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Hypocalcaemia, Alcohol Drinking and Viroimmune Responses in ART Recipients

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Abstract

Metabolic perturbations associated with HIV and antiretroviral therapies are widespread. Unfortunately, research has predominantly focused in cardiometabolic problems, neglecting other important areas. In fact, the immune-calcium-skeletal interface has been understudied despite its potential relevance in people living with HIV (PLWH). Using a case-control methodology, 200 PLWH receiving medical care were enrolled and stratified according to hazardous vs. non-hazardous alcohol intake (HAU vs. non-HAU) and calcium (Ca) levels by analyzing baseline data. The group was chosen to represent relatively "pure" HAU with minimal drug use and no psychiatric diagnoses. With these narrow parameters in place, we found evidence that HAU significantly increases TNF- α levels compared to Non-HAU (2.8 ± 0.6 vs. 1.9 ± 0.3 pg/ml, $p=0.05$) and decreases blood Ca levels (9 ± 0.6 vs. 9.4 ± 0.5 , $p=0.03$). Our analyses also suggest that chronic inflammation, as indicated by increased TNF- α levels, is associated with hypocalcemia (hypoCa

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<8.6). Despite the limited prevalence of hypoCa, these findings are clinically significant given that hypoCa PLWH exhibited decreased CD4 (253 ± 224 vs. 417.7 ± 281 , $p=0.02$), B cells (147 ± 58 vs. 248 ± 151 , $p=0.03$) and NK cells (146.8 ± 90 vs. 229 ± 148 , $p=0.008$) and elevated CD8 (902.5 ± 438 vs. 699 ± 510 , $p=0.09$) compared to those with normal calcium. Furthermore, calcium effects on viral load were also evident with hypoCa exhibiting the highest loads ($140,187 \pm 111$ vs. 35622 ± 7770 HIV copies, $p=0.01$). Multivariate analyses confirmed the significance of hypoCa in predicting viro-immune parameters. This paper provides the first evidence that hypoCa accounts for some of the variation in viroimmune measures in HAART recipients and suggests that hypoCa may be mediating alcohol's deleterious effects.

Keywords

Alcohol; Antiretroviral therapy; Calcium; HIV; Viral Load

INTRODUCTION

Since a predominant area of focus in the newly expanded antiretroviral therapy (ART) era has been cardiovascular problems such as insulin resistance, hyperlipidemia, and fat redistribution, other emerging metabolic alterations have been, for the most part, neglected. For example, data indicate that ART carries the potential to alter calcium (Ca) metabolism (Gutierrez and Masia, 2011; McComsey et al., 2010; Ofotokun et al., 2011; Ofotokun and Weitzmann, 2010).

Similar to ART, animal studies have revealed a significant inverse relationship between calcaemia and ethanol consumption (Broulik et al., 2010; Keiver et al., 2005). Research shows that chronic heavy alcohol use can impair calcium absorption and affect calcium-regulating hormones. However, no study of this nature has been conducted among people living with HIV (PLWH) despite their high prevalence of hazardous alcohol use (HAU).

Hypocalcemia (hypoCa)'s health consequences will likely be significant, given that calcium is one of the most critical signaling molecules inside mammalian cells (Glancy and Balaban, 2012). Animal and laboratory experiments demonstrate that calcium balance needs to be conserved within narrow limits to maintain normal immune functioning, thereby supporting clinical concerns (Feske, 2007). In fact, mutant T cells deficient of calcium influx exhibited defective T cell-mediated immune responses and impaired interleukin-2 production and T cell proliferation (Feske, 2007; 2011). Of concern, the resulting immune suppression and modulation of transcription factor activities could contribute to regulating HIV gene expression. However, these "viroimmuno-calcium interfaces" evidenced by laboratory studies have been largely neglected within the HIV literature.

This dearth of information prompted us to investigate the prevalence and potential viroimmune consequences of hypoCa in individuals with and without HAU. Based on current literature and our prior research studies, we postulated that alcohol would induce a chronic pro-inflammatory response that may compromise calcium homeostasis. As depicted in Figure 1, we also hypothesized that calcium abnormalities encountered in PLWH + HAU would result in further viroimmune dysregulations. In order to test our model, we performed

a cross-sectional analysis of data from our ongoing alcohol study among PLWH to examine the immuno-skeletal interface in PLWH.

METHODS

Study Population

The Platelets Mediating Alcohol and HIV Damage Study (PADS) is a large, single-site cohort consisting of 400 PLWH who are at least 18 years old and under regular care at Miami's primary open-access public health system. Our choice of PLWH in an open-access public health system with standard treatment protocols has the advantage of minimizing social, medical, and treatment inequalities.

Non-ambulatory patients and those presenting with major medical co-morbidities were excluded. Participants were questioned about the following medical disorders and study staff reviewed medical records to confirm their eligibility: CNS opportunistic infection, head injury with or without loss of consciousness, tumors, major psychiatric disease, developmental disorders, severe malnutrition, or cardiovascular or immune-based disease, (i.e., malignancies, autoimmune diseases or arthritis). Participants were excluded if they had chronic renal failure, intestinal pathology, or thyroid problems.

To reduce the confounding effects of illicit drug use, the DSM-IV-TR questionnaire was applied and those participants dependent on or injecting illicit psychoactive substances were excluded. In addition, based on medical records, participants who had cirrhosis, active viral hepatitis, or liver enzymes two standard deviations above normal values were not eligible. Otherwise, the subject was enrolled.

Participants' Assessment Protocol

Those participants who provided written informed consent and a signed medical release form were consecutively enrolled and followed over a period of six months. Upon entry into the study, we obtained sociodemographic, alcohol use and medical history information (e.g., ART details) using computerized structured questionnaires. Blood was drawn in fasting subjects in order to best evaluate immunological, metabolic and nutritional profiles. HIV disease staging was established using standard Centers for Disease Control and Prevention criteria. The definition of ART for analyses followed published guidelines (DHHS, 2003).

Alcohol Use

At each visit, PLWH reported alcohol intake in the past six months using two standardized and validated brief screening questionnaires: the Alcohol Use Disorders Identification Test (AUDIT) and the Alcohol Dependence Scale (ADS) (Babor et al., 2001; Skinner and Horn, 1984). The AUDIT includes three questions on alcohol consumption, three on drinking behavior and dependence, and four on the consequences or problems related to drinking. The ADS assesses alcohol withdrawal symptoms, impaired control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol and salience of drink-seeking behavior.

Alcohol consumption scores were computed by averaging cross products of quantity and frequency of beer/wine and hard liquor reported on the AUDIT and ADS responses. This information is highly relevant, given that we previously observed significant differences in immune and biochemical profiles depending on the type of alcoholic beverage consumed. Then, based on the National Institute of Alcohol Abuse and Alcoholism guidelines criteria, men who reported >14 drinks/week or >4 drinks in one day and women >7 drinks/week or >3 drinks in one day were classified as HAU, while those who reported fewer drinks were categorized as non-HAU. Participants drinking more than 5 standard drinks in a day were considered binge drinkers (Sobell and Sobell, 1995).

Calcium

All PLWH were evaluated for serum calcium and albumin levels. Serum alkaline phosphatase was measured with the use of an auto-analyzer at the University of Miami's laboratory. All data points falling outside the expected reference ranges were indicated individually by the laboratory. The expected calcium reference range was between 8.6 and 10.2 mg/dL and that for alkaline phosphatase was between 38 and 126 U/L.

Outcome Variables

The main study outcomes related to HIV and ART response were: number of years since first diagnosis, viral load, CD4, and TNF- α . HIV viral burden was quantified using the Amplicor HIV monitor test (Roche Diagnostic System). The lower threshold for detection at the time of the study was 400 copies/ml. The percentage and absolute numbers of T lymphocyte subpopulations CD3+/CD4+, CD3+/CD8+, CD3+/CD19+ (B cells), and CD3+/CD56+ (NK) cell counts and percentages were determined by flow cytometry per National Institute of Allergy and Infectious Diseases laboratory protocols. TNF- α was quantified using a commercially available kit (R&D Systems, Minneapolis, MN, USA). This immunoassay is a 3.5-hour solid phase ELISA that recognizes natural human TNF in serum and plasma. This ELISA system is characterized by its high sensitivity detecting low amounts of TNF- α (0.1 pg/ml).

The date of initiation of ART, antiretroviral medications used, and prior exposure to ART therapies were all recorded. Adherence was measured using pharmacy records along with the ACTG questionnaire. This measure consists of 11-items that were specifically developed for HIV medication adherence. The measure asks participants to report the number of doses missed during the week and the weekends.

Statistical Analyses

The data were analyzed using SPSS 18.0 for Windows (IBM, Inc., Chicago, IL, USA) and *p* values <0.05 were considered statistically significant. Data are reported as means $\pm$ SD or counts and percentages. Subsequent to descriptive statistical analyses, group differences on continuous measures were compared with independent t-tests and one-way analysis of variance (ANOVA) procedures. Group differences on dichotomous categorical measures were estimated with Fisher's exact test. Viral load results (RNA copies per mL) were log-transformed before analysis. Sociodemographic and clinical variables (e.g., age, race/

ethnicity, gender, and adherence) were evaluated for possible inclusion in the univariate analyses and in the multivariate model as covariates.

Univariate analyses were used to calculate odds ratios (OR) and 95% confidence intervals (CI). Given the potential confounding effects of heavy drinking, our outcome variables were adjusted by total amounts of alcohol consumed. Outcomes and observed covariates that were significantly associated with viro-immune responses ($p < 0.05$) in univariate analysis were then included in a multivariable model. Furthermore, potential patient predictors, such as gender, race/ethnicity, CDC disease stage, and body mass index (BMI), were selected based on findings in the HIV literature, and were added to the model (Egger et al., 2002). Non-significant variables ($p \geq 0.05$) were removed, beginning with the least significant, until the final full model was determined. Model statistics included adjusted OR, 95% CI, and their corresponding p values.

RESULTS

Group Characteristics

Among the 400 PLWH participants, 27% were females and 73% were males ranging in age from 21 to 50 years (42 ± 6 years). Most participants were African-Americans (67%) or Hispanics (25%) with a smaller proportion of Caucasians (4%) and Caribbeans (4%). Overall nutritional status, determined by levels of serum albumin, was within the normal range for 99% of the participants (4.3 ± 0.4); malnutrition (serum albumin < 3.5 g/dl) was observed in only 1% of the group.

The cohort was characterized by normal liver enzymes levels suggestive of preserved liver function. Drugs of abuse, particularly marijuana and crack, were reported by only one third of the sample and mostly as recreational use over the weekends.

HIV Characteristics

Most participants had received a diagnosis of HIV infection > 1 decade previously (13.9 ± 8 years). Although all enrolled participants were prescribed ART (mostly Truvada 44%, Atripla 22%, alone or in combination with Norvir 32% or Kaletra 13%), 5% of the subjects decided not to start the prescribed regimen or discontinued it for side effects. Notably, adherence was shown to be high during the week (93%), although it dropped significantly during the weekend (83%). Despite being on ART, nearly one-quarter of the cohort (22%) had CD4+ counts ranging below 200. Of the remaining participants (78%), one-third (33% of the cohort) had more than 500 CD4+ cell counts (306 ± 14 cells/ml). Half of the group had undetectable viral load. Based on the literature, we explored gender differences in viro-immune parameters and confirmed that despite similar viral loads (2.6 ± 1.3 vs. 2.7 ± 1.4 , $p = 0.5$), women exhibited significantly higher CD4+ (387 ± 257 vs. 502 ± 319 , $p = 0.000$) and B (207 ± 129 vs. 290 ± 170 , $p = 0.01$) cell counts. Crack but not marijuana use was moderately correlated with CD4+ ($r^2 = -0.2$, $p = 0.01$), but not with viral load ($r^2 = -0.09$, $p = 0.03$).

Alcohol Use

Almost half of the study population was comprised of HAU (48%), with a median intake of 28 ± 4 alcoholic drinks weekly for HAU. Liquor was the most commonly consumed alcoholic beverage (45%), and most liquor users (60%) only drank liquor. One-third of the participants consumed beer, and 21% preferred wine. Noteworthy, HAU were twice as likely to use liquor or beer (OR=2.6, 95% CI 1.4–4.5, $p=0.0001$) as the main alcoholic beverage compared to nondrinkers. Conversely, non-HAU were more likely to report wine usage (OR=1.7, 95% CI 1–3.0, $p=0.04$).

As depicted in Table 1, alcohol use groups were very similar in sociodemographic, behavioral, and clinical characteristics. However, HAU were more likely to be poly-drug users. They also had lower body mass index. Interestingly, the mean BMI for both groups were shown to be in the overweight range. With regards to treatment, we did not find any evidence that antiretrovirals and/or adherence differed between the HAU and non-HAU groups.

Calcium

Mean calcium levels were within the normal range (9.3 ± 0.5 mg/dL). Fifteen percent of PLWH had albumin corrected-hypoCa defined as plasma levels of 8.6 mg/dL or below. We had no cases of hypercalcaemia. Although a sizable proportion of the participants had normal calcium values, levels were significantly lower in HAU than in non-HAU PLWH (8.9 ± 0.6 vs. 9.4 ± 0.5 , $p=0.03$). HAU were six times more likely to suffer hypoCa than non-HAU (OR=5.5, 95% CI: 1–23, $p=0.01$). Drugs of abuse did not affect the risk of developing hypoCa. Although mean calcium levels tended to be lower in women than in men (9.3 ± 0.47 vs. 9.2 ± 0.4 , $p=0.07$), the prevalence of hypoCa was shown to be similar. Nevertheless, all women with hypoCa were HAU.

Since total calcium is closely related to albumin levels, we explored the potential role of malnutrition in all study members and found no significant differences. No significant differences in nutritional status were noted between subjects with hypoCa and those with normal values. No significant differences in intake of multivitamins were observed between calcium groups (25% versus 45%, $p=0.2$).

Since the literature frequently relates HIV-associated hypoCa to pharmacotherapy, we carefully reviewed medical and pharmacy records. However, no participant was prescribed medications such as foscarnet, ketoconazole, aminoglycosides or pentamidine that may be associated with calcium disarrangements. No evidence was found to suggest that antiretrovirals and/or adherence were correlated with hypocalCa.

Chronic Inflammation

All PLWH had detectable plasma TNF- α levels. Values ranged from 0.03 pg/ml to 10.7 pg/ml (2.3 ± 0.3 pg/ml) with 10% of the population having more than 9 pg/ml. Compared to non-HAU, HAU exhibited significantly higher TNF- α levels (2.8 ± 0.6 vs. 1.9 ± 0.3 pg/ml, $p=0.05$). Given our prior findings showing immune differences by the type of alcohol beverage consumed, we explored if similar effects would be observed for TNF- α responses.

As depicted in Figure 2, wine consumers were notable for their low TNF- α levels. On the contrary, both liquor and beer HAU exhibited significantly higher TNF- α levels than their non-HAU counterparts.

Given epidemiological evidence relating TNF- α with calcium, we assessed its potential association and found significant differences in TNF- α levels between individuals with hypoCa versus those with normal levels (2.7 ± 2 vs. 10.7 ± 8 pg/ml, $p=0.001$). Additional analyses indicated that men with high levels of TNF- α were nine times more likely to have hypoCa (95% CI 1.6–43, $p=0.001$). Similarly, women with high TNF- α levels were more likely to have hypocalcaemiaCa (OR=5.7, 95% CI 1–33, $p=0.01$).

Calcium and Viro-Immune Measures

There was a significant and robust correlation between calcium values and immune measures. As illustrated in Figure 2, PLWH with hypocalCa exhibited significantly lower B cells. (248 ± 152 vs. 102 ± 63 , $p=0.02$) than those with normal calcium levels. These subjects also had significantly lower CD4+ counts (300 ± 160 , versus 190 ± 160 , $p=0.04$). Univariate analyses indicated that subjects with hypoCa were twice as likely to have less than 200 cell counts (OR=2.2, 95% CI 1–5.8 $p=0.04$). The hypoCa group exhibited lower NK cell percentages than their normal calcaemic counterparts (231 ± 132 vs. 78 ± 40 , $p=0.03$). The hypoCa group tended to have a higher percentage of CD8+ cells than their normal calcemia counterparts (55 ± 14 versus 52.8 ± 11.9 , $p=0.06$). Concurrent with this immune suppressed profile, the hypoCa group exhibited the highest viremia (3.4 ± 1.5 vs. 2.7 ± 1.3 $34,430 \pm 8,784$, $p=0.02$).

Multivariate Analyses

Although there is no perfect marker of cumulative disease severity, CD4+ cell count and viral RNA serve as useful surrogate markers of current disease activity and status and thus were used as our dependent variables of interest. We modeled absolute CD4+, and HIV RNA as continuous variables, rather than grouping them into 2 or more categories, as using continuous variables typically improves power, decreases residual confounding, and minimizes bias. Table 2 shows our final CD4+ prediction model: Being a male ($p=0.0001$), having a low calcium level ($p=0.05$), exhibiting higher TNF- α values ($p=0.001$) and consuming liquor ($p=0.04$) were significant predictors of current CD4 + T cell count. No significant interaction occurred among any of these variables. Age, race, time since infection, other drugs of abuse, liver enzymes, and ART regimen did not influence CD4+ count.

As depicted in Table 3, in the multivariate analysis detectable viral load was more likely in participants who presented with low calcaemia, poor adherence over the weekend, and higher CD8+ cell counts.

DISCUSSION

The success of HAART depends not only on its ability to yield an undetectable plasma viral load but also on reaching more than 500 CD4+ cell count. Otherwise, patients will be at an increased risk of clinical progression to AIDS-related and non-AIDS-related illnesses.

Accordingly, clinicians, researchers, and public health officials are interested in identifying any potential risk factor(s) associated with incomplete immune and virological responses. This study demonstrates for the first time in PLWH that hypoCa could be associated with various immune abnormalities in individuals receiving ART. In addition, multivariate analyses confirmed the relevance of calcium homeostasis on viral load, suggesting that calcium could be a modifiable factor to be screened and targeted among individuals with poor viro-immune responses.

Generally, after ART initiation, patients experience a rise in CD4+ cell count as a result of either redistribution and/or an expansion of naive CD4+ cells. Of concern, hypoCa was associated with low CD4+ counts was observed in our population. Our findings that calcium levels are significant predictors of T cell counts are not at all surprising, given that calcium homeostasis controls several cellular process including proliferation and apoptosis (Zhong et al., 2006, Qu Bin et al., 2011). In addition, hypoCa has been associated with thymus atrophy (Perris AD 1970, Fossard C 1977)).

While the hallmark of HIV-1 infection is a steep decrease of CD4+ counts, B cells also play a critical response; one that has largely been overlooked by HIV researchers in the field. Of special concern, our data indicated that calcium deficits affect the B cell lines. In order to contain HIV, the immune response needs to generate a massive production of antibodies with the sufficient breadth and potency to control HIV-1. It was not unexpected that calcium was a significant predictor of viral load. Furthermore, several groups have demonstrated that critical virus-host interactions depend on cellular calcium. Noteworthy, a decrease in Calcium has been associated with an inhibition of protein trafficking and a thwarting of anti-viral responses. In addition, evidence indicates that HIV is able to induce alterations in calcium.

Unfortunately, calcium alterations not only impact B cells, as NK functions and apoptosis are also dependent on an increase in intracellular free calcium concentration (Kagi et al., 1996; Leibson, 1997; Trinchieri, 1989). Zhong and colleagues showed that T cells with a high level of cytosolic calcium more readily undergo apoptosis compared to those with lower levels. In addition, natural cytotoxicity is initiated by adhesion receptors that are known to prompt a intracellular calcium rise associated with extracellular calcium influx that drives NK cells' effects. The rise in intracellular calcium is also needed for the granule release involved in the killing process (Kagi et al., 1996; Ostergaard and Clark, 1987). Therefore, permanent or temporal calcium depletions induced by HAU may compromise NK functionality resulting in increased risks of cancer and viral infections that are highly dependent on NK for their clearance.

The viral response to ART is highly variable, spanning from a rapid viral suppression to a long-term replication. A major determinant of the pace of disease progression is the level of HIV-1 replication, which is regulated by a complex network of molecules. In line with prior laboratory reports, our data indicated a close relationship between calcium levels and viral load.

Of equal importance, our data reveals that hazardous alcohol users are at increased risks of developing hypoCa. These findings are generally consistent with animal studies showing a direct effect of alcohol administration (on total calcium levels (Diez et al., 1997; Krishnamra and Limlomwongse, 1983; Krishnamra et al., 1987; Peng et al., 1972; Peng and Gitelman, 1974). The mechanism by which ethanol decreases calcium levels is still relatively unknown. However, it has been hypothesized that ethanol induces a shift in calcium from the extracellular to intracellular compartments within soft tissue and bone (Peng and Gitelman, 1974). If this is the case, then transient increases in free cytoplasmic calcium may induce T cell activation (Negulescu et al., 1994; Timmerman et al., 1996). Furthermore, the depleted calcium stores in the endoplasmic reticulum may trigger the opening of calcium channels in the plasma membrane, and thereby promote calcium influx from outside of the cell (Guse, 1998; Lewis, 2001). This process is known as capacitative calcium entry (CCE) or store-operated calcium entry.

An increased production of pro-inflammatory cytokines such as TNF- α is one of the many adverse consequences of HAU that can negatively impact calcium levels. Our multivariate analysis demonstrates that TNF- α is a strong predictor of T cell counts. Therefore, our data suggest that interventions to reduce alcohol consumption and increase dietary calcium could be attempted to prevent immune deterioration that subsequently leads to unsatisfactory immune recovery.

Although our research group along with others has documented the deleterious effects of alcohol on both immune and viral load status, the underlying molecular mechanisms are poorly defined. If alcohol induces an increase in free cytoplasmic calcium, then it also promotes T cell activation, which is in line with the increased TNF- α levels observed among HAU (Negulescu et al., 1994; Timmerman et al., 1996). The consequences of such chronic activation may include immune exhaustion, apoptosis and increased viral replication.

Conversely, if hypoCa is unrelated to intracellular influx, but rather to an overall depletion, then a deleterious immune effect will also be observed given that an intracellular calcium increase is needed to promote several immune functions.

Study Limitations

Although our study had a strong design, our findings are limited to the analyses of baseline data, which inevitably restricts our power to draw stronger conclusions. Since our study is based on a clinical population with strict inclusion criteria, our findings might not be generalizable to other groups. Further analyses will be performed once follow-up visits are concluded; meanwhile, studies with broader inclusion criteria and longer follow-up visits will be helpful to support our hypotheses and findings.

Conclusions

Our results are important because we quantified not only the relationship between hypoCa and CD4+ but also with B and NK cells, providing a more informative immune assessment. Early detection of hypoCa and establishment of appropriate treatment measures may be effective solutions in the ongoing prevention of viroimmune failures. In addition, calcium screening may also be relevant to a population whose rates of HAU are higher, and the

skeletal integrity is already seriously compromised in a sizable proportion of cases (67% among PLWH vs. 15% among the uninfected population) (Brown and Qaqish, 2006).

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Figure 1.
Proposed Model.

Figure 2.
Alcohol Differences and TNF- α .

Table 1

Baseline Clinical and Demographic Characteristics of the Cohort According To Alcohol Groups

VARIABLE	Hazardous Alcohol	Non-Hazardous Alcohol	<i>p value</i>
	Users	Users	
	N=200	N=200	
Age in years	42.8±6	41±6	NS
Male	77%	72%	
Female	23%	28%	NS
African-Americans	69%	68%	
Hispanic	26%	28%	NS
Caucasian	5%	4%	
Yearly Income			
	<\$10,000	89%	88%
	\$11,000-20,000	9%	10%
	>\$20,000	2%	2%
Tobacco	74%	64%	NS
Crack	33%	12%	0.002
Marijuana	31%	20%	0.02
Albumin gr/dl	4±0.4	4.1±0.5	0.9
Body Mass Index	27.6±7	29±8	0.02
Liver enzymes			
AST iu/l	27.4±8.9	25.4±9.3	0.8
ALT iu/l	23.7±6.8	19.2±8.6	
Alkaline Phosphatase iu/l	102.2±48	104±47	
Years living with HIV	13.5±7.7	14.3±7.4	0.3
CD4 counts/mm ³	445±300	416±270	0.3
Viral load	29,747±12,607	46,910±12,944	0.4
Log HIV copies/ml	2.7±1.2	2.7±1.3	
Adherence	77%	79%	0.9
Multivitamins	25%	45%	0.2

Data are either mean ± SD or percent of the group. NS= non-significant

Table 2

Prediction model.

	Calcium	<i>p</i> value	Mean (SD)
TNF	≥ 8.60	0.05	2.1 ± 2.60
	< 8.60		3.6 ± 4.2
Number B cells	≥ 8.60	0.02	250.32 ±
	< 8.60		202.03 ± 149.8
NK number	≥ 8.60	0.02	230.08 ± 151.9
	< 8.60		155.5 ± 96.7
Log Viral load	≥ 8.60	0.02	2.7 ± 1.2
	< 8.60		3.3 ± 1.5
CD4 Baseline	≥ 8.60	0.04	413.2 ± 262.7
	< 8.60		387.8 ± 359.8
CD4 % Baseline	≥ 8.60	0.0	22.2 ± 11.4
	< 8.60		18.3 ± 14.2
CD8 % Baseline	≥ 8.60	0.06	50.8 ± 12.8
	< 8.60		56.3 ± 16.2
CD8 Counts	≥ 8.60	0.06	891.2 ± 419.9
	< 8.60		908.9 ± 556.8

Table 3

Multivariate Regression Model Examining Predictors of CD4 of Absolute Counts

Unstandardized Coefficients		Standardized Coefficients		
B	Std. Error	Beta	t	Sig.
(Constant)	−378.435	330.218	−1.146	.253
TNF- α	−17.714	5.391	−.194	.3286
Liquor	12.628	6.175	.119	2.045
Calcium	68.348	34.669	.113	1.971
Gender code	131.116	32.313	.229	4.058

Table 4

Multivariate Analyses Predictors of HIV load

Unstandardized Coefficients		Standardized Coefficients			
Beta	Std. Error	Beta	t	Sig.	p
(Constant)	4.778	2.184		2.188	.030
CD8 +	.041	.007	.396	5.808	.000
Calcium	-.433	.212	-.144	-2.043	.043
TNF- α	.070	.028	.175	2.538	.012
Adherence weekend	-.096	.239	.028	-.402	.688

Model adjusted for age, gender and race