

HCV genotype distribution among HIV co-infected individuals in Argentina: Relationship with host and viral factors

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Summary

Coinfection with hepatitis C virus (HCV) in individuals infected with HIV is associated with a higher incidence of liver injury, hepatic decompensation, and decreased survival than that observed in an HIV-monoinfected population. While prevalence studies on HIV/HCV coinfection have been performed in the U.S. and in some European countries, little is known about HCV genotype distribution in Latin America. The main objective was to evaluate the HCV prevalence and genotypes among HIV co-infected patients, and their relationship with HCV viral load, serum ALT level and T lymphocyte CD4+ cell count. These data pursue to increase the knowledge from South America about a pressing problem from HIV-infected patients. Retrospectively collected specimens from 593 HIV-positive individuals in Argentina were tested for anti-HCV. These were analyzed for HCV-RNA qualitatively and quantitatively. The HCV genotype was determined by the RFLP method. One hundred and twenty-nine (21.7%) HIV-infected individuals were anti-HCV positive; 65.9% of them exhibited detectable HCV-RNA. Genotype 1 (43, 1a/c; 9, 1b;

and 5, 1a/c+1b) was present in 57, while 1, 14 and 13 were infected with genotype 2, 3 or a mix, respectively. Co-infected individuals were more likely to be male, without significant differences in age and CD4+ cell counts than HIV-monoinfected individuals. HCV infection prevalence in patients co-infected with HIV highlights the impending public health impact of this problem. Considering the increasing rate of HCV genotypes with lower response rates to treatment among HIV co-infected patients, antiretroviral therapy success might be jeopardized by HCV coinfection.

Distribución de los genotipos de HCV entre individuos co-infectados con HIV en Argentina: su relación con factores del huésped y del virus

Resumen

La coinfección con el virus de hepatitis C (HCV) en individuos infectados con HIV está asociada con una mayor incidencia de injuria y descompensación hepática, y un menor tiempo de supervivencia respecto de la población mono-infectada por HIV. Mientras que diferentes estudios de prevalencia de la coinfección HIV/HCV se han llevado a cabo en Estados Unidos y países de Europa, la información de la distribución de genotipos de HCV en Latinoamérica es escasa. El objetivo de este estudio fue evaluar la prevalencia de HCV y la distribución de sus genotipos entre pacientes co-infectados con HIV, y su relación con la carga viral de HCV, los niveles séricos de ALT y el recuento de linfocitos T CD4+. Estos datos pretenden incrementar el conocimiento desde la región de Sudamérica acerca de este acuciante problema en pacientes infectados con HIV.

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Retrospectivamente se colectaron especímenes desde 593 pacientes infectados con HIV en Argentina en quienes se investigó la presencia de anticuerpos anti-HCV. Se pesquisó además la presencia de RNA viral de HCV tanto cualitativa como cuantitativamente. El genotipo de HCV se determinó por la técnica de RFLP. Ciento veintinueve (21.7%) individuos infectados con HIV fueron positivos para anti-HCV; 65.9% de ellos exhibieron RNA de HCV detectable. El genotipo 1 (43, 1a/c; 9, 1b; y 5, 1a/c+1b) se presentó en 57 individuos, en tanto que 1, 14 y 13 estaban infectados por los genotipos 2, 3 o mezcla de ellos, respectivamente. Predominó el sexo masculino entre los individuos con coinfección, en tanto que no se advirtieron diferencias significativas respecto de los pacientes infectados sólo con HIV en lo referido a edad y recuento de linfocitos T CD4+. La prevalencia de infección por HCV en pacientes coinfectados con HIV resalta el impacto de esta problemática en la salud pública. Considerando la creciente tasa de genotipos de HCV con menor respuesta al tratamiento entre los pacientes coinfectados con HIV, el efecto beneficioso de la terapia anti-retroviral podría verse opacado ante la coinfección con HCV.

Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) share the same routes of transmission, which explains the high rate of HCV and HIV co-infection.

For HCV, at least six major genotypes and more than 50 subtypes have been identified.¹ HCV genotyping is widely used in acute and chronically infected patients as a predictive marker of disease progression and response to interferon therapy.² We are reporting the status of HCV infection and its genotypic distribution among chronic liver disease patients co-infected with HIV-1 in a single institution in Buenos Aires, Argentina. This molecular epidemiological assessment is essential for planning proper control measures and therapy against HCV for which an effective vaccine is not available.

In June 2005, it was estimated that approximately 0.64% of blood donors in Argentina (n=147,475) were infected with HCV (National Project Program for Viral Hepatitis Control, Hepatitis and Gastroenteritis Laboratory, National Institute of Microbiology "Dr. C. Malbrán, Argentinean Epidemiological Bulletin June, 2005, [\[.hepatitisviral.com.ar\]\(http://www.hepatitisviral.com.ar\)\).](http://www-</p>
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Similarly to U.S. reported findings^{3,4} the prevalence of HIV/HCV coinfection in Argentina varies according the risk group analyzed. Fainboim et al.⁵ studied a population of HIV infected patients (n=484) comprising mainly injecting drug users –IDU– (n=234), men-who-had sex with men –MSM–(n=99), and heterosexual –HT– risk (n=142) and found 92.3%, 14.1% and 33.1% anti-HCV prevalence, respectively. Two other reports exhibited high prevalence of coinfection (32% among 174 young heterosexual HIV-infected patients;⁶ 88.3% among 77 HIV-infected people's street-recruited injection drug users).⁷

With the advent of highly active antiretroviral therapy (HAART), the effect of HCV in co-infected patients became apparent because treatment markedly reduced the development of HIV-related disease complications; thus, patients were living long enough for HCV-related disease manifestations to occur.^{8,9} Data from the Swiss HIV Cohort study¹⁰ also indicates that HCV co-infection accelerates HIV disease. A study in co-infected haemophiliacs¹¹ indicated that the progression of HIV disease was related to HCV RNA viral load.

This cross-sectional study is intended to describe the prevalence of HCV infection in 593 HIV-1 infected patients, consecutively tested for HIV-1 viral load during an 8 months period. Likewise, the HCV genotype distribution is determined and analyzing its relationship with biochemical markers of liver function, virological and immunological parameters, as well. These data are compared with previous Argentinean epidemiological data on HCV monoinfected patients.

Methods

• 1. Study design

Between September 2004 and April 2005, serum samples were collected from 593 patients (age, 39 ± 8.6 [mean ± standard deviation, SD] years; 66% men and 34% women) with HIV infection who attended the Unit of Infectious Diseases at Fernández Hospital. This is a university public hospital located in a residential area of Buenos Aires city, Argentina. The main route of HIV infection was heterosexual contact (n: 233, 39.3%), followed by men who ha-

ve sex with men (n: 184, 31%), injecting drug use (n:75, 12.6%), and unknown (in 12.3%). No patient had received HCV specific anti-viral treatment. Blood specimens were drawn after obtaining written informed consent and IRB approval. HIV status was confirmed by Western blot. The serum specimens had been maintained at -80 °C from the time of collection until they were retrieved for this study. HCV specific antibodies were firstly tested by MEIA AXSYM; secondly, those samples where the s/co (signal to cut-off) was <5 were further tested by HCV 3.0 Murex (both from Abbott Laboratories, Diagnostic Division). Following CDC guidelines, the screening-test--positive average s/co ratios >3.8 are highly predictive of RIBA positivity (>95%) and would be highly predictive of the true anti-HCV status.¹² In addition, the impaired HCV-antibody response in patients coinfecting with HIV was reported to correlate with a higher rate of RIBA indeterminate results.¹³ The presence of HCV RNA was determined for all samples proven reactive in any serological test. Two different qualitative approaches were used: an in house assay previously described¹⁴ and commercial RT-PCR-based AMPLICOR HCV test kit, version 2.0 (low detection level, 50 IU/ml) (Roche Molecular Systems). For quantitative purposes, a commercial assay (Bayer VERSANT® HCV RNA 3.0 Assay –bDNA- test kit; range from 615 to 7,700,000 HCV RNA IU/ml) was carried out. Specimens that contained HCV RNA positive were genotyped, using the restriction fragment length polymorphism (RFLP) method previously described.¹⁴ CD4+ cell counts were measured using a Coulter Counter (manual method; Beckman Coulter Inc., Fullerton, CA, USA). Serum alanine aminotransferase (ALT) level was determined in all patients by using a commercial kit (ALT, BioSystems, Spain) following the manufacturer's instruction. Normal ALT levels were ≤35 U/l when testing was done at 37°C.

Serum samples were tested for HBsAg and anti-HBc by enzyme immunoassay (Abbott, USA). Those samples that exhibited reactivity for any of the previous HBV serological markers were also tested for HBeAg, anti-HBe, IgM and total anti-HBc, and anti-HBs by ELISA tests (Abbott, USA).

• 2. Description of assays

RNA extraction, RT-nested PCR of the 5'UTR,

HCV viral load determination, and RFLP analysis. RNA was extracted from 200µl of serum using guanidinium isothiocyanate and acidic phenol followed by reverse transcription (RT)-nested PCR amplification of the 5'UTR as previously described.¹⁵ Genotyping of HCV was based on restriction fragment length polymorphism –RFLP-,¹⁶ employing endonuclease digestion of 250 bp amplicons from the 5' untranslated region (UTR) after conventional reverse transcription-nested PCR (RT-nested PCR) as modified by the authors.¹⁵ Briefly, 15 µl of 5' UTR RT-nested PCR amplicons from all studied samples were cleaved with 10 U of the following enzymes used for combined digestions: *HaeIII/RsaI*, *ScrFI/HinfI*, and *BstNI/HinfI*. Thereafter, according to the genotype, a fresh aliquot of each amplicon was digested with either *BstUI* (for type 1 subtyping) or *ScrFI* (for subtyping of types 2 and 3). This procedure was applied to the whole population analyzed. For HCV subtype assignment, it is necessary to consider the RFLP limitations with regards to differentiate among some of them. For this reason they are named as 1a/c, 2a/c, 3a/c/d/e, and 3b/f.¹⁶ The digestion products were further resolved by UV light visualization of ethidium bromide stained gels after electrophoresis.

The HCV AMPLICOR 2.0 assay (Roche Diagnostics) was carried out following the manufacturer's instructions. Its lower detection limit is 50 IU/ml.¹⁷

The Versant HCV bDNA 3.0 assay (Bayer Diagnostics, Berkeley, California) was performed as per the manufacturer's instructions.

• 3. Statistical analyses

Because data were not normally distributed, differences in age and CD4+ cell count between the HIV-monoinfected subjects and the HIV/HCV-co-infected subjects were evaluated, using a Wilcoxon rank sum test.¹⁸ Student t test with Yate's correction was used to determine differences in distribution of males and females in the two subpopulations. The non-parametric Kruskal-Wallis test¹⁹ was used for viral load median comparison. Statistical significance was defined as $p \leq 0.05$.

Results

• 1. Study population

No differences were found between the HIV-monoinfected (285/593, 48%) and HIV/HCV-co-infected (129/593, 22%) populations with respect to gender, age or CD4 cell count.

Overall 41.6% (246) had markers of HBV infection. Sixty-five cases (11%) showed markers for both viruses and 285 (48%) did not have serological markers of HBV or HCV infections. Considering the objective of the present study, those mentioned HIV infected patients with concomitant presence of HBV-HCV (n=65) were not included for further analysis considering that HBV influences biochemical markers of liver function, immunological and HCV-related virological parameters under a pure HCV coinfection.

Abnormal ALT (> 35 mU/ml) was found in 104 out of 593 (17.5%) HIV infected patients: 77 (74%) were between one to two times over the normal limit and 27 (26%) were at least twice normal values more. Abnormal values of ALT were statistically associated with male gender, chronic HBV coinfection, or HCV coinfection. Isolated anti-HBc (total antibodies to hepatitis B core) was associated in univariable ($p=0.001$) analysis but not in multivariable analysis ($p=0.64$). There was no association with HAART ($p=0.84$) nor with the presence of HCV RNA ($p=0.18$), in coinfecting patients. Serological markers only for HBV were found in 55/104 samples (52.8%) (9 chronic carriers, 24 isolated anti-HBc and 22 resolved HBV) – data not shown-, only for HCV in 54/104 samples (51.9%). Positive HCV RNA PCR was detected in 44/54 samples, 81.4% characterized as genotype 1a, no differences in level of abnormal ALT were seen among different genotypes.

Among the group of non coinfecting patients, HAART, age, LT CD4+ count and HIV viral load were not statistically associated with abnormal ALT ($p=0.59$).

•2. HCV positive results

Of the 593 specimens tested, 129 (21.7%) were positive for serum HCV antibodies tested by MEIA and/or EIA (Abbott), but there was a lower percentage of detectable HCV viremia among coinfecting persons (85/129 -65.9%) by both in house and commercial qualitative tests. False negative

results (HCV-RNA positive; anti-HCV: no reactive), associated with acute infection or with low CD4 counts, are uncommon in HIV-1 coinfecting patients.²⁰ Those HIV-monoinfected patients (n=285) with none serological evidence of HCV (and/or HBV) infection require exploring the RNA-HCV presence by RT-PCR (under progress) considering that they could show a higher proportion of detectable serum HCV RNA in comparison with patients with only HCV infection.²⁰ Thus, the founded HCV prevalence in HIV-1 coinfecting patients could be underestimated.

There was no statistical association between CD4 count ($p=0.21$), HIV viral load ($p=0.37$), use of HAART ($p=0.64$) or detectable HCV viremia. ALT more than two times about the normal value was statistically associated with positive serum HCV RNA ($p=0.002$) and normal ALT was associated with negative serum HCV RNA ($p=0.001$).

HCV genotypes were obtained for all 85 HCV RNA-positive specimens. Fifty-seven specimens were genotype 1. Of these, 43 were 1a/c, 9 were 1b, and 5 were 1a/c + 1b. The remaining specimens were genotype 2a/c (1 patient) and genotype 3 (14 patients). Among patients infected with HCV genotype 3, 10 were infected with the subtype 3a/c/d/e. The remaining 4 were infected with subtype 3b/f.

Thirteen patients had mixed infections, implying at least two different HCV viral types (1, 2, and/or 3). No statistically significant differences were found regarding age, sex and risk behaviour among patients infected with the different HCV subtypes.

The frequency of abnormal ALT serum level was 43.2% in coinfecting patients without any significant difference among the founded HCV genotypes. This figure is substantially higher than reported in HIV monoinfected patients (8.1%, $p<0.05$). (table 1, 2)

HCV viral load and median CD4+ T cell counts were not significantly different among the HCV genotype groups (table 2). Although patients coinfecting with HCV genotype 1b showed a trend to have lower cell count than did patients infected with non-1b HCV, this association was not statistically significant ($p=0.07$, Kruskal-Wallis test).

Table 1. Characteristics of the 593 HIV-infected individuals in the study population and in the HIV/HCV-co-infected and HIV-monoinfected groups.

Variable	HIV-monoinfected (n = 285)	HIV/HCV-coinfected (n = 129)	All subjects (n = 593)
Age			
Mean (\pm SD)	38 (8.7)	38 (6.1)	39 (8.6)
Median (range)	37 (19-64)	38 (24-59)	38 (19-71)
Sex			
Male (%)	49.8 ^a	69.6 ^a	65.6
Female (%)	50.2	30.4	34.4
Treatment status at time of specimen collection			
On HAART treatment (%)	65.4	79	70
CD4+ (T cells/mm ³)			
Mean (\pm SD)	440 (275.3)	354 (243.0)	425 (276.6)
Median (range)	404 (2-1717)	330 (5-1384)	374 (2-1717)
Abnormal ALT			
n	23 ^b	54 ^b	104
%	8.1	43.2 ^c	17.5 ^c
^a p = 0.0002	^b p = 0.0000	^c p = 0.0000	

Table 2. Demographic, biochemical, immunological and HCV related viral findings in the studied population according to the HCV genotype.

HCV genotype	n (%)	Median age (range)	Number of patients with abnormal ALT serum level	Median HCV viral load, in log UI/ml	Median CD4+ cell count	Number of patients receiving HAART	HIV-infection associated-risk			
							IDU	MSM	HT	o(?)*
1a/c	43 (50.6)	36 (27-49)	23	5.9	279	35	25	3	9	6
1b	9 (10.6)	39 (34-46)	4	5.7	176	9	3	1	3	2
1a/c+1b	5 (5.6)	41 (38-44)	2	6.4	273	5	1	3	1	0
2a/c	1 (1.2)	45	0	6.5	212	1	1	0	0	0
3a/c/d/e	10 (11.8)	38 (26-42)	8	5.6	262	7	7	0	0	3
3b/f	4 (4.7)	46 (41-57)	1	5.7	311	3	2	1	1	0
Mixed	13 (15.3)	38 (24-59)	6	6.0	463	9	5	1	7	0

*IDU: injecting drug users; MSM: Men who have sex with men; HT: heterosexual; O (?): other and, unknown

Discussion

Hepatitis C infection is a devastating problem for the individual patient and poses a huge financial burden on the society, particularly in developing countries with limited resources devoted to public health.

The reported sero-prevalence of HCV infection in HIV-infected patients from Buenos Aires varied from 32 to 88%,^{5,6,7} whereas the prevalence was lower (21.7%) in this report. This might be explained by the lower contribution of injecting drug users (12.6%) included in this study.

In our population, after comparing HIV mono-infected (n=285) with HCV co-infected (n=129) patients, no significant differences between these two groups were found with respect to age or CD4 cell count, although male gender was significantly more prevalent in co-infected patients (table 1), in agreement with previous report.²¹ By contrast, two other studies^{10,22} reported lower CD4+ cell counts and conflicting results for age and gender which may reflect differences in study populations.

The influence of HIV co-infection and HCV genotype distribution on HCV viral load and ALT levels in chronically infected patients remains unclear. The significantly higher prevalence of abnormal ALT serum level found in HCV co-infected patients indicates the presence of necro-inflammatory active liver disease and probable HCV viral replication in dually infected patients (tables 1 and 2). A marked increase in the transaminase level has been previously suggested to be associated with genotype 3 coinfecting subjects,²³ but this association neither was not further consistently reported²⁴ nor observed in the present study.

Among HCV viremic HIV co-infected patients (n=85), the HCV subtype distribution denotes prevalence of genotype 1a/c (43/85, 50.6%) and mixed infections (13/85, 15.3%) followed by 3a/c/d/e (10/85, 11.8%) and 1b (9/85, 10.6%) subtypes. This is a dissimilar distribution than previously reported by the author in HCV mono-infected patients.¹⁴ HCV genotypes 1a and 3a have been associated with a history of intravenous drug use,^{25,26,27} which was the route of HCV infection in a half of the HCV-HIV co-infected population studied by us. In agreement with other reports,^{28,29} a plausible shift in the HCV genotype predominance is seen. Genotype 1b was previously the main subtype in this region^{14,26,30} and subsequently there was an HCV genotype shifting from 1b toward 3a and 3b.

This phenomenon could be related to an increased rate of intravenous drug use.³¹ As responsiveness to HCV anti-viral treatment varies significantly with genotype,³² the observed predominance of genotype 1 and mixed HCV infections may affect overall anti-HCV treatment.

A high prevalence of HCV mixed infections (involving at least two different HCV types) were observed among HIV co-infected patients (15.3%). Intravenous drug users with mixed infections could be expected,³³ but in our study we found a slightly higher association with heterosexual patients who denied intravenous drug use or exposure to blood-products (table 2). This observation could be ascribed to the compartmentalization phenomenon frequently seen in HCV infection where viral variants or genotypes are distributed non randomly among the different sites of replication. Infection of immune cells could be a mechanism by which HCV evades the host response. PBMCs could harbor different or mixed HCV genotypes.³⁴ Considering that some of these cells could have a prolonged life span, the genotype they harbored would have appeared in plasma in the presence of autonomous HCV replication. This extrahepatic scenario for HCV replication highly contributes to viremia.³⁵

In co-infected patients it was proposed that HCV genotype 1 was associated with lower absolute and percentage CD4+ T cell measurements.³⁶ We could not analyze this because most patients infected by HCV genotype 1 were receiving HAART (49/57 patients) and had a CD4 median value of 243.5 (range: 5-1384). Only those patients co-infected with HCV genotype 1b exhibited a tendency to have a lower CD4+ T cell count with a median value of 176 (range: 14-518; table 2), in spite to exhibit similar mean HIV-1 load (data not shown).

In conclusion, epidemiologic data on HIV-HCV infections in Argentinean patients from an urban setting in a middle income country strongly indicates the need for continuing with HCV screening, both in blood transfusion centers as well as in people with HIV. Although derived from a small set of patients, the results of this study show a tendency for the proportion of HCV genotypes to differ between HIV infected and uninfected patients. Finally, while HAART in Argentina has resulted in a similar impact on morbidity and mortality as seen in industrialized countries, analysis of a larger HIV/HCV co-infected cohort could provide useful insights for establishing specific HIV/HCV treatment guidelines.

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