Abstract

**Objectives:** The anti-microbial peptide cathelicidin, LL-37, is produced in response to active vitamin D to enhance innate immunity, exert immunomodulatory effects, and decrease pro-inflammatory cytokines. LL-37 also inhibits HIV replication in vitro. To date, no studies have investigated plasma LL-37 concentrations in HIV-infected patients in vivo. This study sought to investigate the relationship between plasma LL-37, and 25-hydroxyvitamin D (25(OH)D) concentrations, and HIV-related variables in HIV-infected youth.

**Methods:** HIV-infected subjects and healthy controls ages 1-25 years old were prospectively enrolled. Fasting plasma LL-37 was measured in duplicate with ELISA. Fasting plasma 25(OH)D was measured using an automated chemiluminescent assay. Non-parametric tests were used to assess differences between groups. Spearman coefficients were used to assess correlations with LL-37. Multivariable regression analysis was performed to determine variables independently associated with LL-37.

**Results:** HIV+ subjects (36 ART-experienced; 27 ART-naïve) and 31 healthy controls were enrolled. ART-experienced subjects were similar to the two other groups (ART-experienced: median (interquartile range (IQR)) age= 17.9 years (14.6,20.2), 94% black, 58% male), except that ART-naïve had more males (85%). Plasma 25(OH)D concentrations did not differ between groups (ART-experienced: median (IQR) 25(OH)D= 15.5 ng/mL (10.8,19.6)). 83% of ART-experienced were currently on ART (81% HIV-1 RNA <80 copies/mL). ART-experienced had a median current/nadir CD4 count of 431/183 cells/mm³, respectively vs. 297/296 cells/mm³, respectively in ART-naïve. In ART-experienced, CD4 restoration after ART (ΔCD4= current-nadir CD4) was 229 cells/mm³ with 14.5 median years of HIV infection. There was no difference in median(IQR) LL-37 between the HIV+ group and controls (HIV+: 58.3 µg/mL (46.4,69.5) vs. controls: 51.3 (40.8,98.2), P=0.57); however, ART-experienced had higher levels than ART-naïve (ART-experienced: 66.2 µg/mL (55.4,77.0) vs. ART-naïve: 48.9 µg/mL (38.9,57.9), P<0.001). In univariate analysis, LL-37 was positively correlated with 25(OH)D in controls (R=0.43;P=0.02), but not in the HIV+ group as a whole or when the ART-experienced and ART-naïve groups were considered separately. LL-37 was not correlated with age, body mass index, CD4 nadir, or HIV-1 RNA in any group. However, LL-37 was positively correlated with current CD4 count in ART-experienced (R=0.37;P=0.02) but not in ART-naïve. LL-37 was positively correlated with ΔCD4 in ART-experienced (R=0.40;P=0.02). After adjustment for age, race, sex, and HIV duration, the association between LL-37 and current CD4 count remained significant (P=0.03) but not between LL-37 and ΔCD4 (P=0.09).

**Conclusions:** Plasma LL-37 was positively associated with plasma 25(OH)D concentrations in healthy controls but not in HIV+ subjects. Plasma LL-37 was higher in ART-experienced HIV+ subjects compared to ART-naïve, and plasma LL-37 was associated with CD4 counts and immune restoration after ART. These findings suggest that HIV+ status and/or HIV-related variables may interfere with the expected positive relationship between vitamin D and LL-37, and LL-37 may play an important role in immunomodulation and immune restoration in HIV disease.