The Breast Milk's *Hsa-miR-195-5p* is a Potential Biomarker for the Protection against Mother-to-Child Transmission of HIV-1

Linda Mouafo Mekue[1-3], Céline Nguefeu Nkenfou[2,4*], Lorena Zentilin[5], Jules-roger Kuiaté[1], Mauro Giacca[5] and Alexis Ndjolo[2,6]

[1]Faculty of Science, University of Dschang, P.O. Box-56 Dschang, Cameroon

[2]Chantal BIYA International Reference Centre, P.O. Box-3077, Yaounde, Cameroon

[3]Laboratory of Animal Physiology and Health, Institute of Agriculture Research for Development (IRAD), P.O Box-2123 Bambui, Cameroon

[4]Higher Teacher Training College, University of Yaounde I, P.O. Box-47, Yaounde, Cameroon

[5]International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano 99, 34149 Trieste, Italy

[6]Faculty of Medicine and Biomedical Science, University of Yaounde I, P.O. Box-1364, Yaounde, Cameroon

*Corresponding author: Céline Nguefeu Nkenfou, Assistant Professor, Higher Teacher Training College

University of Yaounde, Cameroon, Tel: 675573519; E-mail: nkenfou@yahoo.com

Abstract

Background: Some factors have been described to impact mother-to-child transmission of HIV. It is known that it is a combination of factors that can explain transmission or protection. Other factors such as miRNA can be used as a marker for understanding and predicting the pattern of many diseases. The aim of this study was to look at host or viral miRNA that can be used as a marker to predict the transmission or prevention of the HIV-1 from mother-to-child.

Methods and findings: The breast milk and plasma samples of 13 transmitters, 56 non-transmitters and 15 HIV non-infected mothers were collected. The differential expression levels of seven human miRNAs (*Hsa-miR-29a-3p, Hsa-miR-29b, Hsa-miR-28-3p, Hsa-miR-125a-5p, Hsa-miR-149-3p, Hsa-miR-195-5p, Hsa-miR-191-5p*) and one viral miRNA (*HIV-miR-N367*) were analyzed by miRNA real-time PCR. Breast milk's *Hsa-miR-195-5p* (p=0.009) and *Hsa-miR-191-5p* (p=0.003) were upregulated in the non-transmitter mothers. Plasma Hsa-miR-28-3p (p=0.04) was upregulated in non-transmitter mothers as well. The *Hsa-miR-195-5p* negatively correlate with the CD4+ count lower than 300 (r=-0.91, p=0.005) and was identified as a miRNA having the potential to distinguish between transmitter and non-transmitter mothers.

Conclusion: This study has shown for the first time the implication of breast milk's *Hsa-miR-195-5p* in protecting newborns from acquiring HIV infection. This miRNAs will be further evaluated in a larger population of transmitters and non-transmitters in order to set a cutoff for clinical exploitation.

Introduction

Mother-to-child transmission (MTCT) of human immune-deficiency virus (HIV) remains a critical problem in some sub Saharan countries. The introduction of the "Option B+" in the prevention of Mother-to-child transmission of HIV infection (long-life tri-therapy administered as soon as the pregnant women have been diagnosed as infected and nevirapine (NVP) or azidothymidine (AZT) to the baby right after birth until 6 weeks of age), MTCT rates have reduced considerably [1-3]. However in the natural history of MTCT, more than half of the children exposed to the virus do not become infected. Then the question remains why some children are susceptible to HIV-1 infection while others are not? Why are some children infected despite effective tri-therapy administration? Therefore, identifying factors responsible for this observation would play a significant role in reducing MTCT of HIV.

MicroRNAs (miRNAs) are small non-coding RNAs and important post-transcriptional regulators of gene expression. They regulate gene expression by imperfect base pairing to complementary sites (miRNA binding sites) in the corresponding target genes. The main mechanism of action in silencing the genes results in translational repression and mRNA cleavage or alteration of its stability [4]. Since the silencing mechanism does not involve perfect complementary base-pairing, multiple genes can be targeted for repression by a single miRNA Shalgi [5]. MiRNA can be cellular (found inside the cells) or circulating (found in different fluids such as serum, plasma, urine, breast milk or saliva). These circulating miRNA are important in cell-to-cell communication [6]. Studies have demonstrated that miRNAs participate in the host immune responses to viral infections, including HIV [7-9]. As an overall mechanism, miRNAs act by interfering with HIV viral replication. They can directly bind to the viral RNA or target the cellular factors that support survival of HIV virus [7,10,11]. For example, these HIV restriction miRNAs can target the 3'-UTR of HIV transcripts, potentially rendering productive infection of HIV into latency in resting CD4+ T lymphocytes [7]. It has been reported that monocytes from peripheral blood are enriched with HIV restriction miRNA, which contribute to the resistance of monocytes to HIV infection. They are down regulated upon monocyte differentiation into macrophages [9]. Several cellular miRNAs (miR-28, 29a, 125b, 150, 198, 223, and 382) have been identified to target a set of accessory genes of HIV [9,12,13].

MiRNA impact in MTCT of HIV infection has not yet been extensively studied to the best of our knowledge. A recent review speculated on the implication of the viral miRNAs in MTCT, based on the findings of miRNA implication in HIV-1 infection, hence there was no conclusive implication [14]. Another study did not find any effect of miRNA in microparticles from the placenta and the HIV infection [15].

In this study, we aimed to analyze the miRNA expression profile of seven host miRNAs and one viral miRNA (*Hsa-miR-29a-3p, Hsa-miR-29b, Hsa-miR-28-3p, Hsa-miR-125a-5p, Hsa-miR-149-3p, Hsa-miR-195-5p, Hsa-miR-191-5p* and *HIV-miR-N367*), chosen based on published potential associations with HIV-infection, or their impact in other diseases. This work was done in order to identify potential miRNA biomarker, impacting the vertical transmission of the HIV-1.

Methods

Patients

Breast milk and plasma from a total of 84 breastfeeding mothers aged 17-42 years were collected in three hospitals (Chantal BIYA Mother and child centre, Efoulan District Hospital, Cité Verte District Hospital) from the city of Yaoundé-Cameroon between January 2013 and December 2015. Written informed consent was obtained from each participant before obtaining the samples. Breast milk was collected by self-pumping. The plasma samples were obtained from 64 out of 84 mothers having agreed to give their breast milk. The study population was made of HIV infected and non-infected mothers. The HIV infected mothers were subdivided into transmitters and non-transmitters. These samples were kept at -80°C until testing.

Total RNA extraction and quality control

Total RNA from each breast milk and plasma sample was isolated with TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, USA) and purified with miRNeasy Mini kit (Qiagen, Inc., Valencia, USA), following standard protocols provided with the kits by manufacturer. RNA quality and quantity were assessed using a NanoDrop spectrophotometer at a wavelength of 280 nm (ND 1000; Thermo Fisher Scientific, Inc., Wilmington, USA). RNA integrity was determined by gel electrophoresis (1% agarose).

CD4 count and Viral load testing

The techniques used for the analysis of these parameters in the plasma samples of different mothers were already described previously by Mouafo [16].

Reverse transcription quantitative polymerase chain reaction (RT qPCR)

To obtain cDNA, the poly A tails were added to the miRNAs and later on reverse transcribed with miRCURY LNATM RT kit (Qiagen Sciences, Maryland, USA) as per the standard protocol provided by the manufacturer. For this purpose, 2 µl of RNA (5 ng/µl) were mixed with 1 µl of enzymes mix (poly (A) polymerase and reverse transcriptase), 2 µl of 5x reaction buffer and 5 µl of nuclease free water (NFW). The above reaction mix was incubated at 42°C for 60 min, then the reverse transcriptase was inactivated at 95°C for 5 min and the mix was immediately cooled at 4°C. The expression profile of miRNA was determined using a miRCURY LNA SYBR Green PCR Kit (Qiagen Sciences, Maryland, USA) using instructions provided by manufacturer. For this, 4 µl of the diluted cDNA (1/80 dilution in NFW) were mixed with 5 µl PCR Master Mix and 1 µl PCR primer mix. The forward and reverse primers were designed by Qiagen and registered under the catalog number: YP00204306, YP00204698, YP00204679, YP00204339, YP00204119, YP00204093, YP00205869 and YP00205769, for *Hsa-miR-191-5p, Hsa-miR-29a-3p, Hsa-miR-29b-3p, Hsa-miR-125a-5p, Hsa-miR-28-3p, Hsa-miR-149-3p, Hsa-miR-195-5p* and *HIV-miR-N367* respectively. The thermocycling conditions were as follows: 95°C for 10 min; 45 cycles of 95°C for 10 sec and 60°C for 60 sec. The experiments were performed with SYBR Green dye (Qiagen Sciences, Maryland, USA).

Ethical considerations

The National Ethic Committee reviewed the proposal for ethical consideration and approval was given under N °2013/11/375/L/CNERH/SP. The approval to execute the project was obtained from the Division for Health Operations

Research, N°D30-63/L/MINSANTE/SG/DROS/CRC/CEA2/DTLC. All participants have signed a consent form.

Statistical analysis

The 5S RNA gene was used as a reference gene. Samples were analysed in duplicate and the melting curve analysis was used to determine the quality of the qPCR results. Expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method [17] and data were presented as mean ± standard error of the mean. $p<0.05$ was considered to indicate a statistically significant difference.

Results

Demographic characteristics

The HIV infected mothers group was made of 13 transmitters and 56 non transmitters. A control group of 15 healthy mothers (HIV non-infected) was also analysed. The characteristic features of the mothers are summarized in **Table 1**. **Table 1** shows CD4 count and viral load for 64 mothers whose plasma samples were available. We defined transmitters as those mothers who transmitted HIV infection to their babies, and non-transmitters as those who did not.
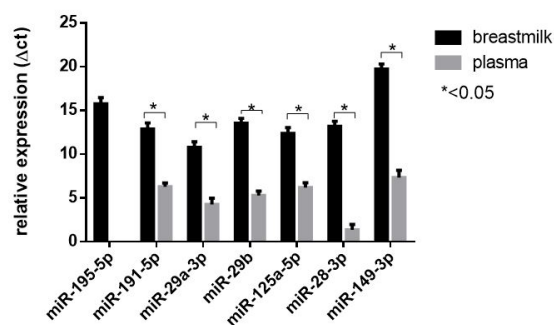
Expression profile of miRNA in the breast milk and the plasma

Expression profiles of eight miRNAs were compared in the two body fluids collected. One miRNA, the *HIV-miR-N367* was expressed neither in the breast milk, nor in the plasma. The *Hsa-miR-195-5p* was expressed only in the breast milk and not in the plasma. For the other six miRNAs, the relative expression of each was significantly higher in the plasma than in the breast milk. Data are presented in **Figure 1** as the mean ± standard error of the mean (SEM).

Table 1 Demographic and clinical characteristics of the mothers.

Variables		Age (Years) ± SD	CD4 in cells/ml (range)	Viral load in Log10 RNA copies/ml (range)
HIV infected	T[a] (n=13)	26,17± 6.6	382 (231-999)	4.4 (ND[d]-5.26)
	NT[b] (n=56)	28.5± 5.6	519.5 (135-1004)	3.77 (ND-6.39)
HIV non-infected	C[c] (n=15)	29.5± 7.7		
Continuous variables are presented as (Mean ± SD) and [median (range)]. [a]Transmitter mothers, [b]Non-transmitter mothers, [c]Control group, [d]Not Detected.				

Figure 1 Relative expression of miRNAs in the breast-milk compared to the plasma samples of 64 participants. The miR-195-5p was completely absent in the plasma. The expression levels (mean ± SEM) normalized to RNA 5S are shown. The asterisks denote statistically significant differences ($p<0.05$) (Student's T test).

Expression of miRNA in different groups and implication in MTCT

The expression levels of the various miRNAs studied were normalized using the expression level of 5S RNA, a widely used endogenous reference for the measurement of miRNAs. The breast milk's expression levels of *Hsa-miR-195-5p* and *Hsa-miR-191-5p* (**Figure 2a**) were significantly upregulated in the non-transmitter group compared to HIV non-infected group ($p<0.005$), while in the transmitter group the same miRNAs were down-regulated. Plasma *Hsa-miR-28-3p* level was significantly up-regulated in the non-transmitter and transmitter groups compared to HIV non-infected group ($p<0.05$) (**Figure 2b**). These significant differences were also observed when comparing the expression levels of *Hsa-miR-195-5p*, *Hsa-miR-191-5p* and *Hsa-miR-28-3p* between the transmitters and non-transmitters.

## Correlation between breast milk and plasma miRNAs to CD4 cell counts and viral loads

In order to evaluate the potential of miRNAs as a surrogate marker for monitoring HIV/AIDS infection, the correlation between miRNA expression levels and existing markers such as CD4+ T-cell counts or viral loads was determined. There was no statistical correlation found between the expression (fold-change relative to HIV non-infected mothers) and the CD4 count across transmitters and non-transmitters. But when CD4 values were subdivided into low (CD4<300 cell/μL), medium (300<CD4 <500 cell/μL) and high (CD4 > 500 cell/μL), breast milk's *Hsa-miR-195-5p* negatively correlated with CD4+ cell counts across samples belonging to the group of mothers having a lower CD4+ than 300, with a Pearson correlation of -0.91 and $p=0.005$ (**Figure 3a**).
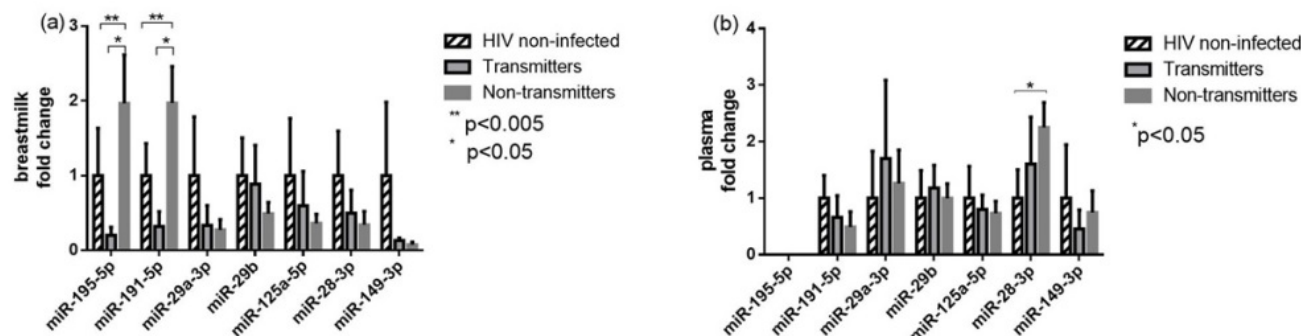
Figure 2 Differential expression of the indicated miRNAs in the breastmilk (**a**) and plasma (**b**) of HIV non-infected (15 samples) and HIV infected mothers (69 samples) (transmitters and non-transmitters). The values in the bar graphs are given as mean ± SEM, and asterisks denote statistically significant differences for the indicated groups (Student's T test).

This infers that in case of pronounced depletion of CD4, the higher expression of *miR195-5p* protect children from been infected. Breast milk's *Hsa-miR-191-5p* negatively correlated with the CD4+ cell counts in the group of mothers having a higher than 500 CD4+, with a Pearson correlation of -0.52 and p=0.01 (**Figure 3b**).
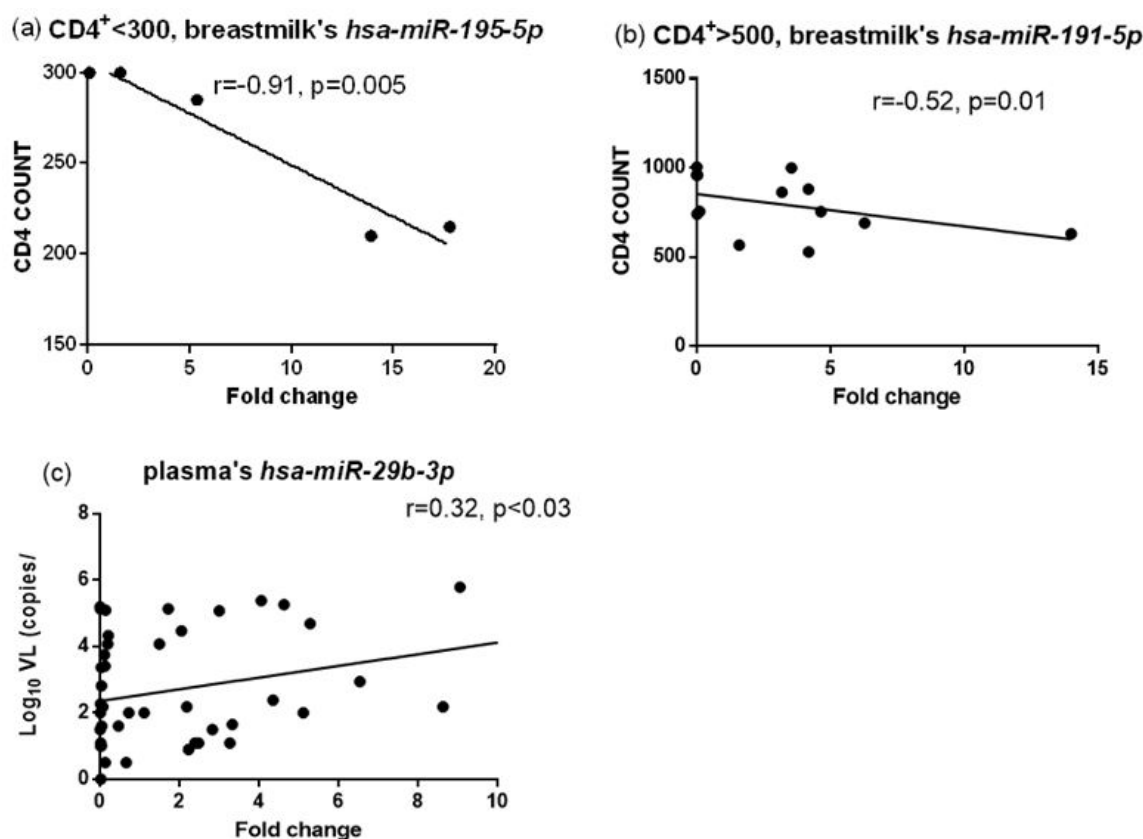
**Figure 3** Correlation of miRNA levels with CD4 counts and viral load. The negative correlations are shown for (**a**) breastmilk's Hsa-miR-195-5p with absolute CD4+ T-cell counts <300 (r =-0.91; p=0.005) and (**b**) the breastmilk's Hsa-miR-191-5p with absolute CD4+ T-cell counts >500 (r =-0.52; p=0.01). A positive significant correlation was noted between plasma's Hsa-miR-29b-3p and HIV viral loads (r =0.32; p<0.03).

In this case, expression level of *miR-191-5p* in case of immune-competency may complete the action of strong immunity to reducing transmission. But these two miRNAs did not correlate with the viral load. Rather, the plasma's *Hsa-miR-29b-3p* positively correlated with the viral load in all the samples with a Pearson correlation of 0.32 and p<0.03 (**Figure 3c**).

Accuracy of miRNA as differential biomarkers

The use of Hsa-miR as a biomarker would depend on their capacity to differentiate transmitters from non-transmitters using the Receiver Operating Characteristic (ROC) curve analysis. The expression value of each miRNA in each sample was calculated by dividing the Ct value of the target miRNA by the Ct-value of the reference gene [18]. Breast milk's *Hsa-miR-195-5p* was the only miRNA capable in differentiating transmitters from non-transmitters with an area-under-the-curve (AUC) value of 0.708 (Standard Error = 0.102, p<0.04) (**Figure 4**). The diagnostic accuracies of other miRNAs were found to be poor and not statistically significant (Data not shown).
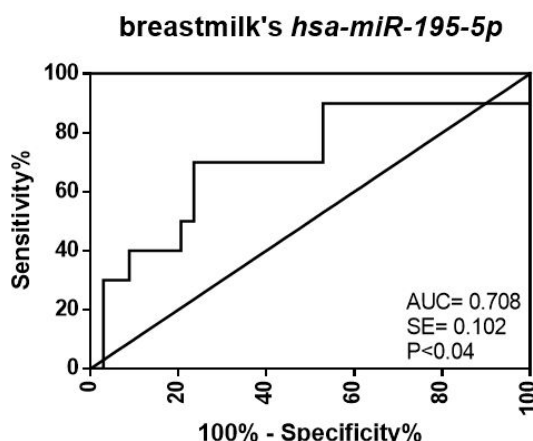
Figure 4 Receiver Operating Characteristic (ROC) curve analysis of breast milk's *Hsa-miR-195-5p* as a distinguisher between transmitters and non-transmitters.

Discussion

The aim of this study was to evaluate the implication of eight miRNAs (*Hsa-miR-29a-3p, Hsa-miR-29b, Hsa-miR-28-3p, Hsa-miR-125a-5p, Hsa-miR-149-3p, Hsa-miR-195-5p, Hsa-miR-191-5p* and *HIV-miR-N367*) in MTCT of HIV-1 infection. These miRNAs were chosen because of their implication in HIV or their implication in several other diseases. Here, we report that breast milk's *Hsa-miR-195-5p*, *Hsa-miR-191-5p* and plasma's *Hsa-miR-28-3p* were upregulated in the non-transmitters; breast milk's *Hsa-miR-195-5p*, *Hsa-miR-191-5p* and plasma's Hsa-miR-28-3p negatively correlate with CD4+ count and breast milk's *Hsa-miR-195-5p* was the only miRNA able to distinguish transmitters from non-transmitters based on ROC. The results presented in this report showed that

breast milk's *miRNA -Hsa-miR-195-5p-* acts as a new biomarker impacting MTCT of HIV infection. This biomarker was shown to be highly expressed in non-transmitter, indicating its potential role as a protecting factor against MTCT of HIV-1 infection.

Although several groups have investigated the impact of host miRNAs on HIV-1 infection, for example by analyzing their expression in permissive and resistant cells [7,9], only a few have looked at viral miRNAs [14,15]. We analysed one of the HIV miRNA, *HIV-miR-N367* which was found neither to be expressed in breast milk nor in plasma. This miRNA was suggested to suppress both Nef function and HIV-1 virulence through the RNAi pathway [19]. Nef has a positive effect on viral infection and replication by promoting the survival of infected cells through down modulation of the surface molecules such as major histocompatibility complex-I (MHC I) and MHC II [20]. Other authors have also reported the ability of RNA viruses such as hepatitis C virus (HCV) and HIV-1 to encode functional miRNAs [21]. The absence of *HIV-miR-N367* in our samples may be due to the limited amount of this miRNA in body fluid samples, as the average viral load in the plasma was 4log (1000 copies/ml) and by deduction lower in the breast milk. Data so far reported were from cell cultures, with viral load over 6log [20].

The primary goal of our study was to discover miRNA biomarkers involved in MTCT of HIV especially in breast milk. Breastfeeding is one of the major routes of transmission of HIV from mother-to-child, so we compared the expression profiles of some selected miRNA in breast milk and plasma. The miRNAs assessed were highly expressed in plasma compared to breast milk. This has been shown in previous studies too [22]. This observation may be explained by the fact that the plasma contains many more cells than any other body fluid. It was also shown earlier that miRNAs are stably expressed in animal serum/plasma [23].

The breast milk's expression levels of *Hsa-miR-195-5p* and *Hsa-miR-191-5p* (**Figure 2a**) were significantly upregulated in the non-transmitters compared to the transmitters and HIV non-infected group. The cellular miR-195 belongs to the microRNA-15 family cluster shown to have potential roles in the cell cycle and apoptosis by regulating the expression of various proteins such as WEE1, cyclin D1, E2F3 and Bcl-2 [24]. Also, miR-195 is predicted to potentially target the DNA helicase protein DDX3 [25] shown to have an essential role in HIV-1 replication for the efficient export of viral mRNA [26]. This miRNA was also stated to suppress the proliferation, migration, and invasion of oral squamous cell carcinoma by targeting TRIM14 [27]. A prospective study reported that tumor-specific miRNAs such as *miR-195* were detected and significantly altered in the circulation by using prospectively collected samples from 127 women, including 83 patients with breast cancer and 44 healthy age-matched controls [28]. Because *Hsa-miR-195-5p* targets the DDX3 protein essential for HIV-1 replication, its role in preventing vertical transmission of HIV can be understood.

Breast milk's *Hsa-miR-195-5p* negatively correlated with CD4+ cell counts <300 cell/µl), and *Hsa-miR-191-5p* negatively correlated with CD4 >500 cell/µl. It is known that CD4 count

and viral load are monitoring markers for HIV infection. Hence we wanted to evaluate studied miRNA as a surrogate marker that can be correlated with CD4 count and viral load. A recent report provides important insights into miRNA and mRNA dysregulation during HIV infection, and showed the cellular transcriptome to be significantly modulated by HIV-1 through miRNAs [29]. We found that some miRNAs correlated with subgroups of CD4 count population. As expected breast milk's *Hsa-miR-195-5p* and *Hsa-miR-191-5p* negatively correlated with the number of circulating CD4 cells, and these miRNAs are those two which were overexpressed in HIV non-transmitter mothers. This finding may confirm their possible implication in protection against transmission. It is known that the decrease in CD4 cells is a favorable factor for the HIV transmission [30]. So *Hsa-miR-195-5p* comes to play to help immune-suppressed body in controlling HIV infection. The plasma's *Hsa-miR-29b* was the only miRNA positively correlated with the viral load. The implication and protective effect of this miRNA in the pattern of HIV have been widely studied [31,32]. The main mechanism of action is the inhibition of Nef expression and HIV replication [33]. The breast milk *Hsa-miR-195-5p*, *Hsa-miR-191-5p* did not correlate with the viral load. This may be due to differential distribution of viral load measure in the plasma and in the breast milk.

Besides targeting the Nef gene, *Hsa-miR-195-5p* targets many other genes, more than 1769 [34], and few of them are presented in **Table 2**.

Table 2 Few genes target by the Hsa-miRNA-195-5p.

Gene target	Name
CDC25B	Cell division cycle 25B
TRIM72	Tripartite motif containing 72
C6orf57	Chromosome 6 open reading frame 57
ZNF622	Zinc finger protein 622
RAF1	V-raf-1 murine leukemia viral oncogene homolog 1
TNFAIP1	Tumor necrosis factor, alpha-induced protein
UBE4B	Ubiquitination factorE4B
TRIM66	Tripartite motif containing 66

List of few genes target by human miR-195-5p, obtained from Target scan human [34]

Breast milk's *Hsa-miR-195-5p* was identified as a new biomarker impacting MTCT. The distinguishing power accuracy was determined from a ROC analysis. AUC values of 0.70 - 0.90 are considered as medium accuracy and 0.90 - 1.00 as high accuracy [33,34]. With AUC values of 0.708, breast milk's *Hsa-miR-195-5p* appears to distinguish transmitters from non-transmitters with a medium accuracy. This function may be further confirmed as it was found that this miRNA was over-expressed in the HIV non-transmitter mothers.

Conclusion

This study has shown for the first time the implication of breast milk's *Hsa-miR-195-5p* in protecting the newborn from acquiring HIV. In future studies, this miRNA will be further evaluated in a larger population of transmitter and non-transmitter in order to set a cutoff for clinical exploitation. Also miRNA array will be performed in order to identify novel miRNAs as biomarker.

References

1. Karnon J, Orji N (2016) Option B+ for the prevention of mother-to-child transmission of HIV infection in developing countries: a review of published cost-effectiveness analyses. Health Policy Plan 31: 1133-1141.

2. Tweya H, Keiser O, Haas AD, Tenthani L, Phiri S, et al. (2016) Comparative cost-effectiveness of Option B+ for prevention of mother to child transmission of HIV in Malawi: Mathematical modelling study. AIDS Lond Engl 30: 953-962.

3. Van Deusen A, Paintsil E, Agyarko-Poku T, Long EF (2015) Cost effectiveness of option B plus for prevention of mother-to-child transmission of HIV in resource-limited countries: evidence from Kumasi, Ghana. BMC Infect Dis 15: 130.

4. Pillai RS (2005) MicroRNA function: Multiple mechanisms for a tiny RNA? RNA. 11: 1753-1761.

5. Shalgi R, Lieber D, Oren M, Pilpel Y (2007) Global and local architecture of the mammalian microRNA-transcription factor regulatory network. PLoS Comput Biol 3: e131.

6. Swaminathan G, Navas-Martín S, Martín-García J (2014) Interplay between microRNAs, Toll-like receptors, and HIV-1: potential implications in HIV-1 replication and chronic immune activation. Discov Med 18: 15-27.

7. Huang J, Wang F, Argyris E, Chen K, Liang Z, et al. (2007) Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. Nat Med 13: 1241.

8. Kumar A (2011) MicroRNA in HCV infection and liver cancer. Biochim Biophys Acta 1809: 694-699.

9. Wang X, Ye L, Hou W, Zhou Y, Wang YJ, et al. (2009) Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. Blood 113: 671-674.

10. Chen AK, Sengupta P, Waki K, Van Engelenburg SB, Ochiya T, et al. (2014) MicroRNA binding to the HIV-1 Gag protein inhibits Gag assembly and virus production. Proc Natl Acad Sci USA 111: E2676-E2683.

11. Klase Z, Houzet L, Jeang KT (2012) MicroRNAs and HIV-1: Complex Interactions. J Biol Chem 287: 40884-40890.

12. Nathans R, Chu C, Serquina AK, Lu CC, Cao H, et al. (2009) Cellular MicroRNA and P bodies modulate host-HIV-1 interactions. Mol Cell 34: 696-709.

13. Sung TL, Rice AP (2009) miR-198 Inhibits HIV-1 Gene Expression and Replication in Monocytes and Its Mechanism of Action Appears To Involve Repression of Cyclin T1. PLOS Pathog 5: e1000263.

14. Uğur AR, Özdemir M (2018) Might miRNAs Be Related to mother-to-child transmission of HIV-1? A Short Review on Putative Viral miRNAs Encoded by HIV-1. J Pediatr Infect Dis 13: 10-14.

15. Moro L, Bardají A, Macete E, Barrios D, Morales-Prieto DM, et al. (2016) Placental Microparticles and MicroRNAs in Pregnant Women with Plasmodium falciparum or HIV Infection. PLos One 11: e0146361.

16. Mouafo Mekue LC, Nkenfou CN, Ngoufack MN, Kuiate JR, Henri Theze J, et al. (2018) Expression Level of Plasma Soluble Toll like Receptor 2 and 6 in the Mother to Child Transmission of Human Immunodeficiency Virus Type 1. Biochem Mol Biol J 4: 10.

17. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods San Diego Calif 25: 402-408.

18. Munshi SU, Panda H, Holla P, Rewari BB, Jameel S (2014) MicroRNA-150 Is a potential biomarker of HIV/AIDS disease progression and therapy. PLos One 9: e95920.

19. Omoto S, Ito M, Tsutsumi Y, Ichikawa Y, Okuyama H, et al. (2004) HIV-1 nef suppression by virally encoded microRNA. Retrovirol 1: 44.

20. Das SR, Jameel S (2005) Biology of the HIV Nef protein. Indian J Med Res 121: 315-332.

21. Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, et al. (2005) Identification of microRNAs of the Herpes virus family. Nat Methods 2: 269-276.

22. Kosaka N, Izumi H, Sekine K, Ochiya T (2010) microRNA as a new immune-regulatory agent in breast milk. Silence 1: 7.

23. Chen X, Ba Y, Ma L, Cai X, Yin Y, et al. (2008) Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 18: 997-1006.

24. Bueno MJ, Malumbres M (2011) MicroRNAs and the cell cycle. Biochim Biophys Acta BBA- Mol Basis Dis 1812: 592-601.

25. Garcia DM, Baek D, Shin C, Bell GW, Grimson A, et al. (2011) Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsy-6 and other miRNAs. Nat Struct Mol Biol 18: 1139-1146.

26. Yedavalli VSRK, Neuveut C, Chi YH, Kleiman L, Jeang KT (2004) Requirement of DDX3 DEAD box RNA helicase for HIV-1 Rev-RRE export function. Cell 119: 381-392.

27. Wang T, Ren Y, Liu R, Ma J, Shi Y, et al. (2017) miR-195-5p Suppresses the Proliferation, Migration, and Invasion of Oral Squamous Cell Carcinoma by Targeting TRIM14. BioMed Res International.

28. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, et al. (2010) Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 251: 499-505.

29. Duskova K, Nagilla P, Le HS, Iyer P, Thalamuthu A, et al. (2013) MicroRNA regulation and its effects on cellular transcriptome in Human Immunodeficiency Virus-1 (HIV-1) infected individuals with distinct viral load and CD4 cell counts. BMC Infect Dis 13: 250.

30. Thorne C, Newell M, Gray L (2001) HIV-infected pregnant women and vertical transmission in Europe since 1986: European collaborative study. AIDS 15: 761-770.

31. Ahluwalia JK, Khan SZ, Soni K, Rawat P, Gupta A, et al. Human cellular microRNA Hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. Retrovirol 5: 117.

32. Reynoso R, Laufer N, Hackl M, Skalicky S, Monteforte R, et al. (2014) MicroRNAs differentially present in the plasma of HIV elite controllers reduce HIV infection in vitro. Sci Rep 4: 5915.

33. Swets JA (1988) Measuring the accuracy of diagnostic systems. Science 240: 1285-1293.

34. Agarwal V, Bell GW, Nam J, Bartel DP (2015) Predicting effective microRNA target sites in mammalian Mtnas. eLife 4: e05005.