DOI: 10.1590/S0004-28032016000400007 ARQGA/1865

FREQUENCY OF THE *MDR1* GENE POLYMORPHISM RS1045642 (C3435T) IN HCV-HIV CO-INFECTED PATIENTS

Giórgia de Souza **MARASCA**[1], André Luiz **MACHADO**[1,2], Nélson Alexandre **KRETZMANN FILHO**[1], Adaliany Cecília da Silva **SOUZA**[1], Angelo Alves de **MATTOS**[1], Dimas **KLIEMANN**[1,2], Cristiane Valle **TOVO**[1,2] and Ana Beatriz Gorini da **VEIGA**[1]

Received 1/7/2016
Accepted 1/8/2016

ABSTRACT - ***Background*** - Due to the high prevalence of co-infection by hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and the severity of these infections, the understanding of the biological mechanisms involved in these processes, including viral behavior and host genetic profile, is of great importance for patient treatment and for public health policies. Some single nucleotide polymorphisms (SNPs) in the human genome, such as the SNP rs1045642 (C3435T) in the *MDR1* gene, have been reported to be associated to the sustained virological response (SVR) to HCV treatment in HCV-HIV co-infected patients. ***Objective*** -The present study analyzes the *MDR1* gene C3435T polymorphism in HCV-HIV co-infected patients. ***Methods*** - A total of 99 HCV-HIV patients were included in the study. The DNA was extracted from blood samples, and the SNP rs1045642 was assessed by Real Time PCR (qPCR). Risk factors for acquiring the virus and the SVR after HCV treatment with pegylated interferon-alpha and ribavirin were also analyzed. ***Results*** - Among the patients, 54 (54.5%) were male and 45 (45.5%) were female. The average age was 46.1±9.8 years. The SVR after HCV treatment was 40%. The frequencies of *MDR1* genotypes CC, CT and TT were 28.3%, 47.5% and 24.2%, respectively. Allele frequencies were 52% for the C allele and 48% for the T allele. No association was found for SNP rs1045642 (C3435T) regarding response to treatment (P=0.308). ***Conclusion*** - In this study, the C3435T polymorphism in the *MDR1* gene appears not to be associated with SVR in HCV-HIV co-infected individuals.

HEADINGS - Hepacivirus. HIV. Single nucleotide polymorphism. P-glycoprotein. Coinfection. Interferon-alpha. Ribavirin.

INTRODUCTION

Hepatitis C is considered a major public health problem. Approximately 3% of the human population is infected with the hepatitis C virus (HCV), which represents approximately 170 million people chronically infected and at risk of developing disease complications, including cirrhosis and hepatocellular carcinoma[17]. In Southern Brazil, the prevalence of HCV in adults (aged 20 to 69 years) is 1.7%[22].

HCV infection has been commonly observed in individuals infected with the human immunodeficiency virus (HIV), mainly because both viruses share transmission routes; notably, injecting drug users constitute a high-risk group for acquiring both HCV and HIV[18,23]. Considering that approximately 400 million people are infected with HIV worldwide, co-infection rates have been increasing significantly; in North America and Europe, about 30% of HIV-infected patients are co-infected with HCV[23]. In Brazil, the highest HIV detection rate is reported in the South region of the country; not surprisingly, the number of HCV-HIV co-infected individuals is also high[18-20]. In our experience, a study performed in a tertiary reference center of attendance for HIV showed a prevalence of co-infection with HCV of 38.2%[26].

After the introduction of highly active antiretroviral therapy (HAART), patients with HIV have been given a higher survival rate, enabling the development of other chronic diseases, such as liver diseases. In addition, in co-infected patients, HCV can increase morbidity and mortality related to HIV[12]. The efficacy of the treatment for hepatitis C can vary among patients, and genetic characteristics play an important role in the response to the drugs used. Some single nucleotide polymorphisms (SNPs) have been associated with HCV and HIV infections, disease outcome and response to treatment[4,7].

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
Financial support: Marasca GS and Souza ACS hold a fellowship from CAPES (Ministry of Education, Brazil).
[1] Programa de Pós-Graduação em Hepatologia, Universidade Federal de Ciências da Saúde de Porto Alegre, RS, Brasil; [2] Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brasil.
Correspondence: Ana Beatriz Gorini da Veiga. Rua Sarmento Leite, 245, sala 309 - UFCSPA - CEP 90050-170 - Porto Alegre, RS, Brasil. Email: anabgv@ufcspa.edu.br

The multi-drug resistance gene 1 (*MDR1*, NT_007933; NM_000927) is well known for its role in the resistance to drugs used in the treatment of different human diseases[21]. Also called *ABCB1*, the *MDR1* gene is located in chromosome 7q21.1 [5] and comprises a promoter region of 29 exons, with a total of 209kb[3,6]. *MDR1* codes for the P-glycoprotein (PgP) 1, a member of the superfamily of ABC transporters (ATP-binding cassette), which includes proteins that transport a wide variety of substrates[8,9]. It has been observed that cells that exhibit a multidrug resistance phenotype also show an increase in the expression of the PgP[27].

In 2000, Hoffemeyer et al. described 15 SNPs for the *MDR1* gene, including C1236T, G2677T/A and C3435T. The latter, which is located in exon 26, has been correlated with the expression levels and activity of PgP[13].

Given the high prevalence of co-infection by HCV and HIV and the numerous complications that co-infected patients present, this work aims to map the C3435T polymorphism of the *MDR1* gene (rs1045642) in HCV-HIV co-infected patients and correlate its distribution with the response to treatment for hepatitis C.

METHODS

This is a cross-sectional study that evaluated patients from a reference Hospital in Southern Brazil in the management of co-infected HCV-HIV patients. Those diagnosed with HCV and HIV, who held monitoring in the outpatient clinic from January 2013 to December 2013 were included.

Interviews were conducted with 99 patients who agreed to participate in the study and signed the Informed Consent sheet. All the 99 patients answered an information form and were submitted to blood collection in 4 mL tubes containing EDTA.

To characterize the population studied, the following variables were evaluated: age, gender, ethnicity, response to treatment with pegylated interferon and ribavirin, genotype of HCV, METAVIR score[2], risk factors for acquiring HCV-HIV, $CD4^+$ levels, treatment regimen for HIV (HAART) and genotype profile for the SNP rs1045642 (C3435T) of the *MDR1* gene.

Patients were treated with pegylated interferon-alfa and ribavirin, and those who had a negative result for HCV-RNA at week 24 after the end of treatment were considered as having sustained virological response (SVR).

All molecular analyses were carried out at the Molecular Biology Laboratory of the Federal University of Health Sciences of Porto Alegre (UFCSPA). After blood collection, the nucleated cell layer (buffy coat) was separated by centrifugation at 500 x *g* for 10 minutes. The buffy coat was stored in 1.5 mL tubes and kept at -80ºC until DNA extraction.

DNA extraction was based on the methodology described by Iranpur et al.[16]. DNA was analyzed in a NanoDrop (NanoDrop Technologies, Wilmington, USA), quantified and stored at -80ºC.

For molecular identification of the C3435T polymorphism of the *MDR1* gene (GenBank accession number: M29445), qPCR was performed on a Step One PLUS thermocycler (Applied Biosystems, Foster City, CA), based on allele-specific assay using a pair of primers and two hydrolysis probes, which have been designed and validated by Applied Biosystms (SNP: rs1045642). The primers were used to identify the DNA sequence containing the SNP, and the probes were classified into "Normal Probe" (used to amplify the C allele) and the "Polymorphic Probe" (to amplify the T allele).

Quantitative variables were expressed as mean, standard deviation, minimum and maximum. The qualitative variables were described by absolute and relative frequencies. To verify if there was an association between the *MDR1* gene polymorphism C3435T and the patient's response to treatment, analysis was performed using the chi-square test where associations with $P<0.25$ were later analyzed under multivariate approach, using logistic regression. To evaluate other associations for the C3435T polymorphism, chi-square test of Pearson was used. The significance level was 5.0% and the analyses were performed using SPSS program v. 21.0.

The study was approved by the local Research Ethics Committee under the Protocol Number 12-089.

RESULTS

This study analyzed 99 patients infected with HCV and HIV. The characteristics of the patients and the distribution of genotypes of the *MDR1* gene C3435T polymorphism are shown in Table 1.

TABLE 1. Characteristics of HCV-HIV co-infected patients

Variables	n = 99
Gender - n (%)	
Male	54 (54.54%)
Female	45 (45.45%)
Age (years) - Mean ± SD	46.17 ± 9.8
Ethnicity - n (%)	
White	76 (76.7%)
Brown	13 (13.1%)
Black	10 (10.1%)
Risk factors for acquisition of HCV-HIV - n (%)	
Unprotected sex	38 (38.4%)
IDU	37 (37.4%)
Blood transfusion	13 (13.1%)
Unknown cause	11 (11.1%)
METAVIR score - n (%)	
With cirrhosis	43 (70.5%)
No cirrhosis	18 (29.5%)
Genotype for the *MDR1* gene	
CC	28 (28.3%)
TC	47 (47.5%)
TT	24 (24.2%)

IDU: injecting drug use; SD: standard deviation. HCV: hepatitis C virus; HIV: human immunodeficiency virus.

The genotype frequencies for the *MDR1* gene polymorphism C3435T in the total sample of co-infected patients were 28 patients (28.3%) for the homozygote genotype CC, 47 patients (47.5%) for the heterozygous genotype CT, and 24 patients (24.2%) for the homozygous genotype TT. The allelic frequency for the polymorphic variant C was 52% and the frequency of the T allele was 48% of the cases studied.

The SVR was 40%, and there was no relation with the HCV genotype. To verify the association of the SNP rs1045642 with response to treatment in HCV-HIV co-infected patients, the bivariate analysis followed by multivariate and logistic regression was used (Table 2). The SVR to treatment for HCV was evaluated in 45 patients, and the variables tested were the HCV genotype, ethnicity, HAART regimen, gender and CD4 cell count. The only variable associated to SVR was the CD4 cell count, as the patients with unsatisfactory SVR showed a CD4 cell count lower than 500 cells/mm^3 (OR 5.05; P=0.049).

TABLE 2. Characteristics of HCV-HIV co-infected patients and response to treatment with pegylated interferon combined with ribavirin

Variables	Response to HCV treatment		Bivariate	Multivariate	
	Unsatisfactory (n=27)	Satisfactory (n=18)	*P*-value[#]	*P*-value[*]	O.R.
SNP rs1045642 Genotype					
TT	7	3			
TC	15	8	0.308	-	-
CC	5	7			
HCV genotype					
1	20	7		0.147	3.37
2	1	3	0.051	0.399	0.227
3	6	4		-	1
Ethnicity					
Black	5	2		0.276	3.037
Brown	6	1	0.239	0.288	3.911
White	16	14			1
HAART					
Non-analogous	14	8	0.585	-	-
Inhibitor	9	9			
Sex					
Female	14	10	1	-	-
Male	13	8			
CD4+ (cells/mm^3)					
<200	1	0	0.077	0.049	5.05
201-500	13	4			
>501	11	13			

[#]Chi-square test (P<0.25); [*]Logistic regression (P<0.05). HCV: hepatitis C virus; HIV: human immunodeficiency virus; HAART: highly active antiretroviral therapy.

DISCUSSION

The treatment of HCV has been revolutionized by the new direct antiviral acting drugs (DAA), however many countries still use pegylated interferon-based regimens; therefore, it is of interest to evaluate the factors associated with SVR using these regimens.

In recent years, several studies about the *MDR1* gene have sought to understand the role of SNPs in this gene and the development of diseases, as well as response to drug treatment. The literature reports an association between the C3435T polymorphism of the *MDR1* gene and various diseases. Rose et al. suggest a regulatory role of *MDR1* gene expression in patients with hepatopathies[24]; other studies about this SNP have shown that the C allele is associated with a better prognosis in patients with hepatitis C and in patients with hepatocellular carcinoma[1,25]. On the other hand, in other pathological conditions such as HIV infection, the T allele appears to confer a better prognosis; HIV-infected patients homozygous for the TT genotype, when treated with HAART, have shown significant increases in CD4 cell count and better response to the treatment of hepatitis C[11,15,29].

The present study analyzed the *MDR1* gene polymorphism C3435T in HCV-HIV coinfected patients. The results showed similarity in the prevalence of the C allele and the T allele in the population studied, with CT being the most prevalent genotype in these patients. Among the patients with HCV infection who underwent treatment with pegylated interferon-alpha and ribavirin, our findings corroborate the results found by Timucin et al., which also showed a higher frequency for the CT genotype among these patients[25]. Some studies claim that the presence of the T allele generates no response to treatment, however we did not find such an association; furthermore, the presence of this allele is also associated with development of some type of cancers such as hepatocellular carcinoma, prostate cancer, ovarian cancer and others[28].

Besides genetic polymorphisms, other variables may have a role in the response to antiviral therapy with pegylated interferon-alpha and ribavirin in patients with hepatitis C[10,14]. In this study, multivariate analysis showed an association between CD4 cell count and response to treatment, where individuals with scores lower than 500 cells/mm^3 are five times more likely to not have a SVR after treatment with pegylated interferon and ribavirin. No association between SVR and *MDR1* SNP rs1045642 genotype was observed.

One limitation of this study was the relatively low number of patients treated with pegylated interferon-alpha and ribavirin, therefore it would be interesting to evaluate the effects of this and other polymorphisms in a larger cohort of HCV-HIV co-infected patients. Additionally, studies including patients from different geographical regions would also be valuable to better understand the role of genetic factors in HCV and improve the treatment and prognosis of patients with HCV-HIV co-infection, mainly in the DAA era.

ACKNOWLEDGMENT

The authors thank the Graduate Program in Medicine: Hepatology of UFCSPA for support; we also thank Grasiela Agnes from the Laboratory of Molecular Biology of UFCSPA for technical assistance, and the Adult Infectious Diseases Sector of Hospital Nossa Senhora da Conceição for assistance.

Authors' contributions

Veiga ABG, Kretzmann Filho NA, Mattos AA and Tovo CV contributed to study conception and design; Marasca GS, Kliemann D, Machado AL, Souza ACS, Veiga ABG and Kretzmann Filho NA contributed to data acquisition, data analysis and interpretation; Marasca GS, Veiga ABG, Mattos AA and Tovo CV contributed to writing of article, editing, reviewing and final approval of the article.

Marasca GS, Machado AL, Kretzmann Filho NA, Souza ACS, Mattos AA, Kliemann D, Tovo CV, Veiga ABG. Frequência do polimorfismo rs1045642 (C3435T) do gene *MDR1* em pacientes coinfectados HCV-HIV. Arq Gastroenterol. 2016,53(4):246-9.

RESUMO - ***Contexto*** - Em virtude da elevada prevalência da coinfecção pelos vírus da hepatite C (HCV) e da imunodeficiência humana (HIV) e às inúmeras complicações que esses vírus acarretam, é fundamental o maior entendimento do comportamento biológico dos mesmos. O polimorfismo de nucleotídeo único rs1045642 C3435T do gene de resistência a múltiplas drogas *MDR1*, no qual ocorre modificação do códon ATC para ATT, parece estar relacionado à resposta virológica sustentada ao tratamento do HCV em coinfectados HCV-HIV. ***Objetivo*** - Mapear o polimorfismo C3435T do gene *MDR1* em pacientes coinfectados HCV-HIV e correlacionar com dados clínicos e laboratoriais. ***Métodos*** - Foram analisados 99 pacientes coinfectados HCV-HIV. A identificação molecular do polimorfismo de nucleotídeo único rs1045642 do gene *MDR1* foi realizada pela técnica de PCR em tempo real (qPCR) alelo-específico com *primers* e sondas específicos para a identificação desse polimorfismo. Fatores de risco para a aquisição do HCV e a resposta virológica sustentada ao tratamento do HCV com interferon-alfa peguilado e ribavirina foram analisados. ***Resultados*** - Dentre os pacientes avaliados, 54 (54,5%) eram do gênero masculino e 45 (45,5%) do gênero feminino. A média de idade foi de 46,1 anos (±9,8). As frequências dos genótipos CC, CT e TT foram 28,3%, 47,5% e 24,2% respectivamente, e as frequências alélicas foram 52% para alelo C e 48% para alelo T. Não houve associação entre o gene *MDR1* e a resposta virológica sustentada (*P*=0,308). ***Conclusão*** - Neste estudo, o polimorfismo C3435T no gene *MDR1* não apresentou associação com a resposta virológica sustentada ao tratamento em indivíduos coinfectados HCV-HIV.

DESCRITORES - Hepacivirus. HIV. Polimorfismo de nucleotídeo único. Glicoproteína P. Coinfecção. Interferon-alfa. Ribavirina.

REFERENCES

1. Baldissera VD, de Mattos AA, Coral GP, de Araujo FB, Marroni CA, de Mello Brandão AB, et al. Evaluation of the C3435T polymorphism in the *MDR1* gene in patients with hepatocellular carcinoma. Ann Hepatol. 2012;11:899-906.
2. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 1996;24:289-93.
3. Bodor M, Kelly EJ, Ho RJ. Characterization of the human *MDR1* gene. AAPS J 2005;7:E01-05.
4. Brasileiro-Filho G, Guimarães RC, Bogliolo L. Distúrbios do crescimento e da diferenciação celular. In: Brasileiro-Filho G, editor. Patologia Clínica. Rio de Janeiro: Guanabara Koogan; 1998. p.148-92.
5. Callen DF, Baker E, Simmers RN, Seshadri R, Roninson IB. Localization of the human multiple drug resistance gene, *MDR1*, to 7q21.1. Hum Genet. 1987;77:142-4.
6. Chen CJ, Clark D, Ueda K, Pastan I, Gottesman MM, Roninson IB. Genomic organization of the human multidrug resistance (*MDR1*) gene and origin of P-glycoproteins. J Biol Chem. 1990;265:506-14.
7. Da Silva GK, Vianna P, Veit TD, Crovella S, Catamo E, Cordero EA, et al. Influence of HLA-G polymorphisms in human immunodeficiency virus infection and hepatitis C virus co-infection in Brazilian and Italian individuals. Infect Genet Evol. 2014;21:418-23.
8. Dean M, Hamon Y, Chimini G. The human ATP-Binding Cassette (ABC) transporter Superfamily. J Lipid Res. 2001;42:1007-17.
9. Dean M, Rzhetsky A, Allikmets R. The human ATP-Binding Cassette (ABC) transporter Superfamily genome. Genome Res. 2001;11:1156-66.
10. Eicher C, Dewweth A, Kirchner B, Warmann SW, Fuchs J, Armeanu-Ebinger S. Development of a drug resistance model for hepatoblastoma. Int J Oncol. 2011;38:447-54.
11. Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, Chave JP, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. Lancet. 2002;359:30-6.
12. Hall CS, Charlebois ED, Hahn JA, Moss AR, Bangsberg DR. Hepatitis C virus infection in San Francisco's HIV-1-infected urban poor. Int J Gen Med. 2004; 19:357.
13. Hoffemeyer S, Burk O, Von Richter O, Arnold HP, Brockmöller J, Johne A, et al. Functional polymorphisms of the human multidrug resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci. 2000;97:3473-8.
14. Hoofnagle JH, Wahed AS, Brown RS Jr, Howell CD, Belle SH, Virahep-C Study Group.Early changes in hepatitis C virus (HCV) levels in response to peg-interferon and ribavirin treatment in patients with chronic HCV genotype 1 infection. J Infect Dis. 2009;199:1112-20.
15. Hulgan T, Donahue JP, Hawkins C, Unutmaz D, D'Aquila RT, Raffanti S. Implications of T-cell P-glycoprotein activity during HIV-1 infection and its therapy. J Acquir Immune Defic Syndr. 2003;34:119-26.
16. Iranpur VM, Esmailizadeh AK. Rapid extratction of high quality DNA from whole blood stored at 4ºC for long period. Protocol Online 2010. Available from: http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html
17. Martins T, Narciso-Schiavon JL, Shiavon, LL. Epidemiologia da infecção pelo vírus da hepatite C. Rev Assoc Med Bras. 2011;57:107-12.
18. Mendes-Corrêa MCJ, Barone AA. Hepatitis C in patients co-infected with human immunodeficiency virus: a review and experience of a Brazilian ambulatory. Rev Inst Med Trop S Paulo. 2005;47:59-64.
19. Ministério da Saúde, Brasil. Boletim Epidemiológico Hepatites Virais. Ano III, nº 1 2012. 173p.
20. Ministério da Saúde, Brasil. Boletim Epidemiológico HIV-AIDS. Ano III, nº 1 2014. 82p.
21. Ng WF, Sarangi F, Zastawny RL, Venot-Drebot L, Ling V. Identification of members of the P-glycoprotein multigene family. Mol Cell Biol. 1989;9:1224-32.
22. Pereira LM, Martelli CM, Moreira RC, Merchan-Hamman E, Stein AT, Cardoso MR, et al. Prevalence and risk factors of Hepatitis C virus infection in Brazil, 2005 through 2009: a cross-sectional study. BMC Infect Dis. 2013;13:60.
23. Rockstroh JK. Should HIV/HCV Co-infected patients with severe hepatitis be treated for hepatitis C? Presse Med. 2005;34:1585-8.
24. Rose JE, Libbrecht L, Geuken M, Jansen PL, Roskams TA. High expression of *MDR1*, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. J Pathol. 2003;200:553-56.
25. Timucin M, Alagozlu H, Ozdemir S, Ozdemir O. Association between ABCB1 (*MDR1*) Gene polymorphism and unresponsiveness combined therapy in chronic hepatitis C virus. Hepat Mon. 2012;13:27522.
26. Tovo CV, Dos Santos DE, de Mattos AZ, de Almeida PR, de Mattos AA, Santos BR. Ambulatorial prevalence of hepatitis B and C markers in patients with human immunodeficiency virus infection in a general hospital. Arq Gastroenterol. 2006;43:73-6.
27. Van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P, et al. *MDR1* P-Glycoprotein Is a Lipid Translocase of Broad Specificity, while *MDR3* P-glycoprotein specifically translocates phosphatidylcholune. Cell. 1996;87:507-17.
28. Yan PW, Huang XE, Yan F, Xu L, Jiang Y. Influence of *MDR1* gene codon 3435 polymorphisms on outcome of platinum-based chemotherapy for advanced non small cell lung cancer. Asian Pac J Cancer Prev. 2011;12:2291-4.
29. Zhu P, Zhu Q, Zhang Y, Ma X, Li Z, Chen J, et al. ABCB1 Variation and Treatment Response in AIDS Patients: Initial Results of the Henan Cohort. PLoS ONE 2013;8:e55197.