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## BACKGROUND

After the advent of highly active antiretroviral therapy, liver disease became a leading cause of morbidity and mortality in patients with HIV-1 infections (1). Remarkably, abnormal liver enzymes are common in patients with HIV-1, even in the absence of viral hepatitis or alcohol abuse. Consequently, we need biomarkers of liver disease progression to assess for advanced fibrosis. Dysregulated levels of specific plasma circulating micro(mi)RNAs have been associated with a variety of diseases, including cancer, diabetes, obesity, cardiovascular disease and HCV infection (2).

## OBJECTIVE

In the present study, we conducted a large-scale genome-wide screen to construct a profile of circulating plasma miRNAs related to liver injury in patients infected with HIV-1 that were not co-infected with hepatotropic viruses.

## METHODS

Large-scale deep sequencing analyses of small RNA expression on plasma samples from 144 patients infected with HIV-1 that had elevated levels of alanine aminotransferase (ALT), focal nodular hyperplasia or HCV co-infections (Table 1). Healthy donors and HCV mono-infected individuals were also explored.

**Table 1.** Clinical and biochemical characteristics of the whole study population according to infection status.

	Healthy donors	HCV mono-infected	n-value <sup>1</sup>	HIV-1 mono-infected	n-value <sup>1</sup>	HIV-1/HCV co-infected	n-value <sup>1</sup>
N	21	17	-	53	-	47	-
Age, yr	37 (38-44.5) <sup>2</sup>	54 (44.5-70.5)	0.0011	55 (50-60)	<0.0001	54 (51-57)	<0.0001
Gender, Male, N (%)	6 (28)	7 (41)	-	38 (71)	-	34 (72)	-
Fibroscan (kPa)	7.9 (5.3-10.65)	-	-	8.5 (6.3-12.6)	-	-	-
Alkaline Phosphatase (U/L)	54 (48-65)	75 (60-115.2)	0.001	85 (66-111)	<0.0001	67 (57-84)	0.0004
ALT (U/L)	15 (12-19.5)	36 (25-51)	<0.0001	31 (19-70)	<0.0001	72 (38-107)	<0.0001
AST (U/L)	15 (15-20.5)	35.5 (25.5-46)	<0.0001	31 (21-50)	<0.0001	48 (33-64)	<0.0001
GGT (U/L)	-	39 (21-128)	-	47 (27-105)	-	78 (38-173)	-
Serum Albumin (g/L)	-	42 (38.5-43)	-	46.1 (42.1-47.65)	-	44.2 (41.9-47.5)	-
Total protein (g/L)	73.4 (71.1-76.6)	71.7 (68.2-74.6)	0.1727	71.9 (68.6-74.4)	0.0765	73 (70.2-79)	0.9418
Total bilirubin (umol/L)	8 (6.4-12.85)	10 (8-17)	0.1421	8 (5.3-13.5)	0.7852	10.1 (7.5-13)	0.2564
HDL (mmol/L)	1.45 (1.3-1.8)	-	-	1.2 (1-1.5)	-	1.13 (0.85-1.47)	-
LDL (mmol/L)	2.56 (1.26-3.36)	-	-	3.38 (2.84-3.95)	-	2.27 (2.01-3.35)	-
Cholesterol (mmol/L)	4.5 (3.6-5.45)	4.1 (3.55-4.85)	0.2645	5.2 (4.5-6)	0.0374	4.3 (3.8-5.3)	0.5771
Triglycerides (mmol/L)	0.9 (0.5-1.05)	0.9 (0.75-1.1)	0.4359	1.5 (1.2-2)	<0.0001	1.45 (1.05-2.2)	<0.0001
Creatinin (umol/L)	69 (61-484.5)	75 (65-96.5)	0.5971	73.5 (65-96.5)	0.1139	62 (78-84)	0.0001
Glucose (mmol/L)	-	5.5 (4.85-6.55)	-	4.8 (4.5-5.5)	-	5 (4.6-5.6)	-
Platelet counts (x10 <sup>9</sup> )	253.5 (189.5-348.5)	190 (144.5-223)	0.0039	189 (151-227)	0.0042	189 (151-227)	0.0003
CD4 cell counts (cell/mm <sup>3</sup> )	-	-	-	454 (285-750)	-	513 (362-741)	-
HCV RNA Viral load log <sub>10</sub> (IU/ml)	-	6.05 (5.75-6.71)	-	-	-	5.69 (4.78-6.59)	-
Undetectable HIV viral load, N (%)	-	-	-	42 (79.2)	-	30 (63.8)	-
Patients on ART, N (%)	-	-	-	48 (90.6)	-	44 (93.6)	-

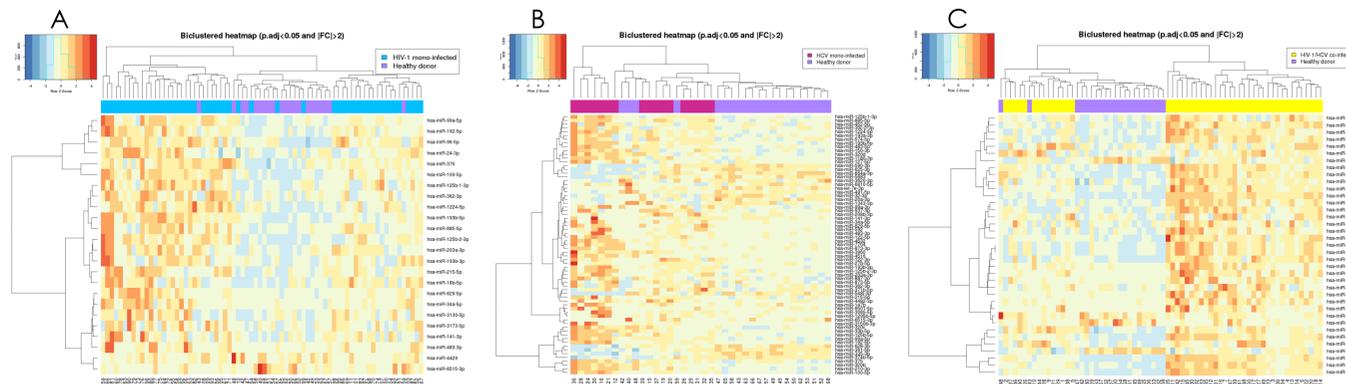
<sup>1</sup>Mann Whitney test, <sup>2</sup>Median, IQR

## RESULTS

We identified 1425 mature miRNAs in the study samples (median, 556 miRNAs; interquartile range [IQR]: 521-618).

Compared to healthy donors, patients with HIV-1 or HCV mono-infections showed significantly altered (fold change >2, adjusted p <0.05) expression of 25 and 70 miRNAs, respectively (Figure 1A and B).

## RESULTS



**Figure 1.** Expression profile of dysregulated circulating miRNAs. A heat map shows the miRNAs with significant (fold change >2 and adjusted p <0.05) differential expression. Red: up-regulated miRNAs; blue: down-regulated miRNAs; yellow: no change. **A)** Patients with HIV-1 mono-infections (n=53, blue) compared to healthy control donors (n=21, magenta). Twenty-four miRNAs were up-regulated and 1 was down-regulated. **B)** Patients with HCV mono-infections (n=17, magenta) compared to healthy control donors (n=21, purple). Fifty-two miRNAs were up-regulated and 18 were down-regulated. **C)** Patients with HIV-1/HCV co-infections (n=47, yellow) compared to healthy control donors (n=21, purple). Thirty-eight miRNAs were up-regulated and 6 were down-regulated.

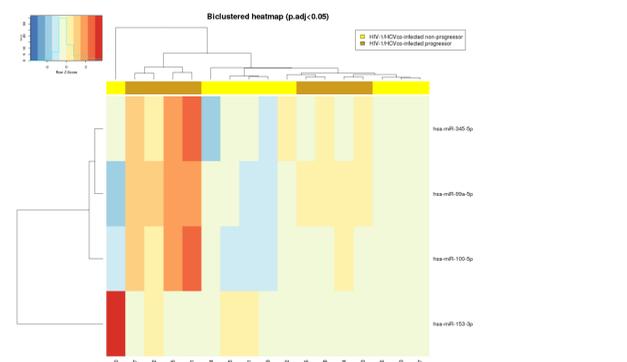
Of the 25 altered miRNAs found in patients with HIV-1, 19 were also found in patients mono-infected with HCV. Moreover, 13 of the 14 most up-regulated miRNAs (range: 9.3–3.4-fold increase) in patients with HCV mono-infections (miR-193b-5p, miR-483-5p, miR-1224-5p, miR-125b-1-3p, miR-885-5p, miR-100-5p, miR-192-5p, miR-592, miR-193b-3p, miR-125b-2-3p, miR-629-5p, miR-99a-5p, and miR-203a-3p) were also up-regulated in patients with HIV-1 mono-infections (Figure 1 A and B). Twelve of these miRNAs (all except miR-629-5p), were also up-regulated in the HIV-1/HCV co-infection group (median 4.6 FC; IQR: 4.02-5.91) (Figure 1C), which validated the results obtained in the HIV-1 and HCV mono-infected groups. Of these 13 miRNAs, 11 (all except miR-629-5p and miR-1224-5p), were significantly and positively correlated with alanine aminotransferase and aspartate aminotransferase levels in most study samples (p < 0.05), including those from healthy donors.

We studied 9 patients with HIV-1/HCV co-infections that displayed no liver fibrosis (F0/1) at sampling time, but later progressed to liver fibrosis (F4) (Table 2). The miRNA expression levels of this group of patients were compared to those of 8 patients with HIV-1/HCV co-infections that did not progress to liver fibrosis (Figure 2).

**Table 2.** Clinical and biochemical characteristics of the HIV-1/HCV co-infected patients.

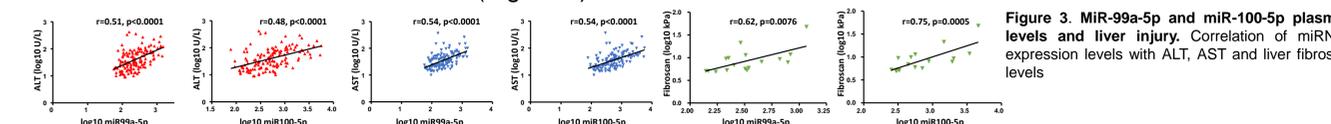
	HIV-1/HCV co-infected patients		p-value <sup>1</sup>
	F0, Non-Progressor	F0, Progressor	
N	9	8	-
Age, yr	57 (56-58) <sup>2</sup>	50.5 (49-52.5)	0.0111
Gender, Male (%)	4 (44.4)	6 (62.5)	-
Fibroscan (kPa) <sup>3</sup>	6.10 (4.85-7.35)	11.50 (10.95-13.60)	<0.0001
Alkaline Phosphatase (U/L)	67 (57-89)	67.5 (63-92)	0.9626
ALT (U/L)	51 (32-71)	98 (55-122)	0.0592
AST (U/L)	39 (33-43)	62.5 (42.5-64)	0.0927
GGT (U/L)	76 (31-220)	67 (42.5-88.5)	0.8884
Serum Albumin (g/L)	45.1 (43.3-48.6)	46.9 (44.3-48.5)	0.4634
Total protein (g/L)	73 (69.8-78.9)	71.7 (70.9-78.05)	0.6058
Total bilirubin (umol/L)	10.2 (6.2-15.25)	10.1 (10.1-15.75)	0.743
HDL (mmol/L)	1.46 (1.18-1.73)	1.23 (0.87-1.93)	0.6095
LDL (mmol/L)	2.35 (2.27-3.19)	2.36 (1.75-3.84)	0.4257
Cholesterol (mmol/L)	4.5 (4.25-5.95)	4.2 (3.9-4.4)	0.1139
Triglycerides (mmol/L)	1.7 (1.2-2.05)	1.25 (0.9-1.3)	0.1139
Creatinin (umol/L)	87 (79.5-88.4)	75 (70-97.5)	0.3704
Glucose (mmol/L)	4.9 (4.45-5.05)	4.9 (4.45-5.3)	0.6058
Platelet counts (x10 <sup>9</sup> )	192 (188.5-227)	143.5 (117.5-196)	0.0745
CD4 cell counts (cell/mm <sup>3</sup> )	564 (476-781)	481 (368-554)	0.1388
HCV RNA Viral load log <sub>10</sub> (IU/ml)	5.78 (4.49-5.93)	5.44 (4.70-5.64)	0.6126
Undetectable HIV viral load, N (%)	6 (66.6)	6 (75)	-

<sup>1</sup>Mann Whitney test, <sup>2</sup>Median, IQR, <sup>3</sup>At the end of follow up



**Figure 2.** Expression profile of dysregulated circulating miRNAs. Patients with HIV-1/HCV co-infections without liver fibrosis (F0/1) at sampling time, but with liver fibrosis (F4) at later times (n=9, brown) compared to patients with co-infections that did not progress to liver fibrosis (n=8, yellow). Three miRNAs were up-regulated and 1 was down-regulated.

MiR-99a-5p and miR-100-5p were not only significantly correlated with ALT and aspartate aminotransferase (AST) levels but also with liver fibrosis levels (Figure 3).



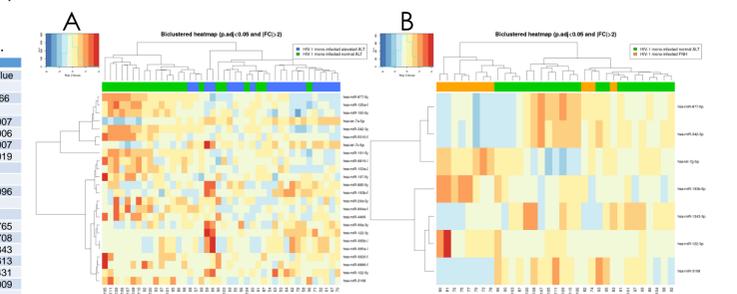
**Figure 3.** MiR-99a-5p and miR-100-5p plasma levels and liver injury. Correlation of miRNA expression levels with ALT, AST and liver fibrosis levels

The comparison of HIV-1 mono-infected samples with elevated ALT or focal nodular hyperplasia with those HIV-1 samples with normal ALT levels (Table 3) revealed that miR-122-3p and miR-193b-5p were highly and significantly up-regulated in these two patient groups (Figure 4).

**Table 3.** Clinical and biochemical characteristics of the HIV-1 mono-infected patients.

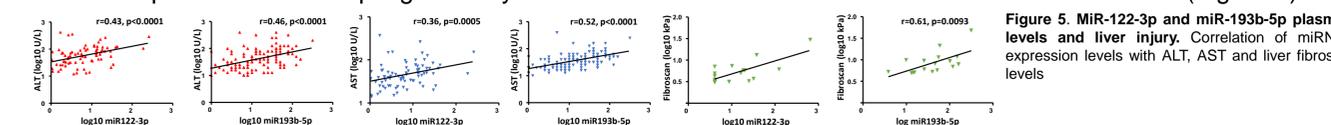
	HIV-1 normal ALT	HIV-1 elevated ALT	p-value <sup>1</sup>	HIV-1 FNH <sup>2</sup>	p-value <sup>1</sup>
N	22	20	-	11	-
Age, yr	58 (54-61.5) <sup>2</sup>	20 (43.5-53)	0.0003	60 (54-69)	0.566
Gender, Male, N (%)	12 (54)	19 (95)	-	7 (63)	-
Alkaline Phosphatase (U/L)	83 (64-93)	84.5 (62-108.5)	0.6233	116 (103.5-138.5)	0.0007
ALT (U/L)	19 (14-22.5)	75.5 (52.5-112)	<0.0001	35 (27.5-54)	0.0006
AST (U/L)	21 (17-24.5)	51.5 (43-71)	<0.0001	35 (28-49.5)	0.0007
GGT (U/L)	24.5 (14.5-38.5)	83 (43.5-214)	0.0001	68 (47-102.5)	0.0019
Serum Albumin (g/L)	46 (42.05-47.2)	46.8 (41.9-48.3)	0.34	-	-
Total protein (g/L)	70 (67-73)	73.7 (71-77.1)	0.0179	-	-
Total bilirubin (umol/L)	6.05 (5-9.5)	6 (5.5-17.05)	0.0697	13.5 (7.65-20.2)	0.0096
HDL (mmol/L)	1.35 (1-1.55)	1.05 (0.76-1.42)	0.0487	-	-
LDL (mmol/L)	3.3 (2.6-3.7)	3.4 (2.6-4.15)	0.5472	-	-
Cholesterol (mmol/L)	5.35 (4.45-6.1)	5.65 (4.6-6.15)	0.571	4.6 (4-5.2)	0.2765
Triglycerides (mmol/L)	1.7 (1.2-2.1)	1.55 (1.1-2.15)	0.7433	1.2 (1-1.45)	0.0708
Creatinin (umol/L)	69 (62-86.5)	82.5 (70.5-91)	0.0394	70 (58.5-82)	0.7343
Glucose (mmol/L)	4.9 (4.45-5.65)	4.9 (4.45-5.65)	0.2622	5.5 (4.75-6.7)	0.0013
Platelet counts (x10 <sup>9</sup> )	198.5 (167-233)	207.5 (167.5-260)	0.7818	125 (99-221.5)	0.0431
CD4 cell counts (cell/mm <sup>3</sup> )	533 (353.5-970.5)	462.5 (378-684)	0.4203	249 (179.5-283.5)	0.0009
Undetectable HIV-1 viral load, N (%)	17 (77.3)	16 (80)	-	9 (81.8)	-
Patients on ART	19 (86.4)	18 (90)	-	11 (100)	-

<sup>1</sup>Mann Whitney test, <sup>2</sup>Median, IQR, <sup>3</sup>Focal nodular hyperplasia



**Figure 4.** Expression profile of dysregulated circulating miRNAs. **A)** Patients with HIV-1 mono-infections with normal levels of ALT (n=22, green) compared to healthy control donors (n=21, purple). Seven miRNAs were up-regulated and 1 was down-regulated. **B)** Patients with HIV-1 mono-infections with focal nodular hyperplasia (FNH; n=11, orange) compared to healthy control donors (n=21, purple). Twenty-nine miRNAs were up-regulated and 6 were down-regulated.

MiR-122-3p and miR-193b-5p significantly correlated with aminotransferases and liver fibrosis levels (Figure 5).



**Figure 5.** MiR-122-3p and miR-193b-5p plasma levels and liver injury. Correlation of miRNA expression levels with ALT, AST and liver fibrosis levels

## CONCLUSIONS

- Our results reveal that HIV-1 infection impacts liver miRNA metabolism, and up-regulated plasma levels of miRNAs that were previously associated with liver damage, even in the absence of an HCV co-infection.
- Two of the identified miRNAs (miR-99a-5p and miR-100-5p) are significantly associated with liver fibrosis progression in patients with HIV-1/HCV co-infections, even before liver fibrosis is detectable.
- MiR-122-3p and miR-193b-5p are significantly associated with elevated ALT levels and focal nodular hyperplasia in HIV-1 mono-infected patients.
- This study demonstrates the potential of miRNAs as biomarkers of liver injury progression in patients with HIV-1 infections.

## REFERENCES

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2. Anadol et al. Circulating microRNAs as a marker for liver injury in human immunodeficiency virus patients. *Hepatology* 2015;61:46-55.

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