











Short Communication
Human and Medical Genetics

MicroRNA-296-5p is differentially expressed in individuals with and without HIV-1 infection

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Abstract

MicroRNAs are considered as potential biomarkers, agents, or therapeutic targets; few studies have addressed the expression of miRNAs in treatment-naïve patients infected with HIV-1. The aim of this study was to assess plasma relative circulating miRNA expression profiles in treatment-naïve Mexican patients with HIV/AIDS and healthy individuals using a commercial array. A low CD4+ T cell count and high viral load were found in all patients. Decreased relative miRNA-296-5p expression was observed in patients; moreover, this was the only miRNA that showed differences between the two groups. Thus, we measured the absolute expression of miR-296-5p by qPCR, confirming the result with statistically significant differences ($P < 0.05$). There is evidence that miR-296-5p regulates the expression of the *PIN1* gene, which encodes the peptidylprolyl Cis/Trans isomerase NIMA-Interacting-1, that is involved in different stages of the biological cycle of HIV-1, this relationship is corroborated by bioinformatics analysis and ELISA assay was used to measure plasma levels of PIN1. The decreased expression of miR-296-5p found in naïve patients with HIV infection suggests a regulatory activity of this miRNA on virus replication, making it a potential therapeutic agent against HIV. Finally, miR-296-5p could be inhibiting the virus transcription by regulating genes different than *PIN1*.

Keywords: miR-296-5p, HIV-1, naïve, *PIN1*.

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Acquired immunodeficiency syndrome (AIDS) has no cure; however, it can be controlled by antiretroviral therapy (UNAIDS, 2018). The search for new therapies that control the virus led to new research lines, e.g., microRNAs (miRNAs), which are chains of noncoding RNAs of 20–25 nucleotides that regulate gene expression by binding to complementary bases on specific sites of mRNAs, thus inhibiting their translation or degrading them (Allantaz *et al.*, 2012).

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In HIV/AIDS pathogenesis, some cellular miRNAs have an antiviral action during the infection and replication of the virus. The identification of miRNA expression differences between patients with HIV treatment-naïve or healthy conditions will clarify the mechanisms of regulation of viral replication (Swaminathan *et al.*, 2014; Liao *et al.*, 2017). Few studies have addressed miRNA expression in HIV-infected patients in their basal state (without antiretroviral treatment). The aim of this study was to assess plasma relative circulating miRNA expression profiles in treatment-naïve Mexican patients with HIV/AIDS and healthy individuals.

This study was approved by the Ethics and Research Committee of the participating institution (R-2014-1305-11). Written informed consent was obtained from all subjects.

Thirteen male individuals were divided into two groups, as follows. Group 1: 10 treatment-naïve HIV-1-positive patients who were being followed at the Laboratorio de Inmunodeficiencias y Retrovirus Humanos, Centro Médico Nacional de Occidente of the Instituto Mexicano del Seguro Social; and Group 2 (control): three voluntary individuals without HIV-1 infection. Subsequently, for the absolute expression, 10 new volunteers without HIV infection were selected (Group 3).

Because of the small sample size, the standardization criteria were strictly fulfilled by increasing the internal validity through the homogenization of the groups, using strict internal controls, and applying normalization processes.

Individuals with hepatitis B or C, influenza, tuberculosis, diabetes, cardiovascular disease, or cancer were excluded from the study. All participants were male because it has been shown that the hormonal changes that occur in women can modify miRNA expression (Klinge, 2015; Chen *et al.*, 2016; Rao *et al.*, 2016).

Clinical and demographic data were collected for HIV-1-positive patients, CD4⁺ and CD8⁺ cells were quantified by flow cytometry (Cytomics FC500, Beckman Coulter), and viral load was determined using an Arthus® HI Virus QS-RGQ kit on a QIASymphony® SP/AS sample extraction and preparation apparatus, and a Rotor-Gene Q® real-time PCR machine.

Total RNA was isolated from plasma using a miRNeasy Serum/Plasma kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The miRNA isolation efficiency was controlled based on the recovered amount of the *Caenorhabditis elegans* miR-39 added during the extraction. The RNA concentration was evaluated by spectrophotometry using NanoDrop 2000/2000c (Thermo Fisher Scientific, Waltham, MA, USA); the purity of the RNA was obtained based on the A260/A280 ratio. RNA integrity was evaluated by electrophoresis on 1.5% agarose gels with formaldehyde, and RNA samples were stored at -80°C until use.

A miScript II RT kit was used for cDNA synthesis and miScript miRNA PCR Array and miScript SYBR® Green PCR kits (QIAGEN) were used to analyze the relative expression of the 84 miRNAs that were most relevant to pathological conditions and were detectable and differentially expressed in serum, plasma, and other bodily fluids. Quantitative real-time PCR (qPCR) conditions were as per the manufacturer's instructions and a Rotor-Gene Q® instrument was used to perform this experiment.

The expression of miRNAs was analyzed using the miScript miRNA PCR Array Data Analysis Tool (QIAGEN) (<http://pcrdataanalysis.sabiosciences.com/mirna/arrayanalysis.php>). The relative miRNA expression levels were calculated using the Cq comparative method. Changes in the expression levels of miRNAs were calculated using the $2^{-\Delta\Delta Cq}$ equation (Fold Change). Mann-Whitney U-test (two-tailed *P* values) was used to examine the differential expression of miRNAs between groups. Significance was set at *P* < 0.05. Expression data were presented as means ± standard error of the mean (SEM).

The expression levels were normalized using the stable miRNAs identified in the array (miR-200b-3p, miR-92a-3p, miR-193a-5p, and miR-103a-3p). The analysis was performed by combining the GenEx version 6 (<http://www.biomcc.com/genex-software.html>) and RefFinder (<http://leonxie.esy.es/RefFinder/>) algorithms which evaluate the relative expression to identify the best internal references (Vandesompele *et al.*, 2002; Marabita *et al.*, 2016).

The clinical and demographic data were as follows. Group 1: age, 30.7 ± 8.4 years; CD4⁺ Cells, 181.4 ± 157.3 and CD8⁺, 947.63 ± 690.8 cells in blood (numbers of cells per microliter); viral load, 1,703,873 ± 3,330,887 copies of HIV per milliliter; Group 2: age, 36.3 ± 9.3 years. The time elapsed since diagnosis was 0–5 months in nine patients and 8 years in one patient. Four of ten patients in Group 1 were classified as stage 2 and six of them as stage 3, according to the Centers for Disease Control and Prevention guidelines (CDC, 2018).

Of the 84 miRNAs evaluated in the array, only miR-296-5p was significantly underexpressed in group 1 (0.093 ± 0.033; *P* = 0.0225) compared with the group 2 (control group healthy) (1.000 ± 0.298) (Figure 1). Therefore, the absolute expression of miR-296-5p was analyzed by qPCR in all individuals in group 1 and 3. The assays were performed in duplicate for each sample using a standard curve, in which a serial dilution of enriched synthetic miR-296-5p (10⁹–10³ copies) was performed. Group 1 exhibited 2.28 × 10¹⁰ ± 3.16 × 10¹⁰ copies of miR-296-5p, and the Group 3 had 2.62 × 10¹¹ ± 1.34 × 10¹¹ copies of miR-296-5p (*P* < 0.05).

For the identification of the miR-296-5p target genes, the public database miRTarBase was accessed because it gathers functional studies of miRNA – target interactions, which are validated experimentally (<http://miRTarBase.cuhk.edu.cn/>) (Chou *et al.*, 2018; Huang *et al.*, 2020); at least 10 genes showed strong evidence of interaction with miR-296-5p, of which peptidylprolyl cis / trans isomerase, the gene that interacts with NIMA 1 (*PINI*), is the only one involved in three different points of the biological cycle of the virus (disassembly or denaturation of the viral capsid, the reverse transcription of the viral RNA and the integration of the HIV-1 cDNA into the host genome). The program miRanda-mirSVR

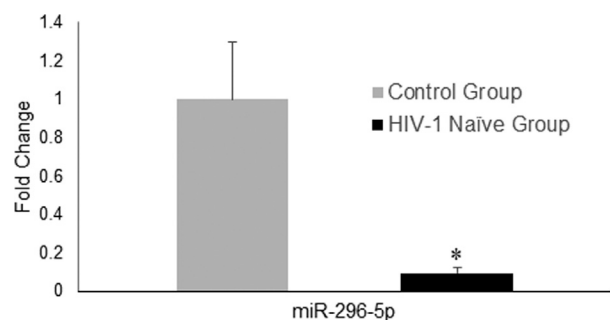


Figure 1 - MicroRNA-296-5p relative expression in plasma from healthy individuals and treatment-naïve HIV-1-positive patients. The expression was normalized with miR-200b-3p, miR-92a-3p, miR-193a-5p, and miR-103a-3p. * Indicates statistically significant differences (*P* < 0.05) by Mann-Whitney U-test.

Conflict of Interest

The authors declare this article content has no conflict of interest.

Authors Contributions

BMTM and MCMM designed the study, managed and conducted the experience, analyzed the data and read the manuscript, JCB contributed and validated experiments, performed bioinformatic analyzed, prepared and analyzed the data, wrote and edited the manuscript, JMP contributed in experiments and participated in the data conversion, MED GVGE supervised the planning and execution of the research, participated in the validation of the data, EVV and AMPR involved in sample collect, collected for all clinical data and classifying patients. All authors read and approved the final version.

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