

Presence of Proviral DNA In Peripheral Blood Leukocytes of People With HIV, With Treatment Adherence, Undetectable Viral Load and CD4 Lymphocytes in Normal Values

Karina Angelica Rodríguez Argáez, Karla Rossanet Dzul- Rosado, Henry Noh-Pech, César Lugo-Caballero and Fernando I Puerto*

Laboratorio de Enfermedades Emergentes y Reemergentes, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi". Universidad Autónoma de Yucatán. Mérida, México

***Corresponding author:** Fernando I Puerto, Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Merida, Yucatán, Mexico

ARTICLE INFO

Received: 📅 February 20, 2024

Published: 📅 March 01, 2024

Citation: Karina Angelica Rodríguez Argáez, Karla Rossanet Dzul- Rosado, Henry Noh-Pech, César Lugo-Caballero and Fernando I Puerto. Presence of Proviral DNA In Peripheral Blood Leukocytes of People With HIV, With Treatment Adherence, Undetectable Viral Load and CD4 Lymphocytes in Normal Values. Biomed J Sci & Tech Res 55(2)-2024. BJSTR.MS.ID.008689.

ABSTRACT

Background: With these data from 2021 from Yucatan Mexico has an incidence of 21.7x100000 inhabitants and ranks 5th at the national level of incidence. Based on a previous finding of the presence of proviral DNA in 24% of patients on antiretroviral treatment, with undetectable HIV viral load (> 20 viruses per μ L), TCD4+ >500 x mL, and treatment adherence;

Objectives: We decided to conduct this study in patients who strictly meet the criteria for adherence to treatment and undetectable HIV viral load (> 20 viruses per μ L), TCD4+ >500 x mL. for finding proviral DNA.

Study Design: The following data was obtained from each patient: age, sex, education, marital status and sexual orientation and history, antiretroviral treatment, other sexually transmitted diseases, risky practices, route of HIV transmission, time of HIV infection, last count of CD4 T lymphocytes, and HIV viral load and PCR was carried out on total leukocytes, stained with ethyde bromide

Results: Thirty-nine people patients with HIV under ART or HAART participated. It is worth mentioning that this group of patients has never presented an AIDS picture; shortly after serological diagnosis and confirmation by RT-PCR, the patients began the treatment. The proviral DNA was detected in 15 patients (38.46%).

Conclusions: The HIV proviral DNA indicates the persistence of the virus in patients with treatment and even in controlled patients, with an adequate response to treatment in this study.

Keywords: Proviral DNA; HIV; PCR; Antiretroviral Treatment; Controlled Patients; Mexico

Abbreviations: AIDS: Acquired Immunodeficiency Syndrome; HIV: Human Immunodeficiency Virus; HAART: Highly Active Antiretroviral Therapy; ART: AntiRetroviral Therapy

Background

Acquired Immunodeficiency Syndrome (AIDS) is a set of clinical manifestations leading to the deterioration of the immune system. AIDS is caused by the Human Immunodeficiency Virus (HIV), is a major global public health problem. [1-3]. In Mexico, during 2021, 13,139 became newly infected with HIV (with 328,791 cases accumulated since 1983). In Yucatan, there were 438 new cases, of which 396 were in men and 42 in women. With these data from 2021, Yucatan has a

national incidence of 19.2 x 100,000 inhabitants and ranks 5th at the national level of incidence [4] Although it has not yet been possible to eradicate HIV, antiretroviral therapy (ART) can control the replication of the virus so that people with HIV can lead healthy and productive lives. Since 1996, the use of highly active antiretroviral therapy (HAART) as a combination of antiretroviral therapy dramatically suppresses viral replication and has significantly reduced the morbidity and mortality of patients with HIV [5,6]. Until June 2016, 18.2 million people with HIV/AIDS worldwide received ART. Between 2000 and

2015, new HIV infections decreased by 35% and HIV/AIDS-related deaths by 28%, indicating that approximately 8 million deaths were averted. It is estimated that expanding ART to all people with HIV/AIDS could help to prevent 21 million HIV/AIDS-related deaths and 28 million new infections by 2030 [7]. Plasma HIV RNA viral load and TCD4+ lymphocyte count are currently the conventional tests used to assess HIV patients and their response to treatment.

Objectives

Based on a previous finding of the presence of proviral DNA in 30% of patients on ART. We decided to conduct this study in patients who strictly meet the criteria for adherence to treatment and undetectable HIV viral load (> 20 viruses per µL), TCD4+ >500 x mL.

Study Design

CAPASITS located in the OHorán Hospital at Av. Itzaes x Av. Jacinto Canek in the Colonia Centro, CP 97000 (The Ambulatory Center for the Prevention and Care of AIDS and Sexually Transmitted Infections) serves approximately 6,000 people with HIV, all They have free attention, medications, consultations and psychological therapy. Of these people, 600 comply with a strict adherence to treatment and all their control and health tests are normal or negative, they are called model patients. After the authorization in 2020 of the research protocol by the ethics committee and research committee, 66 people were invited to participate in our study, however, only 39 agreed to participate in this study. The participants found to be eligible signed the informed consent previous explanation by the study counselors. Thirty-nine people with HIV, of legal age, under AntiRetroviral Therapy (ART) or Highly Active AntiRetroviral Therapy (HAART), belonging to a Self-Help Group of People Living with HIV in Mérida, Yucatán, were enrolled. The following data was obtained from each patient: age, sex, education, marital status and sexual orientation and history, antiretroviral treatment, other sexually transmitted diseases, risky practices, route of HIV transmission, time of HIV infection, time of treatment, last count of CD4 T lymphocytes, and HIV viral load [performed during the last 6 months]. A 5 mL sample of peripheral venous blood with anticoagulant was taken. The DNA was purified from total leukocytes and plasma with QIAamp Viral DNA Mini Kit (Qiagen). The RT-PCR was performed with the following primers [8],

- 1) GP40. TCTTAGGAGCAGCAGCGAAGCAACTATGGG
- 2) GP41. AACGACAAAGGTGAGTATCCCTGCCTAA
- 3) GP46. ACAATTATTGTCTGGTATAGTGCACAGCA
- 4) GP47. TTAAACCTATCAAGCCTCCTACTATCATTA

The gels were processed in 2% agarose, stained with ethidium bromide, and observed under ultraviolet light. Molecular weight control and positive and negative control were used.

Results

Thirty-nine people patients with HIV under ART or HAART participated. It is worth mentioning that this group of patients has never presented an AIDS picture; shortly after serological diagnosis and confirmation by RT-PCR, the patients began the treatment. The proviral DNA was detected in 15 patients (38.46%). Ninety-two-point-seven percent of the participants were men, and the mean age was 38 years (SD ±10.8 years). The sociodemographic variables of the sample are presented in Table 1. Regarding the history of HIV infection, patients, including women, reported having acquired HIV through sexual transmission, and the most common risk practices were sexual relations of men with men, unprotected sex, and sex with strangers or with people with HIV (Table 2). In addition to HIV, other sexually transmitted diseases were mentioned by the patients (more than one may coexist), including condylomatosis 17.1%, genital herpes 9.8%, gonorrhea 12.2%, syphilis 17.1%, and chancroid 2.4%.

Table 1: Distribution of sociodemographic variables.

Variable		Results
Age (years)	Average	38
Sex [n, (%)]	Women	3 (7.3%)
	Men	38 (92.7%)
Schooling [n, (%)]	without studies	2 (4.9%)
	Incomplete higo school	0
	Complete high school	4 (9.8%)
	Bachelor incompleta	3 (7.3%)
	Preparatoria completa	10 (24.3%)
	Licenciatura	18 (43.9%)
	Posgrado	2 (4.9%)
Marital status [n, (%)]	Single	26 (63.4%)
	Married	5 (12.2%)
	Free nion	7 (17.1%)
	divorced	0
	widower	3 (7.3%)
Sexual orientation [n, (%)]	Heterosexual	7 (17.1%)
	Homosexual	32 (78.0%)
	Bisexual	2 (4.9%)

Table 2: Risk practices.

Variable		Results
Risk practices	Men-to-men sexual relations	35 (85.4%)
[n, (%)]	sex workers	2 (4.9%)
	Unprotected sex	22 (53.6%)
	Sex with strangers	12 (29.3%)
	Sex with people with HIV	12 (29.3%)

Conclusion

This study worked with a heterogeneous group of men and women of different ages, educational levels, marital status, and sexual orientation. The mean age of the patients was 38 years, which is within the age group (30-39 years) reported with the highest prevalence of HIV infection [9]. Regarding the distribution by sex of the patients in this study, relatively low participation of female patients was observed, with 92.7% males participating, above the 82% reported in the WHO statistics [9]. The vast majority (78%) of the patients in the study reported having a homosexual orientation. One aspect to highlight is the high level of education of the patients in this study since 43.9% reported having a bachelor's degree and 24.3% had completed high school. This phenomenon is observed in people who are part of self-help groups and who organize to demand their rights to universal health. [10] The main finding of this study was the possibility of detecting HIV DNA in leukocytes of patients under HAART treatment in 15 (38.5%) of the 39 participating patients with a normal CD4+ T cell count (≥ 500 cells/ μ l), undetectable viral load, and treatment adherence. We found few studies that try to define the role of proviral DNA, from HIV mutations to excess viral integrase or inadequate treatment; however, we can only know that our patients have treatment adherence [11], which shows us the importance of continuing with these proviral DNA studies. The HIV proviral DNA indicates the

persistence of the virus in patients with treatment and even in controlled patients, with an adequate response to treatment [11].

In this study. Although practically new in our state and little studied in our country, these results do not agree with the obtained in other studies, which this phenomenon is observed in the suppression of treatment or with other serious diseases. [12-14]. Although this study focused on detecting HIV proviral DNA in peripheral blood leukocytes, the possibility of carrying out a detailed analysis of the presence of HIV DNA in different types of blood cells could provide more information about viral reservoirs. Despite the principal cells responsible for harboring HIV proviral DNA are CD4+ T lymphocytes, these cells are not viral reservoirs; in recent years, the participation of other types of cells has been studied such as hematopoietic stem cells, macrophages, lymphoid tissue associated with the intestine, and the central nervous system [13,15-18]. It is important to verify if this proviral DNA is infectious, if it is due to an excess of viral production from the reservoirs, and which patient's unstudied characteristics are associated to proviral DNA and treatment adherence. As a secondary objective, this study analyzed the association between the presence of HIV DNA in leukocytes and different characteristics of the patients; however, no correlation was found with sociodemographic data, or adherence to HAART [15]. Therefore, it is essential to continue with the studies of patients with proviral DNA since we do not know or do not find any answer in the literature (Figure 1).

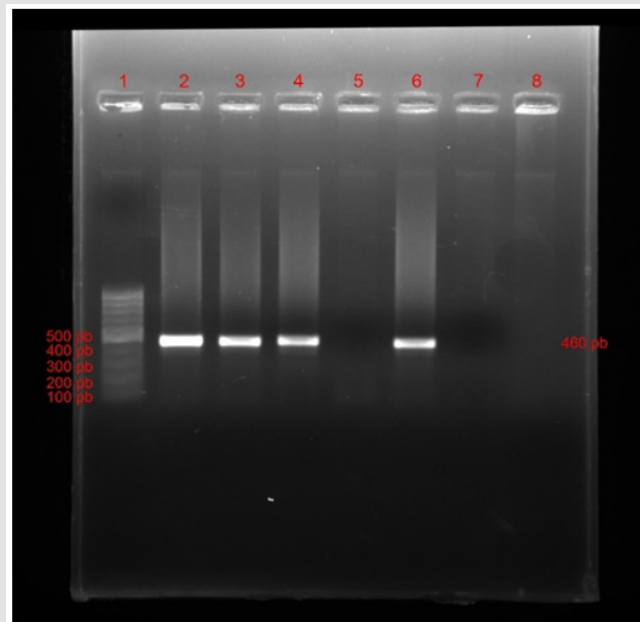


Figure 1: Observe the PCR products, in 2% agarose gel, stained with ethidium bromide, from left to right, in lane 1 is the 100 bp molecular weight marker. In lane 2 the positive control, lane 3, a patient sample with proviral DNA, lane 4, another patient sample with proviral RNA, lane 5, a patient sample without proviral DNA, lane 8 negative test control.

Acknowledgement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

1. FIP: Conceptualization; Formal analysis; original draft; Writing
2. KRDR: Conceptualization; Data curation. Project administration.
3. HNP: Methodology; Supervision; Validation
4. MATC: Conceptualization; Data curation: review & editing.
5. CLC: Conceptualization; Data curation.

References

1. Susan Moir, Tae Wook Chun, Anthony S Fauci (2011) Pathogenic Mechanisms of HIV Disease. *Annu Rev Pathol Mech Dis* 6: 223-248.
2. Bbosaa N, Kaleebua P, Ssemwangaa D (2019) HIV subtype diversity worldwide. *Rev Curr Opin HIV and AIDS* 14(3): 153-160.
3. Nuno RF, Rambaut A, Marc A Suchard MA, Baele Gr, Bedford T, et al. (2014) The early spread and epidemic ignition of HIV-1 in human populations. *Science* 346(6205): 56-61.
4. (2022) Vigilancia Epidemiológica de casos de VIH/SIDA en México (en línea) del 2021 (Consultado 11 marzo 2022). Registro Nacional de Casos de VIH y sida. Disponible.
5. Lozano F, Domingo P (2011) Tratamiento antirretroviral de la infección por el VIH. *Enfermed Infecc Microbiol Clín* 29(6): 455-465.
6. (2023) Visión general de la infección por el VIH. Medicamentos contra el VIH autorizados por la FDA (En línea). Consultado Disponible.
7. (2019) Update of recommendations on first- and second-line antiretroviral regimens HIV treatment. WHO/CDS/HIV/19.15. Policy brief.
8. Yang CH, Gao F, fonjungo PN, Zekeng L, van der Groen G, et al. (2000) Phylogenetic Analysis of Protease and Transmembrane Region of HIV Type 1 Group O. *AIDS Res Hum Retroviruses* 16(11): 1075-1081.
9. (2020) Organización Mundial de la Salud. VIH/SIDA: datos y cifras. [En línea]. 2020. [Consultado el 19 de octubre del 2020] Disponible.
10. Yasel M, Díaz S, Orlando-Narváez, Ballester-Arnal R (2019) Risk behaviors for HIV infection. A review of emerging trends. *Cien Saude Colet* 24(4): 1417-1426.
11. Orozco, Cabestany G (2019) Conversatorio El quehacer de las organizaciones de la sociedad civil en la atención y prevención del VIH-Sida. Retos y perspectivas, Aportes al debate parlamentario.. No. 13, México, Instituto Belisario Domínguez, Senado de la República.
12. Murray JM, Zaunders JJ, McBride KL, Xu Y, Bailey M, et al. (2014) HIV DNA Subspecies Persist in both Activated and Resting Memory CD4+ T Cells during Antiretroviral Therapy. *J Virol* 88(6): 3516-3526.
13. Papasavvas E, Azzoni L, Ross BN, Fair M, Yuan Z, et al. (2021) Intact Human Immunodeficiency Virus (HIV) Reservoir Estimated by the Intact Proviral DNA Assay Correlates With Levels of Total and Integrated DNA in the Blood During Suppressive Antiretroviral Therapy. *Clin Infect Dis* 72(3): 495-498.
14. Huruy K, Mulu A, Liebert UG, Maier M (2018) HIV-1C proviral DNA for detection of drug resistance mutations. *PLoS One* 13 (11): e0207079.
15. Ferreira FA, He Q, Banning S, Roberts-Sano O, Wilkins O, et al. (2021) HIV-1 proviral landscape characterization varies by pipeline analysis. *J Int AIDS Soc* 24(7): e25725.
16. Pace MJ, Agosto L, Graf EH, O'Doherty U (2011) HIV reservoirs and latency models. *Virology* 411(2): 344-354.
17. Eisele E, Siliciano RF (2012) Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity* 37(3): 377-388.
18. Avettand-Fènoël V, Hocqueloux L, Ghosn J, Cheret A, Frange P, et al. (2016) Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications. *Clin Microbiol Rev* 29(4): 859-880.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.55.008689

Fernando I Puerto. Biomed J Sci & Tech Res



This work is licensed under Creative Commons Attribution 4.0 License

Submission Link: <https://biomedres.us/submit-manuscript.php>



Assets of Publishing with us

- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles

<https://biomedres.us/>