Extracellular Vesicles' miRNAs Correlate with Inflammatory Markers in Tanzanians PLWH under Antiretroviral Therapy

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BACKGROUND

- ✓ Although antiretroviral therapy (ART) effectively improves the life expectancy among people living with HIV (PLWH), chronic immune activation and inflammation remain a concern.
- ✓ Recent studies suggest that certain extracellular vesicles-delivered miRNAs have a role in modulating inflammatory responses within the immune system.
- ✓ We thus hypothesized that dysregulation of EVs' miRNAs during HIV infection may be associated with HIV-related inflammation.

METHODS

i. Study participants

Plasma samples from Dar-es-Salaam, Tanzania
(a) Muhimbili National Hospital
(b) Mnazi Mmoja Hospital

- *HIV untreated, n=16*
- *HIV treated, n=30*
- *Health controls, n=10*

ii. Selected Immunoregulatory miRNAs

Selection of 20 Inflammatory associated miRNAs

- **HIV- related Inflammatory miRNAs**: *miR-7-5p, miR-9-5p, miR-16-5p, miR-21-5p, miR-122-5p, miR-144-3p, miR-146a-5p, miR-155-5p, miR-200a-5p, miR-210-3p, miR-221-3p, miR-382-5p and miR-572*
- **Inflammatory miRNAs related to other infections and disorders**: *miR-18a-5p miR-20a-5p, miR-29a-3p, miR-142-3p, miR-192-5p, miR-375-3p and miR-615-5p*

iii. miRNA Quantification by RT-qPCR

Exosome precipitation from plasma
-Total Exosome Isolation Kit

Total RNA extraction
-TRIzol Reagent

MicroRNA Quantification by RT-qPCR

iv. Quantification of clinical parameters

- **IL-6 and IL-10**: Bio-Plex 200 — *HIV untreated, HIV treated, Health controls*
- **sCD14**: Customized Cytometric Bead Array (CBA) — *HIV untreated, HIV treated*
- **hsCRP**: Nephelometric analyzer N-latex CRP II — *HIV treated*

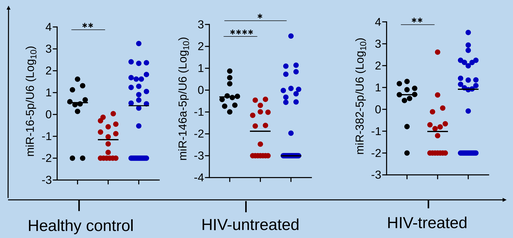
Result 1: Demographic and Clinical profile of participants

	Healthy control (n=10), p1	Untreated (n=16), p2	Treated (n=30), p3	*p* 1 vs. 2	*p* 1 vs. 3	*p* 2 vs. 3
Demographic data						
Median age in years (IQR)	30.5 (25.5-39.25)	37.5 (31.75-43.75)	47 (42.25-53.25)			
Sex-female n(%)	7 (70)	14 (77.8)	26 (86.7)			
Clinical data						
Median baseline CD4 count cells/cm3 (IQR)	N/A	304.5 (130.8-454.8)	145 (32-193)			0.002*
Missing		2	10			
Median current CD4 count cells/cm3 (IQR).	N/A	N/A	510 (342-720)			
Missing			1			
Median years on treatment (IQR)	N/A	N/A	7.5 (5.5-10)			
Missing			8			
Median viral load copies/ml (IQR)	N/A	62684 (13740-244886)	N/A			
Median proviral DNA copies/million PBMCs(IQR)	N/A	N/A	42.8 (0.49-444)			
Median Scd14 pg/ml (IQR)	N/A	17890 (13333-25683)	11318 (9360-13602)			0.0001*
Median hsCRP. pg/ml (IQR)	N/A	N/A	2010 (688.3-4996)			
Median IL-6 pg/ml (IQR)	0.01	0.38 (0.01-1.22)	0.02 (0.01-0.81)	0.0167*	0.0361*	0.3446
Median IL-10 pg/ml (IQR)	2 (1.42-3.19)	0.93 (0.32-1.79)	1.53 (0.89-2.34)	0.0919	0.2456	0.1014

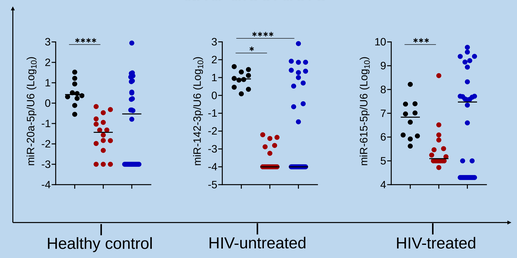
n: sample size, IQR: interquartile range, PBMCs: peripheral blood mononuclear cells, hsCRP: high sensitivity C-reactive protein, N/A: not applicable. *P* value was calculated using Mann Whitney U test

Result 2: Inflammatory miRNAs are differentially expressed in EVs of PLWH

A. HIV-related Inflammatory miRNAs



B. Inflammatory miRNAs r/to other infections and disorders

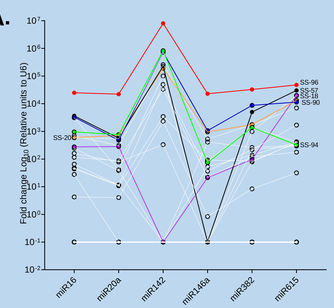


The horizontal bars indicate the median value. Differences between groups were tested for statistical significance by the Mann–Whitney U test

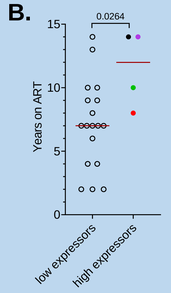
- ➢ A significant decrease in expression levels of six miRNAs (>9-fold) in the HIV-untreated group compared to the healthy control group.
- ➢ Four of the six miRNAs showed a restoration pattern in the treated group with exception of miR-142-3p and miR-146a-5p **(A & B)**

Result 3: Duration on ART is associated with high miRNA expression

A.



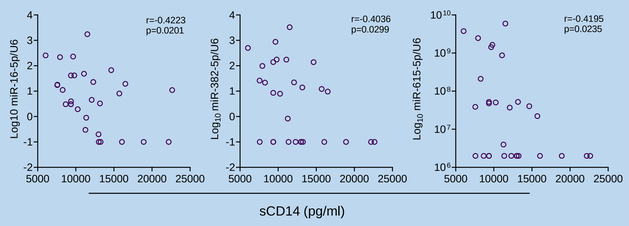
B.



- ➢ A subset of HIV-treated participants was observed to highly express all six dysregulated miRNAs consistently **(A)**
- ➢ Long duration on ART was shown to associate significantly with high miRNA expression **(B)**

Result 4: Correlations between expression levels of EVs' miRNAs and Inflammatory parameters among HIV-treated group

A.



B.

		miR-16-5p	miR-20a-5p	miR-142-3p	miR-146a-5p	miR-382-5p	miR-615-5p	IL-6	IL-10	hsCRP	sCD14
miR-16-5p	Spear.	1	0.958*	0.851*	0.712*	0.982*	0.948*	0.131	0.299	0.027	-0.422*
	p-value		0.0001	0.0001	0.0001	0.0001	0.0001	0.490	0.108	0.889	0.020
miR-20a-5p	Spear.	0.958*	1	0.870*	0.774*	0.953*	0.885*	0.131	0.285	-0.024	-0.231
	p-value	0.0001		0.0001	0.0001	0.0001	0.0001	0.489	0.127	0.899	0.219
miR-142-3p	Spear.	0.851*	0.870*	1	0.679*	0.867*	0.725*	0.086	0.250	0.144	-0.262
	p-value	0.0001	0.0001		0.0001	0.0001	0.0001	0.651	0.183	0.448	0.162
miR-146a-5p	Spear.	0.712*	0.774*	0.679*	1	0.771*	0.638*	-0.016	0.269	-0.057	-0.081
	p-value	0.0001	0.0001	0.0001		0.0001	0.0001	0.933	0.150	0.764	0.671
miR-382-5p	Spear.	0.982*	0.953*	0.867*	0.771*	1	0.924*	0.149	0.317	0.031	-0.404*
	p-value	0.0001	0.0001	0.0001	0.0001		0.0001	0.431	0.088	0.869	0.030
miR-615-5p	Spear.	0.948*	0.885*	0.725*	0.638*	0.924*	1	0.108	0.246	0.002	-0.420*
	p-value	0.0001	0.0001	0.0001	0.0001	0.0001		0.569	0.189	0.993	0.024

Spear Corr.: Spearman Correlation, * denotes Correlation is significant at the 0.05 level

- ➢ miR-16-5p, miR-382 and miR-615-5p significantly correlated inversely with sCD14 **(A)**
- ➢ We also observed positive correlations between the expression levels of the six dysregulated miRNAs in HIV-treated group **(B)**

CONCLUSIONS

- ✓ Collectively, we identified six miRNAs showing substantial dysregulation in EVs in HIV infection.
- ✓ miR-16-5p, miR-382-5p and miR-615-5p could be surrogate markers of chronic inflammation during antiviral treatment in PLWH.
- ✓ The positive correlations between the dysregulated miRNAs suggest shared regulatory mechanisms, providing insights into chronic inflammation underlying pathway.

ADDITIONAL KEY INFORMATION

Contact Information

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