Molecular Signatures of Cervical Cancer Risk in the Context of HIV Infection: Potential biomarkers of immune activation

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Research Article

Keywords: Inflammation, miRNA, cervical cells

Posted Date: October 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3442157/v1

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Abstract

The disparity in cervical cancer incidence and mortality between high-income and low-income countries, exacerbated by the co-occurrence of HIV infection and cervical cancer, presents a complex and distinctive healthcare challenge. Addressing this challenge necessitates a comprehensive investigation into intricate molecular markers for predicting heightened cancer risk. This study assessed the cellular levels of cervical cancer-related specific oncomirs (miR-21, miR-146a, miR-155, miR-182, and miR-200c) and tumour suppressors (miR-let-7b, miR-125b, miR-143, miR-145, and p53) among women living with HIV (HIV+) and those without HIV (HIV-).

Methods: This case-control study was conducted from May 2017 to April 2019 in Abeokuta, Nigeria, and involved two groups: HIV+ (n = 103) and HIV- women (n = 70).

Results: The study revealed significantly higher levels of miR-155 and p53 in HIV+ women compared to their HIV- counterparts (p = 0.046 and 0.033, respectively). Conversely, significantly lower levels of miR-182, miR-200c, and miR-125b were observed in HIV+ women compared to their HIV- counterparts (p= 0.035, 0.045 and 0.004, respectively). Notably, a significant positive correlation was observed between miR-155 and miR-145 in both HIV+ and HIV- women (p < 0.05). Among HIV- women, direct relationships were also observed between miR-155 and miR-125b (p= 0.004), miR-200c and miR-125b (p= 0.033), and miR-200c and p53 (p= 0.003).

Conclusion: This study indicates that HIV upregulates p53 and miR-155, and downregulates miR-125b, miR-182, and miR-200c. This suggests that the upregulation of the tri-miRNA and downregulation of miR-155 through targeted therapy could mitigate HIV-associated immune activation thereby forestalling cervical cancer development.

Introduction

Cervical cancer is the third most common cancer among women [1]. It remains a persistent global health burden, with over 600,000 new cases diagnosed each year and over 340,000 new deaths [2]. Although cervical cancer rates have decreased in many regions due to widespread screening and highly effective HPV vaccines, certain populations, particularly women with HIV, continue to be disproportionately affected. This is especially true in low- and middle-income countries with inadequate access to comprehensive healthcare services [2]. HIV has a profound impact on the risk and progression of cervical cancer. HIV compromises the immune system, reducing the body's ability to control and clear HPV and other oncogenic infections effectively [3]. The stark disparity and intersection of HIV infection and cervical cancer presents a unique and complex healthcare challenge, demanding an in-depth exploration of intricate molecular signatures and innovative approaches to predict, prevent, and mitigate their elevated risk and manage this malignancy. MicroRNAs (miRNAs) are small RNA that down-regulate gene expression by binding to target mRNA. Tumor suppressor miRNAs block uncontrolled cell growth by targeting oncogenes or genes involved in cell cycle regulation, apoptosis, and DNA repair. Conversely,
oncogenic miRNAs may promote tumour progression by targeting tumour suppressor genes [4]. MiRNAs play a crucial role in cervical cancer by regulating gene expression. They can either promote or suppress tumour growth depending on their target genes. Some miRNAs encourage viral replication, while others inhibit interferon production [5]. This study delves into the compelling realm of miRNA expression in cervical cells of HIV+ women, elucidating its profound implications for predicting cervical cancer risk, personalising patient care, and advancing our understanding of the complex interplay between HIV infection, miRNA dysregulation, and cervical carcinogenesis.

**Materials and Methods**

This case-control study was carried out between May (2017) and April (2019). It included HIV uninfected women (HIV−; n = 70) and women living with HIV (HIV+; n = 103) living in Abeokuta, Nigeria.

**Sample Collection, Handling, and Assays**

Participants were screened for cervical cancer using Liquid-based Prep. Cervical scrapings were collected at the Family Planning and HIV clinics at State Hospital Ijaiye, Ogun State. The vials containing the cervical cells were then stored at −20°C in the Department of Physiology, Babcock University, until further analysis. Four mL of peripheral blood was also collected into a plain tube. Following coagulation, two mL was separated into a plain container within 2 h of collection and stored at −20°C in the Department of Chemical Pathology, Babcock University Teaching Hospital, Ogun State. To test for antibodies against HIV1/2, HPV, and EBV ELISA kits (from Qingdao Hightop Biotech Co., Ltd., Qingdao, China, and Calbiotech Inc., El Cajon, CA, USA) were used according to the manufacturer’s instructions. Positive and negative cut-off values for HIV-1/2 antibodies were 1.077 and 1.076, respectively. Individuals with any history of Human papillomavirus and Epstein-Barr virus infection, cancer, especially cervical, breast, and oral cancers were excluded [6].

**RNA Isolation**

The optimized phenol-chloroform method was used for RNA extraction at the Centre for Biocomputing and Drug Development, Adekunle Ajasin University. The Thin Prep. was spun and 50 µL of cell deposit from each sample was added to Eppendorf tubes containing 50 µL Trizol reagent. The mixture was incubated for 5 minutes at room temperature and subsequently spun at 11000 rpm and 4°C for 15 minutes. The supernatants were aspirated into newly labelled tubes. Chloroform (100 µL) was added to the new tubes containing the supernatants. The mixture was vortexed for 15 seconds, incubated at room temperature for 2 minutes, and spun at 11000 rpm and 4°C for 15 minutes. The top clear layer of supernatant was transferred into a new Eppendorf tube. A 100 µL of isopropanol was added to the clear solution. The mixture was again vortexed for 4 seconds, incubated for 15 minutes, and spun for 15 minutes at 11000 rpm and 4°C. The supernatant was removed leaving the RNA pellet. The RNA pellet was washed twice using 200 µL of 70% ethanol for 10 minutes at 11000 rpm and 4°C. Next, the supernatant was decanted. After washing, all tubes were allowed to air dry. Twenty µL of nuclease-free water was
added to the total RNA to form the RNA solution. The total RNA concentration was quantified using a spectrophotometer at 260 nanometres. Based on the OD260/OD280 calculation, the RNA limit of importance was set at 0.05–1.00. Acceptable RNA quality was set at 1.8–2.2 [6].

**Complementary DNA Synthesis**

To establish homogeneity of RNA concentration (Rc) across all samples, the volume of cDNA needed (Va) was divided by the RNA quantity of each sample (Rq). To determine the dilution volume for each sample (Vd), Rc was subtracted from the sample reaction volume (Vs = 10 µL). Thus, the Rc + Vd = 10 µL. The constituents of reagent reaction volume (Vr = 10 µL) were 1 µL of Deoxynucleotide triphosphates (dNTPs: dGTP, dTTP, dCTP, dATP), 2.75 µL of nuclease-free water, 0.25 µL of Reverse transcriptase, 1 µL of Random hexamers, and 5 µL of 5x dye (buffer). The total reaction volume (Vt) was 20 µL (Vt = Vs + Vr). Using a conventional thermocycler, the reaction mixtures for cDNA synthesis were incubated at 37°C, 55°C, 60°C, and 70°C for 60 minutes and stored at -20°C until polymerase chain reaction.

**Reverse Transcriptase Polymerase Chain Reaction**

The primers for miRNA quantification included: miR-Let-7b forward (5′-GTTTCGGGGGTGA GGTAGTA-3′), miR-16 forward (5′-GTTGTCAGCAGTCCTCGT-3′), miR-21 forward (5′-GTTTTGCGCAGCGCCCTG-3′), miR-125b forward (5′-GTTTTGCGCCTCCTTCAGT-3′), miR-143 forward (5′-TTTTTGCGCGCCCTG-3′), miR-145 forward (5′-GTTTTGCGCTCCTCTTCAGT-3′), miR-182 forward (5′-GTTTTGAGTATCGACGTGTGA-3′), universal reverse primer (5′-GTTTCCCT CGTCTTACCCA-3′), wild type p53 forward (5′-GCTCAAGACTGGCGCTAAAA-3′), wild type p53 reverse (5′-GTGAC TCAGAGAGGACTCAT-3′) and miRNA-USLP (Uni- versal stem-loop primer; 5′-AGTGCAGGGTCCGAGGTATTCGCACCAGACCTAG-3′). To perform amplification, optimisation was used. Each sample was mixed with 2 µL of template (cDNA), 3 µL of nuclease-free water, 0.5 µL of forward primers, 0.5 µL of reverse primers (Inqaba Biotechnical Industries Ltd., Pretoria, South Africa), and 4 µL of master mix (Biolabs, Midrand, South Africa). For each sample, all necessary reagents were added to enable a complete enzymatic reaction, followed by PCR. The amplification process was carried out under the following conditions: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. This cycle was repeated 45 times [6].

**Gel Electrophoresis**

The PCR products were separated by electrophoresis in a 0.5% agarose gel, using a 0.5× TBE buffer with a pH of 8.3 and 0.2 µL ethidium as a fluorescent marker. The resulting fluorescent bands of the circulating genes from HIV + and HIV- women were captured in Fig. 1 and analyzed densitometrically using ImageJ software (1.49 V). To calculate the relative expression of miRNAs and the p53 gene, endogenous normalization was performed using miR-16 for miRNAs and β-actin for the p53 gene [6].
**Results**

Figure 2 illustrates distinct miRNA expression patterns between cervical cell samples from HIV + and HIV- women. Notably, a pronounced gel amplicon band of miR-155 was observed in cervical cell samples from HIV + women, contrasting with a similarly elevated gel amplicon band of miR-182, miR-200c, and miR-125b in cervical cells from HIV- women.

**Correlation between biomarkers**

A significant positive correlation was observed between miR-155 and miR-145 in both HIV + and HIV- women at p < 0.05 (Table 1). Among HIV- women, direct relationships were also observed between miR-21 and miR-182, miR-145 and p53, miR-200c and miR-125b (p < 0.05), miR-125b and miR145, miR-143 and miR-145, miR-155 and miR-125b, miR-155 and miR-143, miR-200c and miR-145, and miR-200c and p53 (p < 0.01), miR-125b and miR-143, and miR-155 vs miR-145 (p < 0.001). Conversely, an insignificant inverse relationship was observed between miR-let-7b and p53 (p > 0.05). Among HIV + women, significant direct relationships were observed between miR-let-7b and p53, miR-21 and miR-125b, miR-146a and p53, miR-155 and miR-145, miR-182 and miR-145 (p < 0.05). A significant inverse relationship was observed between miR-146a and miR-143 (p < 0.05) whereas an insignificant inverse relationship was observed between miR-145 and p53 (p > 0.05).

Figure 1 shows a high gel amplicon band of miR-155 from the cervical cell samples of HIV + women compared to the cervical cells of HIV- women. It also showed a high gel amplicon band of miR-182, miR-200c, and miR-125b from the cervical cell samples of HIV- women compared to HIV + women.

The findings of this study indicate that as the levels of some oncogenes like miR-21 and miR-155 rise, the levels of some tumour suppressors like miR-125b, miR-143, and miR-145 increase as well. This study suggests that miR-let-7b and p53 work synergistically in an anti-inflammatory way during HIV infection whereas in the absence of the latter, the activity of miR-let-7b and miR-145 usually dominates that of the p53 gene. It also suggests that miR-143 downregulates miR-146a during HIV infection.

In Fig. 2, there were higher levels of oncogenes such as miR-21 and miR-155 and tumour suppressors such as miR-let-7b, miR-143, and p53 in the cervical cells of HIV- women compared to HIV + women at p > 0.05, < 0.05, > 0.05, and < 0.05, respectively). Whereas higher levels of oncogenes such as miR-146a, miR-182, and miR-200c and tumour suppressors such as miR-125b and miR-145 (p > 0.05, < 0.05, < 0.05, < 0.05, and > 0.05, respectively).
### Table 1
Correlation between oncomirs and tumour suppressors based on HIV status

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV+ Women</th>
<th>HIV- Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-let-7b vs p53</td>
<td>0.574</td>
<td>-0.356</td>
</tr>
<tr>
<td>miR-21 vs miR-125b</td>
<td>0.583</td>
<td>0.392</td>
</tr>
<tr>
<td>miR-21 vs miR-182</td>
<td>0.036</td>
<td>0.624</td>
</tr>
<tr>
<td>miR-125b vs miR-143</td>
<td>0.315</td>
<td>0.958</td>
</tr>
<tr>
<td>miR-125b vs miR-145</td>
<td>0.280</td>
<td>0.804</td>
</tr>
<tr>
<td>miR-143 vs miR-145</td>
<td>0.386</td>
<td>0.786</td>
</tr>
<tr>
<td>miR-145 vs p53</td>
<td>-0.157</td>
<td>0.646</td>
</tr>
<tr>
<td>miR-146a vs miR-143</td>
<td>-0.530</td>
<td>0.278</td>
</tr>
<tr>
<td>miR-146a vs p53</td>
<td>0.572</td>
<td>0.101</td>
</tr>
<tr>
<td>miR-155 vs miR-125b</td>
<td>0.340</td>
<td>0.734</td>
</tr>
<tr>
<td>miR-155 vs miR-143</td>
<td>0.428</td>
<td>0.746</td>
</tr>
<tr>
<td>miR-155 vs miR-145</td>
<td>0.544</td>
<td>0.830</td>
</tr>
<tr>
<td>miR-182 vs miR-145</td>
<td>0.541</td>
<td>0.250</td>
</tr>
<tr>
<td>miR-200c vs miR-125b</td>
<td>0.111</td>
<td>0.591</td>
</tr>
<tr>
<td>miR-200c vs miR-145</td>
<td>0.277</td>
<td>0.772</td>
</tr>
<tr>
<td>miR-200c vs p53</td>
<td>0.498</td>
<td>0.752</td>
</tr>
</tbody>
</table>

Statistical analysis: Pearson's correlation. Significance a = p < 0.05, b = p < 0.01, c = p < 0.001

### Discussion

The results of this study provide important insights into the differential expression of normally downregulated oncomirs (miR-21, miR-146a, miR-155, miR-182, and miR-200c) and normally upregulated tumour suppressors (miR-let-7b, miR-125b, miR-143, miR-145, and p53 expression) and their potential roles in HIV-related cervical carcinogenesis. The findings have significant implications for understanding the molecular mechanisms underlying cervical cancer risk. Since an earlier study demonstrated that miR-143, miR-145, miR-146a and miR-182 play direct roles in immunological processes [7], this study evaluates the potential utility of these miRNAs as predictive biomarkers for cervical cancer risk assessment and their suitability as therapeutic targets.
The differential expression of miR-21 and miR-155 is particularly intriguing. Both miR-21 and miR-155 are often associated with promoting cell proliferation, inhibiting apoptosis, and enhancing cell survival—hallmark features of cancer. Their higher expression in cervical cells of HIV+ women may indicate vulnerability to cervical cancer development. However, it is essential to consider that the functions of miRNAs can be context-dependent, influenced by various factors including target genes and cellular conditions. The study carried out by Gokavi et al. revealed that the inhibition of miR-155 forestalls HIV release in the infected cervical epithelial cell line (ME-180) through the upregulated TGF-β signaling [8]. Bazie et al. opined that elevated miR-155 in plasma large and small extracellular vesicles promotes immune activation and increases the risk of morbidity and mortality among people living with HIV, irrespective of viral replication or antiretroviral therapy status [9]. An experimental study carried out Li et al. revealed that HIV-infected T-cells secrete exosomal miR-155-5p which in turn promotes the proliferation, migration, and invasion of cervical cancer cells through IL-6 and IL-8 activation [10]. Taken together, this study suggests that the elevated levels of miR-155 in HIV+ women drive malignant epithelial transformation through an HIV-release-associated inflammatory mechanism [11]. The pro-inflammatory effect of the elevated levels of miRNA-155 in HIV+ women appears to be countered by corresponding elevated levels of p53 [12,13].

Higher levels of tumour-suppressive p53 and miR-let-7b in cervical cells were evident in cervical cells from HIV+ women. The elevated expressions of the miRNAs in cervical cells of HIV+ women suggest a potential protective mechanism against cervical carcinogenesis. Apart from being anti-inflammatory, miR-let-7b targets oncogenes and inhibits cell growth [12]. An earlier study revealed that miR-let-7b is significantly associated with poor prognosis and short survival in patients with ovarian and hepatocellular cancers [14]. p53 is a pivotal tumour suppressor involved in DNA repair and cell cycle regulation. Literature shows that p53 inhibits HIV replication by inactivating the Tat protein among infected individuals [15]. Noteworthy, our previous study revealed lower levels of miR-155, miR-let-7b, and p53 in the serum of HIV+ women compared with their HIV-counterparts [6]. The reason for the variation or switch of these miRNAs and p53 gene in serum and cervical cells is unknown. The low level of the biomarkers in the serum of HIV+ women despite the high cellular levels could be associated with post-HIV-release degradation through uridylation or target-contact-related decay [16]. However, further studies are warranted to understand the miRNA and mRNA instability in the context of HIV.

Conversely, the lower expression of miR-182, miR-200c, and miR-125b in cervical cells of HIV+ women raises questions about their roles in HIV-associated immune activation and epithelial transformation or their potential protective roles against cervical cancer among HIV+ women. In a prior investigation, a similar expression pattern of miR-182, miR-200c and miR-125b was observed in the serum of HIV-positive women [6]. In contrast, previous research has reported upregulated miR-182 and downregulated miR-146a in HIV-uninfected patients with sepsis, demonstrating significant lymphopenia and depleted CD4 and CD8 T-cells [7]. This suggests that the downregulation of miR-182 is HIV-dependent. On the other hand, individuals diagnosed with melanomas exhibit reduced levels of miR-182 and miR-125b, which have been linked to shorter survival and an elevated risk of metastasis. This observation suggests that, during immune activation, HIV directly downregulate miR-182 and miR-125b through a yet-to-be-identified
mechanism thereby malignant promoting epithelial transformation and disease progression. MiR-182 and miR-200c have been implicated in inhibiting cell migration and invasion, whereas miR-125b is known to target oncogenes and promote apoptosis by regulating host immunological response against bacterial and viral infections [17]. The higher levels of these miRNAs in HIV- women may suggest a tumour-suppressive effect that could mitigate cervical cancer risk in this group. However, further studies are required to elucidate the specific targets and pathways regulated by these miRNAs.

The small sample size used in this study may constitute a limitation. Further research on a large population is essential to validate these findings and unravel the underlying mechanisms behind miRNA variations. It may pave the way for conducting longitudinal studies to assess how changes in miRNA expression patterns over time correlate with cervical cancer development and disease progression and ultimately contribute to improved strategies for cervical cancer prevention, diagnosis, and treatment, especially in populations at elevated risk.

**Conclusion**

The study highlights the complex and dynamic nature of miRNA expression in cervical cells of HIV+ and HIV- women and their potential impact on cervical cancer risk. This study indicates that HIV upregulates p53 and miR-155, and downregulates miR-125b, miR-182, and miR-200c. This suggests that the upregulation of the tri-miRNA and downregulation of miR-155 through targeted therapy could mitigate HIV-associated immune activation thereby forestalling cervical cancer development.

**Declarations**

**Acknowledgements**

The staff members of the Centre for Biocomputing and Drug Development at Adekunle Ajasin University, as well as the HIV counselling and testing and family planning clinics at State Hospital Abeokuta, provided technical assistance for which the author is deeply grateful.

**Data availability**

Data will be made available by the corresponding author upon reasonable request.

**Ethical Consideration and Informed Consent.**

Ethical approvals were obtained from the State Hospital Abeokuta Ethics Committee (SHA/RES/VOL.2/177) and Babcock University Health Research Committee (BUHREC549/18) for this study. The study was conducted in accordance with the Declaration of Helsinki. As recommended by the ethics committees, informed consent was obtained from all subjects involved in the study. The patients have provided written informed consent to publish this paper.

**Conflict of Interest**
References


Figures

Figure 1

Gel amplicons of some miRNA in cervical cell samples of HIV+ and HIV- women
Figure 2

Level of oncogenes and tumour suppressors among HIV+ and HIV- women