Pro-inflammatory Interleukin-18 is Associated with Hepatosteatosis and Elevated Liver Enzymes in People with HIV Monoinfection

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BACKGROUND

Nonalcoholic Fatty Liver Disease (NAFLD) in People with HIV (PWH)

- PWH are at increased risk of development of NAFLD
- Histologic studies suggest increased severity of HIV-associated NAFLD compared to non-HIV patients with NAFLD[1]
- Chronic low-grade inflammation, which is persistent in PWH despite viral suppression, has been associated with the pathogenesis of NAFLD
- PWH have elevated systemic levels of interleukin-18 (IL-18)

Pro-inflammatory IL-18 and Inflammasome in NAFLD

- IL-18, a member of the pro-inflammatory IL-1 family, is regulated by inflammasomes in innate immune cells and hepatocytes in response to pathogens and danger signals[2]
- Inflammasome components in the liver including IL-18 are more highly expressed in NASH compared to NAFLD without inflammation in people without HIV[3]
- Inflammasome activation creates proinflammatory and profibrotic milieu by increasing the expression of secondary pro-inflammatory cytokines and chemokines including TNFα and monocyte chemoattractant protein-1 (MCP-1/CCL2), and activating hepatic stellate cells

Hypothesis: Increased levels of IL-18, as a marker of inflammasome activation, may relate to NAFLD and liver injury in PWH.

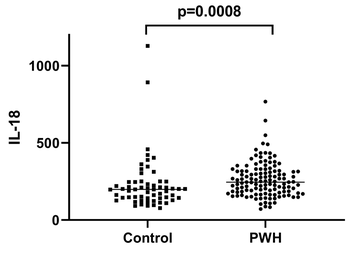
We investigate for the first time relationships between systemic levels of IL-18 and liver transaminases and noninvasive measures of liver fat in PWH

METHODS

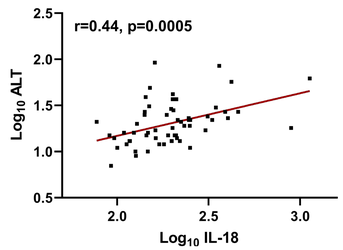
- Observational study including 125 PWH and 59 HIV-negative controls without known hepatitis B, hepatitis C, and significant alcohol consumption (>21 and >14 drinks/week for men and women, respectively). Participants did not have known cardiovascular disease based on the inclusion criteria of the original study.
- Participants had blood drawn fasting in the morning for laboratory assessment including IL-18 and MCP-1 (R&D Systems) by ELISA and computed tomography which was used to characterize hepatic lipid content
- We analyzed the relationships of IL-18 to liver transaminases, CT liver-to-spleen attenuation ratio (lower ratio indicates higher hepatic lipid content), traditional metabolic risk factors, HIV-related parameters, and inflammatory markers.

RESULTS

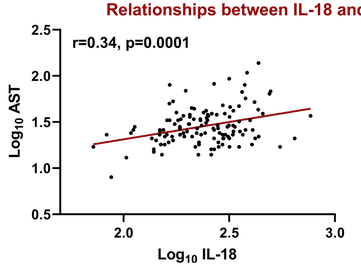
IL-18 Levels in Control vs PWH

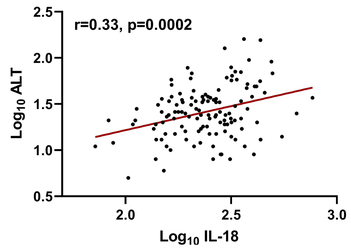


Relationship between IL-18 and ALT among people without HIV



Relationships between IL-18 and liver transaminases in PWH





RESULTS

Relationships of liver, metabolic, and inflammatory indices with IL-18 and MCP-1 in PWH

	Log_{10} (IL-18)		Log_{10} (MCP-1)	
	r	p-value	r	p-value
Liver				
Log_{10} (AST)	**0.34**	**0.0001**	**0.27**	**0.004**
Log_{10} (ALT)	**0.33**	**0.0002**	**0.26**	**0.006**
Liver/Spleen Ratio	**-0.24**	**0.02**	-0.20	0.06
Glucose				
Fasting Glucose	0.08	0.40	0.09	0.33
Log_{10} (Hemoglobin A1c)	-0.09	0.32	-0.001	0.99
Lipids				
Total Cholesterol	-0.02	0.83	-0.06	0.52
LDL	-0.01	0.87	-0.05	0.63
HDL	**-0.28**	**0.002**	**-0.25**	**0.009**
Log_{10} (Triglycerides)	**0.26**	**0.003**	**0.25**	**0.007**
Fat distribution				
BMI	-0.04	0.64	0.06	0.53
Log_{10} (VAT)	0.12	0.19	**0.22**	**0.02**
Log_{10} (SAT)	-0.15	0.09	-0.004	0.97
Inflammatory Markers				
Log_{10} (MCP-1)	**0.33**	**0.0004**	-	-
Log_{10} (IL-18)	-	-	**0.33**	**0.0004**
Log_{10} (LPS)	**0.28**	**0.004**	0.02	0.86
Log_{10} (Caspase-1)	**0.35**	**<.0001**	0.15	0.13
HIV Disease Parameters				
Log_{10} (Viral load)	**0.29**	**0.002**	-0.03	0.74
Log_{10} (CD4+ T-cell count)	-0.17	0.06	0.12	0.21
Log_{10} (CD8+ T-cell count)	0.13	0.16	**0.24**	**0.01**
Log_{10} (CD4+/CD8+ Ratio)	**-0.24**	**0.007**	-0.05	0.61

- Univariate relationships were assessed by Pearson correlation coefficient
- In individuals without HIV:
  - the relationship between log_{10} IL-18 and log_{10} ALT was also significant (r=0.44, p=0.0005)
  - the inverse correlation between log_{10} IL-18 and HDL was also significant (r=-0.27, p=0.04)

Multivariate model for adjusted relationships with log_{10} ALT

Covariate	β	p-value
Log_{10} IL-18	0.43	**0.003**
Age	0.00006	0.98
Gender	0.14	**<0.0001**
BMI	0.01	**0.02**
Log_{10} triglycerides	0.008	0.94
Statin use	0.01	0.73
Log_{10} HIV RNA	-0.04	0.32
Log_{10} CD4 count	0.04	0.75

Multivariate model for adjusted relationships with liver-to-spleen ratio

Covariate	β	p-value
Log_{10} IL-18	-0.62	**0.02**
Age	-0.003	0.54
Gender	0.03	0.57
BMI	-0.01	0.18
Log_{10} triglycerides	-0.11	0.62
Fasting glucose	0.0002	0.95

- Multivariate modeling performed to assess adjusted relationships with ALT including covariates that may be potential confounders relevant to inflammation and liver enzymes
- Similar findings of significant adjusted relationship with IL-18 were also seen for AST (β=0.31, p=0.004) using the same model covariates
- In an additional model including MCP-1, IL-18 remained significantly associated with ALT (β=0.52, p= 0.002) and AST (β=0.36, p= 0.007)
- As liver-to-spleen ratio reflects hepatic lipid content, metabolic variables associated with NAFLD were included in the model

RESULTS

Clinical characteristics of PWH and HIV-negative population

	Chronic HIV (n=125)	HIV-Negative (n=59)	p-value
Demographics			
Age, years	46.5 ± 8.2	45.4 ± 7.1	0.32
Gender, male %	69.6%	66.1%	0.63
Race			0.31
White, %	52.8%	55.9%	
African American, %	31.2%	27.1%	
HIV-related parameters			
Duration of HIV, years	14.3 ± 7.0		
CD4+ cells, cells/m^3	556 [392, 816]		
Suppressed viral load, %	77.1%		
Currently on ART, %	81.6%		
Metabolic parameters			
BMI, kg/m^2	27.9 ± 5.5	27.3 ± 4.9	0.49
VAT, cm^2	111.7 [67.5, 225.7]	103.1 [55.4, 182]	0.33
Fasting glucose, mg/dL	89.6 ± 15.2	89.1 ± 12.6	0.78
Hemoglobin A1c, %	5.5 [5.2, 5.8]	5.5 [5.3, 5.8]	0.61
Total cholesterol, mg/dL	183.6 ± 40.0	180.4 ± 36.4	0.58
LDL, mg/dL	105.0 ± 33.7	108.8 ± 31.0	0.45
HDL, mg/dL	52.1 ± 17.5	50.7 ± 13.7	0.56
Triglycerides, mg/dL	96 [74, 172]	84 [62, 126]	**0.02**
Statin use, %	17.2%	3.5%	**0.005**
Hepatic function markers			
AST, units/dL	26 [21, 37]	23 [17, 29]	**0.009**
ALT, units/dL	25 [17, 38]	19 [14, 27]	**0.005**
Liver-spleen attenuation ratio	1.43 ± 0.45	1.39 ± 0.38	0.61
Liver-spleen ratio < 1 (%)	11.2%	8.7%	0.64
Inflammatory markers			
IL-18, pg/ml	245.8 [179.5, 317.0]	198.6 [141.7, 246.6]	**0.0008**
Caspase-1, pg/ml	49.7 [39.8, 66.2]	50.4 [35.5, 65.7]	0.62
MCP-1, pg/ml	262 [179, 358]	223.5 [170.3, 275]	**0.02**
LPS, EU/ml	0.81 [0.38, 1.15]	0.69 [0.26, 0.87]	**0.06**

CONCLUSIONS

- IL-18 was elevated in PWH and associated with markers of liver injury and NAFLD including liver transaminases and CT liver-to-spleen ratio. Similar association was seen between IL-18 and ALT in controls, consistent with the known role of inflammasomes in steatohepatitis.
- IL-18 was associated with triglycerides in PWH, confirmatory of findings by Falasca et al.[4]
- IL-18 was inversely associated with HDL in both PWH and controls.
- IL-18 was also associated with caspase-1, LPS, and MCP-1, which may underscore the inflammasome's ability to sense danger signals from increased microbial translocation and its known role in activating MCP-1-mediated monocyte recruitment to drive NAFLD progression.
- IL-18 was associated with HIV viral load and inversely associated with CD4/CD8 T-cell ratio, which may suggest that HIV may play a role in chronic low-level inflammation involving inflammasomes.
- The relationship of IL-18 with liver transaminases was independent of factors potentially associated with transaminase elevation or HIV parameters. The relationship of IL-18 with hepatic lipid content was independent of potential metabolic confounders.
- Further studies using liver biopsies are needed to confirm our findings and to elucidate precise mechanisms involving the inflammasome pathways in NAFLD progression in PWH

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