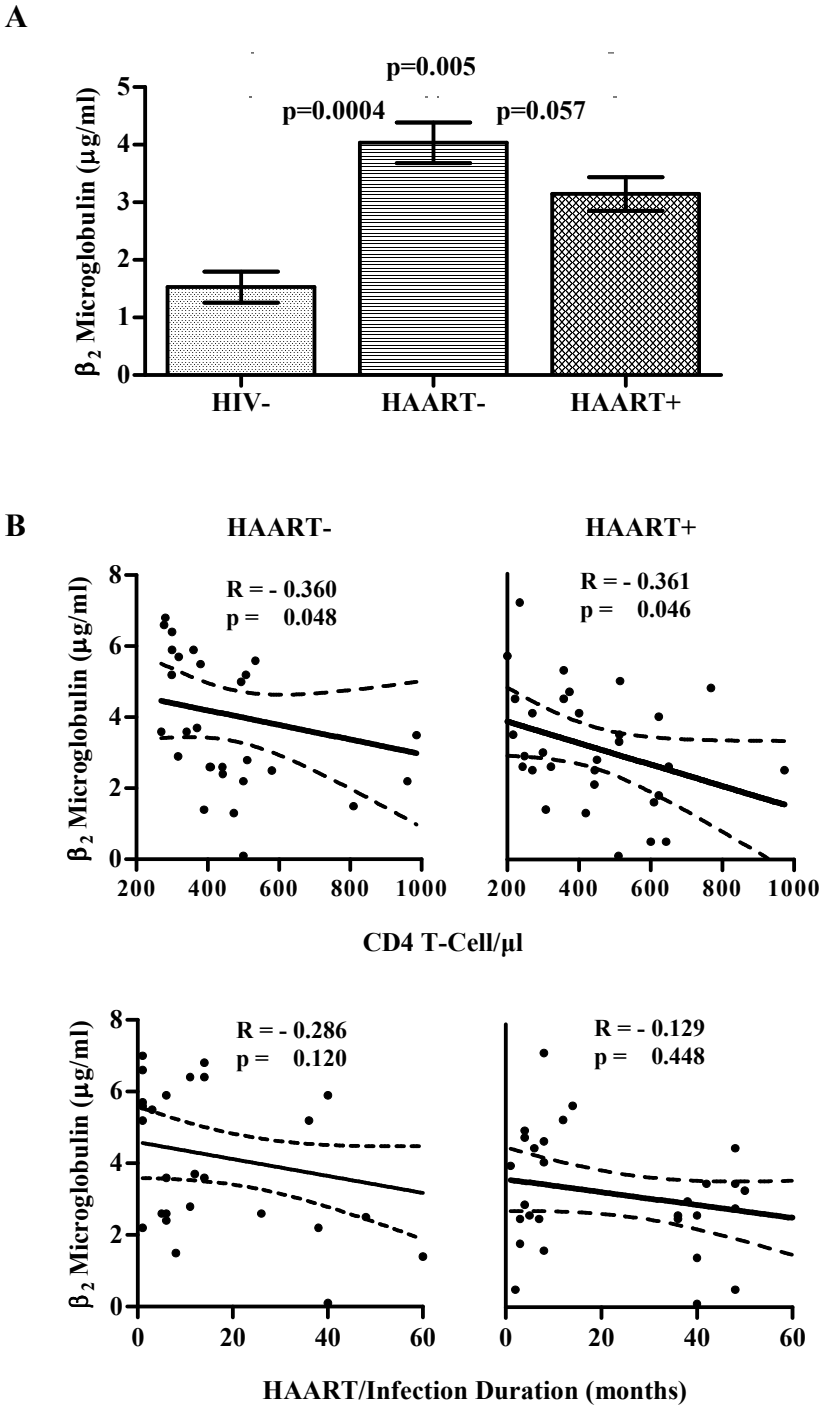

Figure 1. Serum β_2-Microglobulin concentration in HIV-1-infected subjects naïve to HAART (HAART-) and in Subjects under HAART (HAART+) (A): Comparison of serum concentration of β_2M in HAART- (n=31) and HAART+ (n=31) with uninfected control subject (n=10). Each bar-and-error bar plot represents the mean and standard error of serum concentration of β_2M within each group. An unpaired Student 't' test was used to compare the serum concentration of β_2M in HAART- and HAART+, and also with the value in uninfected control. (B): Association between serum concentration of β_2M and blood CD4 T cell (upper panel) and Infection/HAART duration (lower panel). Regression line and Spearman R-value are shown for correlations. The doted lines are 95% confidence band and p-value <0.05 were considered significant.

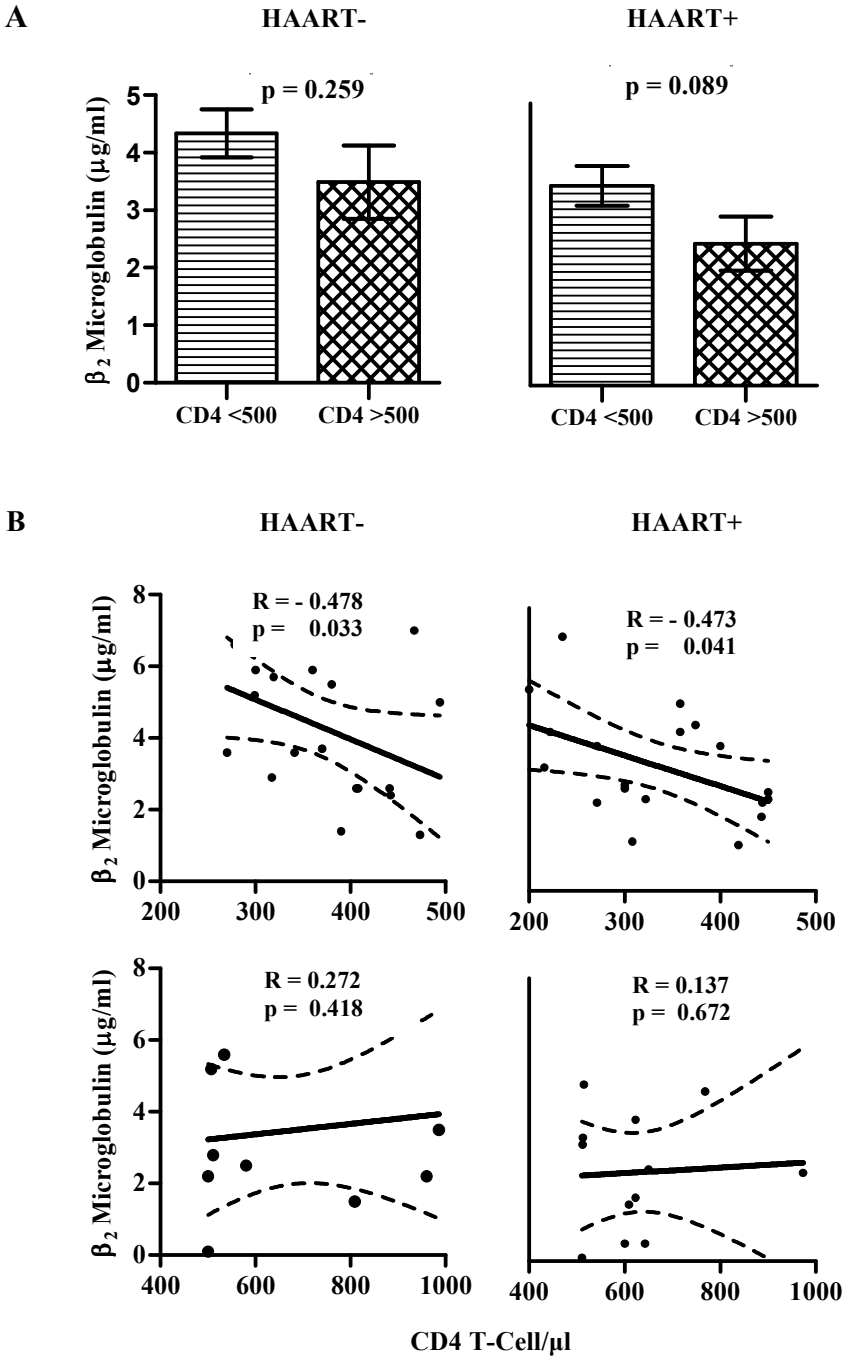

Figure 2. Effects of clinical status on serum β_2M under effective control of HIV-1 disease progression. (A): Comparison of serum concentration of β_2M in subjects naive to HAART having blood CD4 T cells count <500 (n=20) or >500/µl (n=11)(left) [HAART-]. Also in subjects under HAART, having blood CD4 T cells count <500(n=19) or >500/µl (n=12)(right) [HAART+]. Each bar-and-error bar plot represents the mean and standard error of serum concentration of β_2M within each group. An unpaired Student 't' test was used to compare the serum concentration of β_2M in subjects with CD4<500 and those with CD4 >500/µl. (B): Association between serum concentration of β_2M and blood CD4 T-cell in subjects with CD4 T-cell <500/µl (upper panel) or >500/µl (lower panel). Regression line and Spearman R-value is shown for correlations and p-value <0.05 were considered significant.

Table 2. Comparison of Cellular, Serum and Urinary Concentration of β_2-Microglobulin under Effective Control of HIV-1 Disease Progression

β_2-M	HIV-	HAART-	HAART+
N	5	8	10
ng/10^6PBMCs	1.5±0.3[a]	3.3±1.1[b]	2.6±1.0[b]
µg/ml Serum	1.6±0.5[a]	4.5±1.5[b]	2.1±1.0[c]
µg/24-Hr Urine)	86±19[a]	131±66[b]	239±76[c]

The data are the mean±SD. p-values were determined by Student's t test and data with different superscript along the rows are significantly different ($p < 0.05$). PBMCs = Peripheral Blood Mononuclear Cells.

3.6 Additive Effect of Clinical Status and Infection/HAART Durations on Serum β_2-Microglobulin Concentration under Effective Control of HIV-1 Disease Progression

The additive effect of clinical status and infection/HAART durations on serum β_2-microglobulin concentration was investigated by comparing serum β_2-microglobulin concentration in subjects having a blood CD4 T-cell count <500/µl under acute infection/HAART and those with blood CD4 T-cell count >500/µl under chronic infection /HAART. Although, the difference was not statistically significant ($p=0.061$ and $p=0.056$; respectively), evidence of additive effect was apparent (Figure 4).

4. Discussions

Highly active antiretroviral therapy (HAART) has been shown to be efficient in the treatment of human immunodeficiency virus infection. Depending on the composite, it interferes at specific points in the replication cycle of the viral RNA. Previous studies have shown that control of HIV disease progression could be achieved independently by HAART and the host potent HIV specific immunological responses in individuals not undergoing HAART. Asymptomatic HIV-1 infected subjects have a well preserved immune system similar to that of uninfected control (Resino *et al*., 2003) and sustained immunological suppression of viraemia is associated with preserved p24 proliferate responses, regardless of the strength of the cytotoxic T lymphocyte responses (Dyer *et al*., 2008). These studies suggested that control of disease progression in subjects undergoing HAART and HAART naïve subjects is associated with vigorous immune functions.

β_2-microglobulin (β_2M), an invariant chain of the MHC class I molecule, is an 11.8 kDa, non glycosylated protein, composed of 100 amino acid residues. Free β_2M is found in body fluids under physiological conditions because of its shedding from surface or intracellular release. The reference range is 0.8-3.6 µg/ml. β_2M concentration is not only a surrogate for viral or tumour burden or by-product of immune activation, but at high serum concentration (>10 µg/ml) may have negative effect on immune function (Franzetti *et al*., 1988; Xie *et al*., 2003). We hypothesized that similar pattern of serum β_2M would characterize immunological and pharmacological controls of disease progression. Thus, the present study was designed to compare the potential of the two conditions in restoring the impaired levels in serum β_2M consequent to HIV-1 infection in cohorts of subjects with comparable clinical status.

Our results also showed that serum β_2M concentration in HIV-1 infection was significantly higher compared to uninfected subjects ($p=0004$). However, the level was not high enough on its own to warrant an adverse effect on immune function in our study. This is consistent with previous findings (Thakar *et al*., 1992, Xie *et al*., 2003, Pascale *et al*., 1997). As a non-specific marker of immune activation, β_2M has been employed as a marker for cancers, especially lymphoid malignancies, such as non-Hodgkin's lymphoma and multiple myeloma. Consistent with the observation that the major source of serum β_2M is lymphatic tissue in normal subjects, it may occur as a result of immune stimulation, such as in other acute viral infections (Bethea and Forman, 1990). During the continuous turnover of the human leucocyte antigen (HLA) molecules, β_2M is shed from the cell membrane into blood and lymphocytes are the main sources of serum free β_2M. Our data support the general observation that serum β_2M concentrations are elevated in patients with HIV infection.

It was found that the both immunological and pharmacological controls of HIV disease progression tend to restore the abnormalities is serum levels of β_2M consequent to HIV infection. On the basis of clinical status, HAART treatment resulted in decrease in serum β_2M concentration in subjects with CD4 T cell count >500/µl compared to subjects with CD4 T cell count <500/µl, although, not significant ($p=0.259$); whereas, the changes in HAART naïve subjects were not significant ($p=0.089$). This shows that pharmacological control of disease progression has a higher potential than the host immunological response in restoring the impairments in serum concentration of β_2M consequent to HIV infection. Viral replication interferes with normal cellular protein syntheses and functions and ultimately death of infected cells. The process may contribute to increased turnover of membrane β_2M.

One of the immunological responses to viral infection is increased expression of class I MHC molecule and other proteins involved in the antigen processing and presentation. Consequently, increase

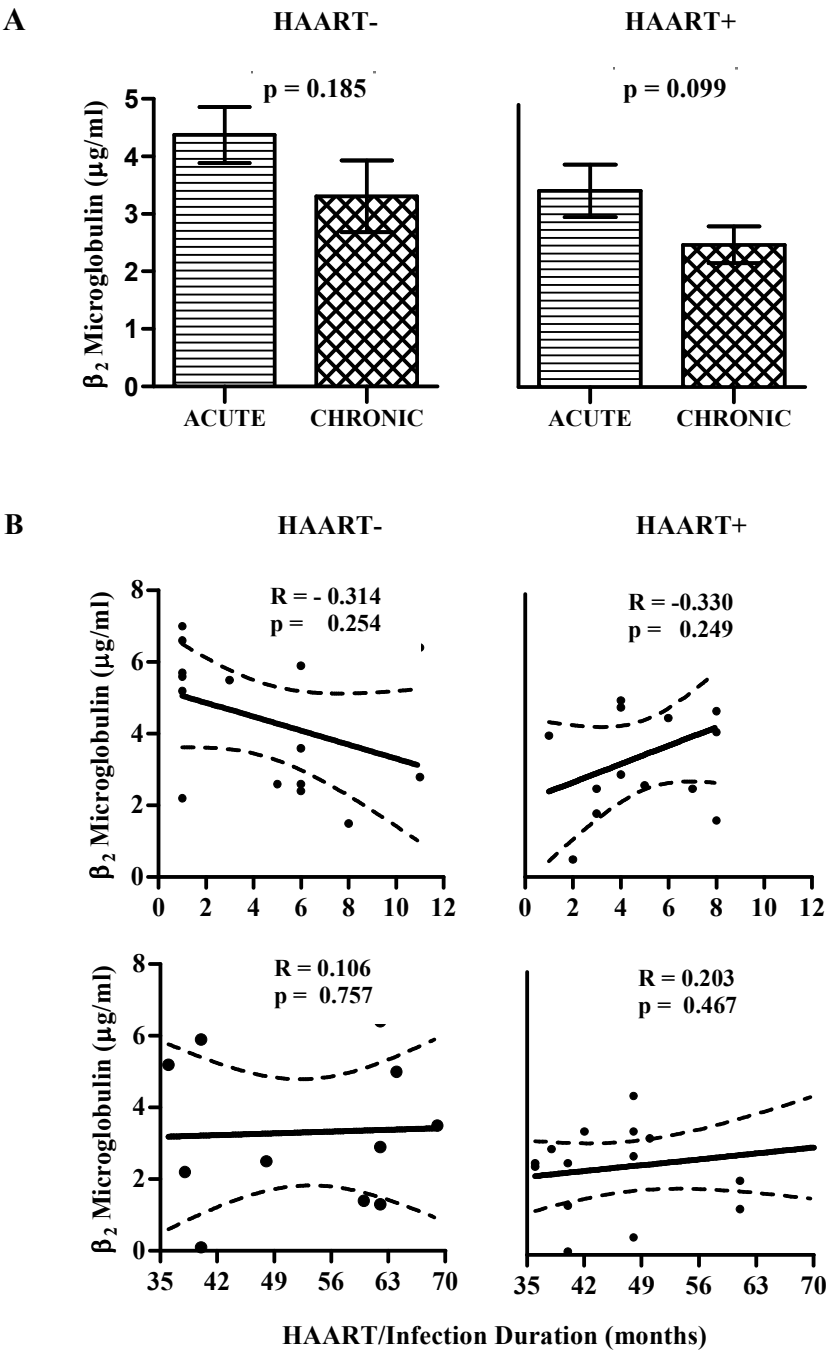

Figure 3. Effects of infection/HAART duration on serum β_2M concentration under effective control of HIV disease progression. (A) Comparison of serum β_2M concentration in subjects under acute (<12 months, n=15) or chronic (>36 months, n=11) infection [left panel]. Also in subjects under acute (<12 months, n=14) or chronic (>36 months, n=15) HAART [right panel]. Each bar-and-error bar plot represents the mean and standard error of serum concentration of β_2M within each group. An unpaired Student 't' test was used to compare the serum concentration of β_2M in acute and chronic HAART/infection. (B): Association between serum concentration of β_2M and acute HAART/infection duration (upper panel) or chronic HAART/infection duration (lower panel). Regression line and Spearman R-value are shown for correlations. The doted lines are 95% confidence band and p-value <0.05 were considered significant.

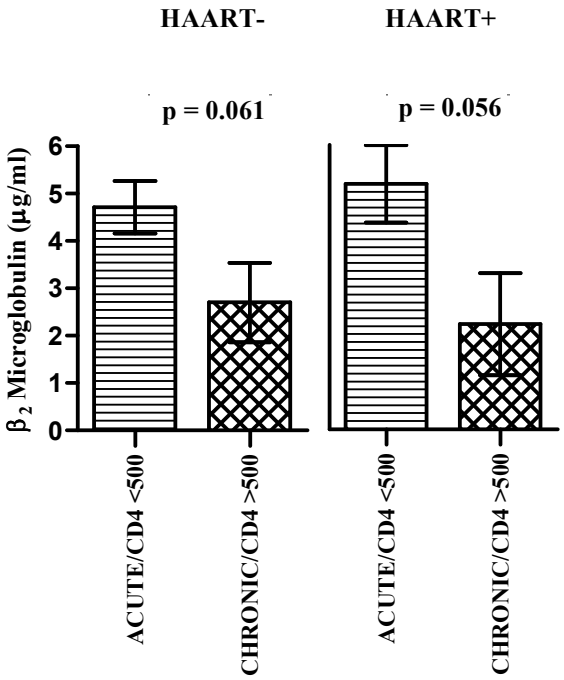

Figure 4. Comparison of serum β_2M concentration in subjects under acute infection duration having a CD4 T-cell <500/µl (n=10) and in subjects under chronic infection duration having a CD4 T-cell >500/µl (n=5) [left]. Also in subjects under acute HAART duration having a CD4 T-cell <500/µl (n=6) and in subjects under chronic HAART duration having a CD4 T-cell >500/µl (n=4) [right]. Each bar-and-error bar plot represents the mean and standard error of serum concentration of β_2M within each group. An unpaired Student 't' test was used for the comparison and p-value <0.05 were considered significant.

in serum concentration of β_2M may also represent an early immunological response to viral infection. HAART has synergistic inhibitory effects on viral replication. The two components of HAART in our study were Lamivudine and Stavudine (NRTIs) and the third Nevirapine (NNRTIs). They competitively and non-competitively inhibit respectively the viral enzyme-reverse transcriptase. Lu and Andrieu (2000) demonstrated that $CD3^+CD4^+$ and $CD3^+CD8^+$ T-lymphocyte compartments were both involved in immunologic recovery in naive as well as in treated subjects and were found to correlate with the decline in serum β_2M concentration. The decline in serum β_2M concentration could simply be the result of the effective viral replication control corresponding to a decrease in viral antigen stimulation under effective control of disease progression.

Interferons (IFNs) have the ability to enhance antigen-processing, expression of class I histocompatibility and substrate specificity of proteosome. Thus, IFNs may be involved in stimulating expression and release of β_2M. However, Decreases in IFN-gamma mRNA level in peripheral blood mononuclear cells are associated with effective highly active antiretroviral therapy in HIV-infected patients (Brazille *et al*., 2003). This could be a possible mechanism by which HAART affect the serum concentration of β_2M. However, decreases serum β_2M concentration may be observed both in the presence and in absence of any modification of plasma viral load (Andrieu *et al*., 1995; Lu and Andrieu, 2000).

We further showed that HAART increases renal excretion of β_2M. Increased urinary β_2M concentration could result from increased glomerular filtration or reduced tubular re-absorption. Tomlinson (1992) showed that increased urinary excretion of β_2M is associated with tubular disease. Recent study by Ando *et al*. (2011) also showed that 25% of HIV-infected subjects receiving HAART had subclinical tubular damage. It is interesting to note that none of the subjects in our present study exhibited evidence of impaired glomerular and tubular functions (assessed by absence of glucosuria and proteinuria; respectively). We therefore suggest that increased the renal excretion of β_2M could be a possible mechanism by which HAART decreases serum concentration in HIV-1 infection.

Our study is the first to compare the impact of pharmacological and host's immunological control of HIV-1-disease progression on serum concentration of β_2M and to report the absence of correlation between blood CD4 T cell count and serum concentration of β_2M in asymptomatic HIV-1- infected subjects with CD4 T-cell count >500/µl**.** This questions the usefulness of serum concentration of β_2M as a biomarker of HIV disease activities in a group of asymptomatic subjects having a CD4 T-cell count >500/µl.

Acknowledgements:

We are grateful to the LIHOC support group members, Ilesa for participating in this study. The Team Leader (Professor TL Olawoye) acknowledges the financial support from The Federal University of Technology Senate Research Grants, Akure. Reference: URC/MAJOR/168.

Corresponding Author:
Dr. AL Adedeji
Department of Biochemistry
Ladoke Akintola University of Technology
PMB 4000, Ogbomoso 210001, Nigeria.
E-mail: aladedeji@lautech.edu.ng

References

1. Salter RD, Benjamin RJ, Wesley PK, Buxton SE, Garret TP, Clayberger C, Krensky AM, Norment AM, Littman DR, Parham P. A binding site for the T-cell co-receptor CD8 on the α_3 domain of HLA-A2. Nature 1990; 345:41-6.
2. Abbas AK, Lichman AH. The major histocompatibility complex. In: Cellular and Molecular Immunology. Pennsylvania: Elsevier & Saunders 2005:65-80.
3. Ando M, Yanagisawa N, Ajisawa A, Tsuchiya K, Nitta K.. Kidney tubular damage in the absence of glomerular defects in HIV-infected patients on highly active antiretroviral therapy. Nephrology and Diagnostic Transplant 2011; 26(10):3224-9.
4. Gupta SM, Ray K, Bala M. Evaluation of beta-2-microglobulin level as a marker to determine HIV/AIDS progression. Journal of Communicable Diseases 2004; 36:166-70.
5. Chitra P, Bakthavatsalam B, Palvannan T. Beta-2 microglobulin as an immunological marker to assess the progression of human immunodeficiency virus infected patients on highly active antiretroviral therapy. Clinica Chimica Acta 2011; 412(11-12):1151-4.
6. Andrieu JM, Lu W, Levy R. Sustained increases in CD4 cell counts in asymptomatic human immunodeficiency virus type 1-seropositive patients treated with prednisolone for 1 year. Journal of Infectious Disease 1995; 171:523-30.
7. Dyer WB, Zaunders JJ, Yuan FF, Wang B, Learmont JC Geczy AF, Saksena NK, McPhee DA, Gorry PR, Sullivan JS. Mechanisms of HIV non-progression; robust and sustained CD4+ T-cell proliferative responses to p24 antigen correlate with control of viraemia and lack of disease progression after long-term transfusion-acquired HIV-1 infection. Retro-virology 2008; 5:112-5.
8. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, Walker BD. Vigorous HIV-1–specific CD4 T- cell responses associated with control of viremia. Science 1997; 278(21):1447-50.
9. Williams KC, Burdo TH. HIV and SIV infection: the role of cellular restriction and immune responses in viral replication and pathogenesis Acta Pathologica Microbiologica et Immunologica Scandinavica 2009; 117(5-6):400-2.
10. Poropatich K, Sullivan DJ. Human immunodeficiency virus type-1 long-term non-progressors: the viral, genetic and immunelogical basis for disease non-progression. Journal of General Virology 2011; 92:247-68.
11. Lederman MM, Valdez H. Immune restoration with antiretroviral therapies: Implication for clinical management. Journal of the American Medical Association 2000; 284:223-8
12. Arminio MA, Sabin CA, Phillips A, Sterne J, May M, Justice A, Dabis F, Grabar S, Ledergerber B, Gill J, Reiss P, Egger M. The changing incidence of AIDS events in patients receiving highly active antiretroviral therapy. Archives of Internal Medicine 2005; 165:416-23.
13. Hanna LE, Nayak K, Subramanyam S, Venkatesan P, Narayanan PR, Swaminathan S. Incomplete immunological recovery following anti-tuberculosis treatment in HIV-infected individuals with active tuberculosis. Indian Journal of Medical Research 2009; 129(5):548-54.
14. Resino S, Correa R, Bellon JM, Munoz-Fernandez MA. Preserved immune system in long-term asymptomatic vertically HIV-1 infected children. Clinical and Experimental Immunology 2003; 132:105-12.
15. Franzetti F, Cavalli G, Foppa CU, Amprimo MC, Gaido P, Lazzarin A. Raised serum beta 2 microglobulin levels in different stages of human immunodeficiency virus infection. Journal of Clinical Laboratory Immunology 1988; 27(3):133-7.
16. Xie J, Wang Y, Freeman ME, Barlogie B, Yi Q. Beta-2 microglobulin as a negative regulator of the immune system: high concentrations of the protein inhibit in vitro generation of functional dendritic cells. Blood 2003; 101(10):4005-12.
17. Thakar M, Rodrigues J, Athalye N, Banerjee K. Serum beta-2 microglobulin levels in HIV seropositive persons. Indian Journal of Medical Research 1992; 95:168-70.
18. Pascale JM, Isaacs MD, Contreras P, Gomez B, Lozano L, Austin E, De Martin MC, Gregory RL, McLaughlin GL, Amador A. Immunological markers of disease progression in patients infected with the human immunodeficiency virus. Clinical Diagnostic Laboratory Immunology 1997; 4(4):474-7.
19. Bethea M and Forman DT. β_2-microglobulin: Its significance and clinical usefulness. Annals of Clinical Laboratory Science1990; 20:163-8.

20. Lu W, Andrieu JM. Cell proliferative response in vivo and in vitro: A viral-suppression-independent mechanism. Blood 2000; 96:250-8.
21. Brazille P, Dereuddre-bosquet N, Leport C, Clayette P, Boyer O, Vilde JL, Dormont D, Benveniste O. Decreases in plasma TNF-alpha level and IFN-gamma mRNA level in peripheral blood mononuclear cells (PBMCs) and an increase in IL-2 mRNA level in PBMC are associated with effective highly active antiretroviral therapy in HIV-infected patients. Clinical and Experimental Immunology 2003; 131(2):304-11.
22. Lu W, Andrieu JM. Cell proliferative response in vivo and in vitro: A viral-suppression-independent mechanism. Blood 2000; 96:250-8.
23. Hofmann B, Wang YX, Cumberland WG, Detels R, Bozorgmehri M, Fahey JL. Serum beta 2-microglobulin level increases in HIV infection: relation to seroconversion, CD4 T-cell fall and prognosis. AIDS 1990; 4(3):207-14.
24. Tomlinson PA. Low molecular weight proteins in children with renal disease. Pediatric Nephrology 1992; 6(6):565-71.

1/27/2012