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Original Article

Comparison of HIV-1 resistance profiles in plasma RNA versus PBMC DNA in heavily treated patients in Honduras, a resource-limited country

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Abstract: The World Health Organization currently does not recommend the use of dried blood spot specimens for drug resistance testing in patients undergoing antiretroviral therapy (ART). Therefore, HIV-1 resistance testing using peripheral blood mononuclear cells (PBMCs) may be of value in resource-limited settings. We compared genotypic resistance profiles in plasma and PBMCs from patients failing ART in two cities of Honduras (Tegucigalpa and San Pedro Sula), a resource-limited country. One hundred patients failing ART were randomly selected from a longitudinal patient monitoring cohort. Plasma and PBMC samples without patient identifier were used for genotypic resistance testing. Sequence data were analyzed, resistance profiles were determined and compared using Stanford HIV Drug Resistance Database algorithm. Specimens with concordant resistance profiles between the two compartments were 88% (95% CI: 80.3% - 94.5 %). Nine specimens (12%, 95% CI: 6.5% - 21.3%) had discordant resistance profiles of clinical significance. Logistic regression analyses indicated that patients on triple therapy were 17.24 times more likely to have concordant drug resistance profile than those on non-triple therapies (OR=17.24, 95% CI: 3.48, 83.33), while patients with increasing number of regimens and years on ART have a decreased rate of concordance (OR = 0.59, 95% CI: 0.32, 1.09 and OR = 0.62, 95% CI: 0.43, 0.88), respectively, than those with less number of regimens and years on ART. Our results show high level of concordance between plasma and PBMC resistance profiles, indicating the possibility of using PBMCs for drug resistance testing in resources-limited settings.

Keywords: HIV-1 drug resistance, RNA, PBMCs, concordance, discordance, resistance profile, resource-limited setting

Introduction

HIV treatment is widely available in the developed world where it was introduced in 1986 whereas it had only started in the developing world around 1998 through different feasibility projects. The "3-by-5" initiative, launched by UNAIDS and the World Health Organization (WHO) in 2003, aimed at providing three million people living with HIV/AIDS in low- and middle-income countries with life-prolonging antiretroviral treatment (ART) by the end of 2005. The Global Fund and the U. S. President's Emergency Plan for AIDS Relief (PEPFAR) have brought HIV treatment to many resource-limited settings. As of December 2011, 6.6 million patients in low- and middle-income countries were receiving combination ART, representing an increase of 1.35 millions over the previous year [1]. Honduras, a country with limited resources, has an estimated adult HIV prevalence rate of 0.7% (range 0.4-1.4%) [2]. Before 2003, ART was available for only a small number of patients through different non-governmental organizations (NGOs) and the private sector. In 2003, the Ministry of Health (MOH) of Honduras

started its widespread ART program [3-5]. In 2009, the country had a coverage around 33% for ART [6]. Unfortunately, there is a constant risk for development of drug resistance (DR) in a patient population taking antiretroviral drugs, because of the high mutation and replication rate of HIV, the chronic nature of HIV infection and the need for lifelong treatment. Thus, there is a need for HIVDR testing for the care of individual patients as well as for surveillance purposes. Genotypic HIVDR testing is usually performed on viral RNA in patient plasma samples, as WHO 2010 revised guidelines do not recommend the use of dried blood spots (DBS) for HIVDR testing for patients failing ART [7]. The reasons behind WHO decision are DBS results variability between assays, the lack of approved commercial assays for DBS testing, and the low volume of blood collected on DBS which can lead to less sensitivity as compared to plasma [8]. Therefore, performing HIVDR testing using DNA from PBMCs may be of value in resource-limited settings where cold chain specimen transport and preservation are challenging. Indeed, suboptimal transport and storage conditions (transport at room temperature for example) will lead to RNA degradation while DNA in PBMCs may still be amplifiable. There were studies in resource-rich countries that had compared resistance profiles in plasma and PBMCs in treatment-naïve and -experienced patients [9-14]. These studies investigated occurrence and accumulation of HIVDR mutations [11], similarity between resistance profiles in treatment-naive and treatment-experienced patients [12, 13, 15-23], as well as the impact of plasma viral load (VL) on the resistance profiles in these two compartments [13]. Overall most studies have indicated a high concordance between resistance profiles in PBMCs and plasma.

In Honduras, two studies investigated and characterized HIVDR in treatment-naïve and -experienced patients [4, 5]. One of the studies mainly focused on determination of the resistance profiles using PBMC DNA, with minor patient population having plasma RNA and PBMCs. The results showed a high concordance rate between resistance profiles in plasma and PBMCs [4]. Because cold chain-required transportation can be a limiting factor for plasma RNA genotyping in resource-limited countries and considering the preliminary findings in Honduras, we decided to investigate the resistance profiles in these two compartments using specimens collected from ART-experienced patients with long treatment history in a larger cohort. The purposes of this study were to determine the HIV-1 resistance profiles in plasma viral RNA and PBMC proviral DNA, and to evaluate the concordance rate between the two resistance profiles as a high degree of concordance would mean that PBMCs from HIV-infected individuals under ART could be an alternative to plasma for HIVDR testing in resource-limited settings.

Materials and methods

Patients

One hundred patients were randomly selected for this study after they had been invited to participate by their medical doctors from two medical facilities, Instituto Nacional del Tórax in Tegucigalpa and Hospital Mario Catarino Rivas in San Pedro Sula, and signed the informed consent. The ethics committee at the National Autonomous University of Honduras and the Regional Medical Ethics Board in Stockholm, Sweden approved the study (Dnr. 2006/1537-31/1). The detailed clinical and demographic data of the patient population had been described elsewhere [4]. In brief, the median time spent on treatment at the time of resistance testing was 3 years [interquartile range (IQR): 2.7 - 4.9 years]. The ART regimens contained a combination of nucleoside RT inhibitors (NRTIs), non-nucleoside RT inhibitors (NNRTIs), and/or protease inhibitors (PIs). Most patients were on three drug combination therapies except for a few that were on dual drug therapies. The median CD4 count was 200 cells/μL (IQR: 85- 422 cells/μL) of blood and the median VL was 4.49 log10 copies/mL. Both VL and CD4 were determined at a maximum of 6 months prior to resistance testing.

Sample collection, plasma viral load (VL) measurement and CD4 cell count determination

From April 2004 to April 2007, patient blood samples were collected into CPT™ Tube- BD Vacutainer Cell Preparation Tubes (Becton Dickinson Vacutainer®, Franklin Lakes, NJ) to obtain plasma and PBMCs. Plasma VL was measured using the Roche Amplicor HIV-1 monitor system, v1.5 (Roche, Rotkreuz, Switzerland)

with a detection limit of 400 copies/mL or the Abbott Real Time PCR HIV-1 system (Abbott Laboratories, Abbott Park, IL, USA) with a detection limit of 50 copies/mL. The CD4 counts were measured using the Dynal® T4 Quant Kit (Dynal Biotech ASA. Oslo, Norway).

Genotypic resistance testing of proviral DNA

Patient PBMC samples were shipped to the Swedish Institute for Infectious Disease Control (SMI) in Stockholm Sweden, for genotypic resistance testing. Resistance testing was carried out from PBMC DNA using an in-house method [4, 24]. In brief, DNA was extracted from PBMCs using the QIAmp DNA kit (QIAGEN, Hilden, Germany). The DNA was directly used in a nested PCR with primers JA269-JA272 which target the first 1,056 nucleotides of the *pol* gene. DNA sequencing was conducted on an ABI Prism™ 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and the raw sequence fragments were sent to Atlanta to be analyzed with the plasma sequences.

Genotypic resistance testing of plasma RNA

After removing all patient identification information, plasma samples from the same patients with PBMCs and labeled with laboratory identifiers were sent to the International Laboratory Branch (ILB) drug resistance laboratory at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, US for testing. The genotyping analysis performed at CDC with de-identified samples was determined as non-human subjects research by the Associate Director for Science at the Center for Global Health, CDC, Atlanta. RNA genotyping was carried out using a broadly sensitive in-house assay [25, 26]. A 1,023 base pairs fragment encompassing the 5' region of *pol* gene was generated by RT-PCR followed with a nested PCR. Sequencing was performed with purified PCR products and run on an ABI Prism™ 3100 Genetic Analyzer (Applied Biosystems Foster City, CA, USA). The raw sequences from plasma and PBMC samples were analyzed using ChromasPro version 1.42 (Technelysium Pty Ltd, Tewantin, Australia) and confirmed by a second operator. Pairwise comparison of each RNA sequence with its counterpart PBMC DNA sequence was conducted using BioEdit version 7.0.9 (Ibis Therapeuthics, Carlsbad, CA, USA).

Interpretation of drug resistance profiles and HIV-1 subtyping

Resistance mutations were identified using the Stanford HIVdb algorithm (version 6.0.11) and drug resistance profiles were classified as sensitive, intermediate or resistant (http://hivdb.stanford.edu). The newly obtained *pol* gene sequences and recommended subtype reference sequences from Los Alamos database (www.hiv.lanl.gov) were used to construct neighbor-joining phylogenetic trees using the MEGA 4 software [27] and the minimum evolution method with Kimura two-parameter model. Reliability of tree topology was determined with bootstrap analyses using 1,000 replicates. The phylogenetic trees were used to determine HIV-1 genetic subtype, to verify that paired plasma and PBMC sequences clustered together and to facilitate detection of possible PCR contamination and sample mix-up. In this project, concordance was defined as concordant drug resistance profile in any pair of sequences from a patient's plasma RNA and PBMC DNA, since mutations could be slightly different between the two compartments and the drug resistance profile would still be the same as not all the mutations identified induce clinically significant drug resistance.

Statistical analyses

Continuous variables were calculated as medians and IQR and categorical variables were expressed as proportion and exact 95% confidence intervals (CI). Bivariate and multivariate logistic regression analyses were used to assess associations of number of years on ART, number of regimens, adherence (good vs. poor), first ART regimen (triple drug therapy vs less than triple drug therapy) and number of mutations by drug class (PIs, NRTIs and NNRTIs) with concordant or discordant drug resistance profiles between the plasma viral RNA and PBMC DNA compartments. The statistical analyses were conducted using SAS/STAT software, Version 9 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 100 samples were included in the study. Due to amplification failure in 9 samples and amplification success in only one compartment in 15 samples, we were able to analyze 76 pairs of sequences obtained from both plas-

ma RNA and PBMC DNA compartments. Among the 15 samples successfully amplified only from one compartment, 12 of them were from PBMC DNA and the remaining 3 were from plasma RNA, and their VL ranged from 3,000 to 7,600 copies/ml. Therefore, PBMCs had a higher sequencing rate, 97% (95 CI: 93.5%-100%) than plasma, 87% (95% CI: 80%-94%).

Although PBMCs sequencing was performed in Sweden and plasma RNA sequencing was generated in CDC laboratory in Atlanta, USA, analysis of all the sequence raw data was performed in CDC laboratory. The quality of sequences generated using PBMC DNA were overall comparable to those generated from plasma RNA. Some of the detected mutations did not induce resistance to the regimens in use at testing time. Phylogenetic analyses further showed that plasma-PBMC pair sequences were highly related, which argues against contamination, mislabeling and/or introduction of artifacts that could negatively impact comparison of the sequences. Phylogenetic analysis revealed that all newly obtained sequences were HIV-1 subtype B (Data not shown). All sequences were

Table 1. Demographic and clinical characteristics of the 76 patients analyzed in the study (data were stratified according to concordant and discordant drug resistance profiles in paired peripheral blood mononuclear cell DNA and plasma RNA)

	Concordant (n = 67)			Discordant (n = 9)		
		Median	IQR[a]		Median	IQR
Age		29	11-39		37	11-49
CD4 count		189	83-415[b]		250	152-558
Number of years on ART		3	2.6-4.7		4.8	3.1-7.9
Number of regimens		2	2-3		3	2-4
Number of PI mutations		0	0-3		2	0-4
Number of NRTI mutations		8	2-12		2	1-4
Number of NNRTI mutations		4	2-6		1	1-5
	n	**(%)**	**Exact 95% CI[c]**	**n**	**(%)**	**Exact 95% CI**
Gender						
Female	34	(50.8)	38.2-63.2	4	(44.4)	13.7- 8.8
Male	33	(49.3)	36.8-61.8	5	(55.6)	21.2-86.3
Adherence						
Good	51	(76.1)	64.1-85.7	6	(66.7)	29.9-92.5
Poor	16	(23.9)	14.3-35.9	3	(33.3)	7.5-70.1
Risk Group						
Blood/MTCT[d]	27	(40.3)	28.5-53.0	4	(44.4)	13.7-78.8
Sexual Contact	40	(59.7)	47.0-71.5	5	(55.6)	21.2-86.3
First ART regimen						
3 drugs	60	(89.6)	79.7-95.7	3	(33.3)	7.5-70.1
<3 drugs	7	(10.5)	4.3-20.4	6	(66.7)	29.9-92.5
Low (≤ 1,000 copies/mL)	19	(28.4)	18.0-40.7	2	(22.2)	2.8-60.0
Intermediate (>1,000 and <100,000 copies/mL)	33	(49.3)	36.8-61.8	5	(55.6)	21.2-86.3
High (≥ 100,000 copies/mL)	15	(22.4)	13.1-34.2	2	(22.2)	2.8-60.0

[a]Interquartile range; [b]One patient data missing; [c]Confidential interval; [d]Mother to child transmission of HIV

Table 2. Summary of mutations detected in the 67 specimens defined as concordant drug resistance profiles.

		No. of specimens	Drug Resistance mutations identified	
			PR	**RT**
No mutation		8	None	None
With mutation	Identical mutations in both plasma and PBMCs for all 59 specimens	59	D30N, M46M, MI54V, I54V A71V, L90M	M41L, E44D, A62V, D67N, T69A/D/N/T, K70R, L74IV, A98G, M184V, G190A/Q, L210W, T215K/N/S/T/Y, K219E/Q, L100I, K103N, V106I, K108I, V118I, V179D, Y188F/L/Y, K238T
	Additional mutations identified only in plasma or PBMCs without impact on DR profiles	8	None	L33FL, K138N, E138EK, A62AV
	Additional mutations identified in plasma or PBMCs with impact on resistance level, but no change from categories[a]	6	None	M41LM, E44DE, K70KR, L74LV, L74IL, K101EK, V108IMV, L210LW, T215NSTY, K219EK, K219KQ

[a]Defined as not changing from susceptible to resistant or vice versa in the two compartments. DR: Drug resistance; PBMCs: Peripheral blood mononuclear cells; PR: Protease and RT: Reverse transcriptase.

submitted to GenBank and the accession numbers are JN215213-JN215364.

In all the analyses, we defined concordance as concordant drug resistance profile in any pair of sequences from a patient's plasma RNA and PBMC DNA since mutations could be slightly different between the two compartments and drug resistance profile would still be the same as not all the mutations identified induce clinically significant drug resistance. Discordance means discordant drug resistance profiles. These analyses were carried out by using the Sensitive, Intermediate, Resistant (SIR) alternative in the Stanford HIValg program.

Table 1 summarizes the demographic and clinical characteristics of the 76 patients analyzed in the study and stratified according to concordant and discordant drug resistance profiles in paired PBMC and plasma samples.

Eighty-eight percent (95% CI: 80.3-94.5) of the samples (N=67) had concordant drug resistance profiles between the two compartments. Among them, 70% (95% CI 59.6-79.8) of the samples (N=53) had either identical drug resistance mutations (N=45) or no drug resistance mutation identified (N=8). Amongst the eight patients without drug resistance mutation, three had poor adherence history (missed three or more drug doses in the last month), while the remaining five had good adherence history (missed less than 3 doses of drug in the last month). Although the remaining 18% samples (N=14) had concordant drug resistance profiles, drug resistance mutations identified were slightly different between the two compartments. For instance, eight samples had one drug resistance mutation (L33FL, K138N, E138EK and A62AV) which was different between the plasma and PBMC samples in their entire drug resistance mutation profiles, while the remaining six specimens contained mutations that changed the predicted level of drug resistance from intermediate to resistant level or vice versa between the two compartments. However, no drug was changed from susceptible to intermediate or resistant level (**Table 2**).

Discordant drug resistance profiles were observed in nine (12%, 95% CI: 6.5-21.3) samples. **Table 3** presents a detailed description of the drug resistance mutations in both compartments for these nine specimens. Five of these

Table 3. Specimens with discordant drug resistance profiles between plasma RNA and peripheral blood mononuclear cell DNA[a]

Sample IDs	Scored Resistance Mutations RNA		Resistance Level/Plasma RNA			Scored Resistance Mutations DNA		Resistance Level/PBMCs DNA		
	PR	RT	Sensitive	Intermediate	Resistance	PR	RT	Sensitive	Intermediate	Resistance
HN-12	None	L74V, K101KPQT, K103R, V179DV M184MV, **L210LW** T215NSTY, K219EK M230LM	None	ETR, D4T, **TDF**	DLV, EFV, NVP, 3TC, ABC, **AZT**, DDI, FTC		L74V, K101P, K103R, V179D M184V, T215Y, K219E, M230LV	**TDF**	ETR, **AZT**, D4T	DLV, EFV, NVP 3TC, ABC, DDI, FTC
HN-25	None	**K103N**	None	None	**DLV, EFV, NVP**	None	None	**DLV, EFV, NVP**	None	None
HN-28	A71V	**M41LM, E44DE**, D67N, K101Q, K103R, G190A L210W, T215Y, K238T	PIs	DLV, ETR, **3TC**, ABC, **FTC**, DDI, TDF	EFV, NVP, AZT **D4T**	A71V	D67N, K101Q K103R, G190A L210W, T215Y K238T	PIs **3TC, FTC**	DLV, ETR, ABC **D4T**, DDI, TDF	EFV, NVP AZT,
HN-37	I54IV, V82FV	A62V, K103R, V179DV, T188FHLY	NRTIs, DRV/r, TPV/r	DLV, ETR, ATV/r, FPV/r, IDV/r, LPV/r, NFV, SQV/r,	EFV, NVP	I54IV, V82FV	A62V, K103R V179DV, **M184MV,** Y188HY	ABC ,AZT ,D4T DDI ,TDF, DRV/r, TPV/ r	DLV, ETR ATV/r, NFV, SQV/r	EFV, NVP, **3TC, FTC**
HN-39		**K103KN**	None	None	**DLV, EFV, NVP**		None	**DLV, EFV, NVP**	None	None
HN-44	I54IV, A71AV, N88DN	T69NT, K70R	NNRTIs 3TC ABC DDI FTC TDF DRV/r FPV/r LPV/r TPV/r	AZT, D4T, ATV/r ,IDV/r, **NFV**, SQV/r	None	D30DN, I54IV, A71AV, N88DN	T69NS, K70R, **Y181CY**	3TC, ABC, DDI FTC, TDF, DRV/r FPV/r, LPV/R, TPV/r	EFV, ETR, AZT,D4T, ATV/r, IDV/r, SQV/r	DLV, NVP, NFV
HN-58	L90LM	K101KQ	NNRTIs, NRTIs, DRV/r, LPV/r, TPV/r	ATV/r, FPV/r, IDV/r, SQV/r, IDV/r ,SQV/r	NFV	L90LM	K101KQ, **M184MV**	NNRTIs, ABC ,AZT, D4T, DDI TDF, DRV/r, LPV/r, TPV/r	ATV/r, FPV/r, IDV/r ,SQV/r	**3TC ,FTC,** NFV
HN-72	D30N, M46L, A71AT, N88D, **L90LM**	D67N ,T69D, K70R, V118I, M184V, T215I , K219Q	DRV/r, TPV/r	ABC, D4T, DDI TDF, ATV/r, **FPV/r,** IDV/r, **LPV/r,** SQV/r	3TC, AZT, FTC NFV	D30N, M46L, A71AT, N88D	D67N, T69D, K70R V118I, M184V, T215F, K219Q	DRV/r, **FPV/r LPV/r**,TPV/r	ABC, D4T, DDI TDF, ATV/r, IDV/r, SQV/r	3TC, AZT, FTC, NFV
HN-77	None	K103KN	**NRTIs**	None	DLV, EFV, NVP		K103KN, **Y181CY, M184MV**	None	ETR	DLV, EFV, NVP, **3TC, FTC**

[a]The differences between the two compartments had clinical implication, leading to components of second-line regimens becoming compartment-dependent. ABC= Abacavir; ETR = Etravirine; DLV = Delavirdine; EFV = Efavirenz; NVP = Nevirapine; AZT = Azidothimydine; 3TC = Lamivudine; D4T = Stavudine; DDI = Didanosine; FTC = Emtricitabine; TDF = Tenfovir; ATV/r = Atazanavir +Ritonavir; DRV/r = Drunavir + Ritonavir; FPV/r = Fosamprenavir + Ritonavir; IDV/r = Indinavir + Ritonavir; LPV/r = Lopinavir + Ritonavir; SQV/r = Saquinavir + Ritonavir; NFV = Nelfinavir; TPV/r = Tipranavir + Ritonavir; Underlined and bold mutations are clinically significant discordant mutations and bold face antiretroviral drugs indicate clinically significant drug changes

Table 4A. Bivariate Logistic Regression Models to investigate association with different mutation profiles in PBMCs and RNA

Independent variable/ Predictor variable	Odds Ratio	95% CI	p-value
Age	0.99[a]	0.94-1.03[a]	0.5192#
Sex (Female vs Male)	1.29	0.32-5.22	0.7231
CD4 count (unit=100 cells/μL)[b]	0.84	0.64-1.12	0.2326
Number of years on ART	0.62	0.43-0.88	0.0081
Number of regimens	0.59	0.32-1.09	0.0907
Number of PI Mutations	0.9	0.72-1.12	0.3485
Number of NRTI Mutations	1.18	1.0-1.39	0.0524
Number of NNRTI Mutations	1.23	0.93-1.62	0.1469
HIV Risk Group (Sexual Contact vs Blood/MTCO)	1.19	0.29-4.82	0.8123
Viral Load			
(Low vs High)	0.70	0.1-6.28	0.9505
(Intermediate vs High)	0.70	0.12-3.94	0.7306
First ART Regimen (Triple therapy vs less than triple therapy)	**17.24**	**(3.48-83.33)**	**0.0005**

[a]Odds Ratios, 95% Confidence Intervals, and p-values calculated from bivariate logistic regression model; [b]Odds Ratio for CD4 count was calculated for an increment change of 100 cells/μL.

Table 4B. Multivariate analysis of factors that may be associated with concordant or discordant drug resistance profiles in PMBCs-DNA and plasma RNA

Variable	Odds Ratio	95% CI	p-value
Age	0.95	0.88-1.03	0.1912
Sex (Female vs Male)	1.22	0.33-5.39	0.6888
CD4 count (units=100 cells/μL)	1.00	0.99-1.00	0.1565
Number of years on ART	0.84	0.55-1.26	0.3874
Number of regimens	1.15	0.53-2.51	0.7173
Number of PI Mutations	0.95	0.74-1.23	0.6947
Number of NRTI Mutations	1.07	0.90-1.27	0.4492
Number of NNRTI Mutations	1.12	0.81-1.55	0.4910
Adherence (Good vs Poor)	1.35	0.22-8.46	0.7493
HIV Risk Group (Sexual Contact vs Blood/MTCO)	1.18	0.09-16.11	0.9007
Viral Load			
(Low vs High)	1.00	0.11-9.59	0.9386
(Intermediate vs High)	0.87	0.15-5.19	0.8503
First ART Regimen (Triple therapy vs less than triple therapy)	**15.63**	**1.79-142.86**	**0.0130**

samples (HN-12, HN-25, HN-28, HN-39, and HN-72) had mutations identified in plasma, not found in PBMCs while the remaining four had mutations in PBMCs which were not present in plasma. These differences in mutation profiles led to differences in drug resistance profiles between the plasma and PBMC compartments that could be of clinical significance.

Bivariate logistic regression analyses indicated that patients on triple drug therapy were 17.24 times more likely to have concordant drug resistance profiles than those on less than triple drug therapies (OR=17.24, 95% CI: 3.48-83.33, P=0.0005) while those patients with increasing number of years on ART were less likely to have concordant drug resistance profiles than those with less number of years on ART (OR=0.62, 95% CI: 0.43-0.88, P=0.0081). An association was not identified when other factors were analyzed, including age, sex, CD4 count change in an increment of 100 cells/μL, number of regimens, number of PI, NRTI and NNRTI mutations, transmission route (sexual contact vs. mother to child transmission, MTCT) low, intermediate and high VL levels (**Table 4A**).

When multivariate logistic regression analysis was used to assess the association of triple drug therapy at first ART with concordant drug resistance profile, the result remained significant (OR=15.36, 95% CI: 1.79-142.86, P=0.013, **Table 4B**).

Discussion

We have compared the drug resistance profiles in plasma and PBMCs in a cohort of patients with a long ART history from Honduras, a country with limited resources. Our data show a high concordance (88%, 95% CI: 80.3-94.5) between PBMC and plasma drug resistance profiles which is in agreement with the literatures where the median concordance rate was 87% (IQR: 83-97) [4, 12, 13, 22, 23, 28, 29] despite the long period exposure to ART median year on ART was three (IQR: 2.8-4.9) and high number of regimens used (median number of regimen was two (IQR: 2-3). Bivariate and multivariate analyses didn't reveal any association of different levels of VL with concordant or discordant drug resistance profiles in the two compartments, which rules out the detection of discordance due to stochastic sampling of different genetic variants. Logistic regression analysis indicated that increasing number of years on ART led to decrease concordant drug resistance profiles. These data could be explained by the fact that due to the integration of the viral strains into cell genomes, PBMC viral population which harbors archived viruses that were resistant to previous therapies would be different from actively replicating viruses found in plasma that are resistant to current medications [13]. Multivariate logistic analyses also indicated that patients starting their treatment with triple drug therapy were 15.6 more likely to have concordant drug resistance profiles than those starting with mono or dual drug therapy. Indeed, starting a highly effective triple drug therapy allows patients to maintain the same treatment regimen for a longer period of time which may lead to infrequent switch of treatment regimens and higher concordant drug resistance profiles.

The high rate of concordance found in our study and reported by others [4, 12, 13, 22, 23, 28, 29] indicates that contribution of drug resistance profiles from integrated proviral DNA in PBMCs does not appear to create significant differences between PBMCs and plasma resistance profiles. Moreover, amongst the 15 specimens that have sequences in only one compartment, the majority (N=12) were obtained from PBMC DNA and only 3 were from plasma RNA, which resulted in the overall genotyping efficiency of 97% (95%, CI: 93.5-100) for PBMCs and 87% (95% CI: 80%-94%) for plasma RNA. These data indicate that genotyping from PBMC DNA appears more efficient than that from plasma RNA. Although both plasma and PBMCs require cold chain conditions for storage and shipment, the fragile and more degradable nature of RNA molecule [30] becomes less attractive for HIVDR testing than DNA molecule in unreliable cold chain conditions for storage and shipment often encountered in resource-limited countries. Therefore, PBMC DNA may represent an alternative for drug resistance testing in ARV-naïve and-experienced individuals in resource-limited settings. In addition, genotyping HIV proviral DNA from PBMCs may be useful in patients with VL <1,000 copies/mL [13, 16].

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