

# EFFECT OF HFE GENE POLYMORPHISM ON SUSTAINED VIROLOGICAL RESPONSE IN PATIENTS WITH CHRONIC HEPATITIS C AND ELEVATED SERUM FERRITIN

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**ABSTRACT** – *Context* - Abnormal serum ferritin levels are found in approximately 20%-30% of the patients with chronic hepatitis C and are associated with a lower response rate to interferon therapy. *Objective* - To determine if the presence of HFE gene mutations had any effect on the sustained virological response rate to interferon based therapy in chronic hepatitis C patients with elevated serum ferritin. *Methods* - A total of 44 treatment naïve patients with histologically demonstrated chronic hepatitis C, all infected with hepatitis C virus genotype non-1 (38 genotype 3; 6 genotype 2) and serum ferritin above 500 ng/mL were treated with interferon (3 MU, 3 times a week) and ribavirin (1.000 mg, daily) for 24 weeks. *Results* - Sustained virological response was defined as negative qualitative HCV-RNA more than 24 weeks after the end of treatment. Serum HCV-RNA was measured by qualitative in house polymerase chain reaction with a limit of detection of 200 IU/mL. HFE gene mutation was detected using restriction-enzyme digestion with *RsaI* (C282Y mutation analysis) and *BclI* (H63D mutation analysis) in 16 (37%) patients, all heterozygous (11 H63D, 2 C282Y and 3 both). Sustained virological response was achieved in 0 of 16 patients with HFE gene mutations and 11 (41%) of 27 patients without HFE gene mutations ( $P = 0.002$ ; exact Fisher test). *Conclusion* - Heterozigosity for H63D and/or C282Y HFE gene mutation predicts absence of sustained virological response to combination treatment with interferon and ribavirin in patients with chronic hepatitis C, non-1 genotype and serum ferritin levels above 500 ng/mL.

**HEADINGS** – Polymorphysm, genetic. Hepatitis C, chronic. Ferritins. Interferons.

## INTRODUCTION

The Brazilian Public Health System provides antiviral treatment free of cost for selected patients with chronic hepatitis C virus (HCV) infection. Current government guidelines indicates 48 weeks of pegylated interferon (PEG-IFN) plus ribavirin (RBV) for patients with HCV genotype 1 and 24 weeks of conventional interferon (IFN) plus RBV for those with HCV genotypes 2 or 3<sup>(7)</sup>. Major predictive factors for sustained virological response (SVR) to IFN based treatment are well established for genotype 1 infected patients, including liver fibrosis stage, viral load, hepatic steatosis, body mass index, insulin resistance and IL-28 polymorphism<sup>(14, 19)</sup>. However, predictive factors for SVR are not so clearly defined for genotypes 2 or 3, since recent studies showed that viral load, insulin resistance

and IL-28 polymorphism failed to predict SVR to IFN based therapy in these patients<sup>(30)</sup>.

Feder et al.<sup>(13)</sup>, in 1996, discovered the HFE gene and two of its polymorphisms, C282Y and H63D, which were clearly associated with higher prevalence of elevated serum ferritin, transferrin saturation, and genetic hemochromatosis. It is well known that 20%-30% of patients with chronic hepatitis C have serum markers of iron overload, but not an increased prevalence of HFE polymorphisms<sup>(4, 5, 10)</sup>. Elevated serum ferritin has long been recognized as a marker of poor response to antiviral treatment in chronic HCV infected patients<sup>(3, 12, 16)</sup>, however the mechanism underlying this effect is not clear and the role of HFE polymorphisms has not yet been established. Interestingly, there is intriguing evidence of an association between HFE polymorphism and higher SVR rates<sup>(8, 31)</sup>, but this issue has not been specifically investigated among

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HCV patients with high serum ferritin, which are recognized as having difficult to treat disease.

Thus, the aim of the present study was to verify the prevalence of HFE gene polymorphisms and its impact on SVR rates among patients with HCV genotypes 2 or 3 and elevated serum ferritin treated with IFN/RBV in the setting of the Brazilian Public Health System. The finding of a reliable predictor of treatment outcome in the present study could help to improve government guidelines and refine treatment strategies, saving costs and avoiding futile therapy.

## METHODS

### Study design

A historical cohort study was conducted to evaluate the association between HFE gene polymorphisms and the response rate to IFN/RBV, using a cross-sectional design. The study was conducted in accordance with the ethics principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines, being approved by appropriate Institutional Review Boards. Written informed consent was obtained from all patients. All authors had access to the data, were actively involved in its analysis and interpretation, and approved the final manuscript.

### Patients

Forty-four patients were recruited from the Viral Hepatitis Outpatient Clinic of Hospital de Clínicas de Porto Alegre, RS, Brazil a tertiary care institution in Porto Alegre, South of Brazil. Key inclusion criteria were: age equal or above 18 years old, detectable HCV RNA by polymerase chain reaction (PCR; Roche Amplicor; lower limit of detection: 50 IU/mL), liver biopsy consistent with the diagnosis of chronic hepatitis C 1 year or less before treatment start, baseline serum ferritin above 500 ng/mL, HCV genotype 2 or 3 infection, complete treatment with IFN 3 MU 3 times a week plus RBV 1000 mg daily for 24 weeks with compliance equal or above 80% of the IFN/RBV dose and intended duration (80/80/80 rule), with a valid PCR result after 6 months of follow-up to establish the occurrence or not of SVR. Demographic data was collected at baseline. Biochemical and haematological parameters were assessed at baseline and every 4 weeks during treatment, and 24 weeks after the end of therapy. Patients with cirrhosis were eligible provided that they had compensated liver disease (Child-Pugh A). Main exclusion criteria were: co-infection with human immune deficiency virus and/or hepatitis B virus, liver disease attributable to a cause other than HCV infection, ultrasound compatible with hepatocellular carcinoma, and suboptimal IFN/RBV treatment (less than 80% of the expected IFN/RBV dose and/or less than 80% of the intended treatment duration).

The study was approved by the ethics committee of the Hospital de Clínicas de Porto Alegre (2004).

### Exposure and outcomes

HFE gene mutations were determined using restriction fragment length polymorphism. Patients were considered

exposed if heterozygous or homozygous for C282Y and/or H63D HFE polymorphisms. Primary outcomes were the rate of end of treatment virological response (EoTVR) and SVR between exposed and non-exposed, defined as undetectable HCV RNA by qualitative PCR assay at the end of treatment and after 24-weeks of follow-up, respectively. Co-primary outcome was the degree of liver fibrosis between exposed and non-exposed, assessed by an experienced pathologist using Metavir score, with cirrhosis considered as Metavir F3 or F4. Hepatic iron deposition was estimated using standard Perls iron stain protocol.

### Statistical Methods

Patients were grouped as positive or negative for HFE gene mutations. Differences in baseline characteristics and outcomes between patients with and without HFE gene mutations were examined by Fisher's exact test (categorical variables), and Kruskal-Wallis (continuous variables). All statistical tests were two-sided and a *P*-value below 0.05 was considered significant. The Statistical Package for the Social Sciences (SPSS 13.0, Chicago, IL) was used for statistical analyses.

## RESULTS

Among the 44 included patients, 40 (90.9%) were male, all were caucasians, with a mean age of  $48.4 \pm 7.7$  years (range 26-63). Thirty-eight were HCV genotype 3 (86.4%) and 6 genotype 2 (13.6%). Metavir fibrosis score was distributed as follows: F0 (*n* = 2); F1 (*n* = 8); F2 (*n* = 1); F3 (*n* = 5) and F4 (*n* = 28). Distribution of iron in the liver was restricted to Kupffer cells, ranging from absent to moderate deposition, with no histological diagnosis of hemochromatosis. Overall, mean baseline serum ferritin was 1.097 ng/mL (standard deviation  $\pm$  552; range 500-2.865). Mean baseline serum transferrin saturation was 50.5% (standard deviation  $\pm$  17.7; range 25%-86%). All patients had elevated baseline serum ALT, with a mean value of 229.6 UI/mL (standard deviation  $\pm$  120.3; range 55-516). HFE gene mutations were detected in 16 patients (36%), with the following distribution: heterozygous H63D (*n* = 11); heterozygous C282Y (*n* = 2), and compound heterozygous H63D plus C282Y (*n* = 3). No patient was homozygous for any of the studied mutations. EoTVR and SVR were observed in 22 (50%) and 11 (25%) of the 44 treated patients, respectively. Comparison of demographic and disease characteristics between patients with versus without HFE gene mutations are depicted in Table 1.

## DISCUSSION

In this study we found an overall SVR rate of 25% among patients with genotype 2 and 3. Remarkably, none of the 16 patients with HFE polymorphisms achieved SVR as opposed to 39% observed among the 28 individuals without this genetic marker. Although our SVR rates were lower than the 64%-79% reported in the IFN/RBV registration trials for HCV genotypes 2 and 3<sup>(15, 27)</sup> it was similar to that obtained in other Brazilian

TABLE 1. Characteristics of the 44 patients according to HFE gene polymorphisms status

	With HFE polymorphisms (n = 16)	Without HFE polymorphisms (n = 28)	P-value
Mean age ± SD (years)	50 ± 6	48 ± 8	0.33
Male gender, n (%)	15 (94)	25 (89)	0.54
HCV genotype 3, n (%)	14 (88)	24 (86)	0.62
Baseline serum ALT ± SD (UI/mL)	221 ± 88	239 ± 138	0.66
Baseline serum ferritin ± SD (ng/dL)	1.171 ± 587	1.060 ± 546	0.68
Baseline serum TS ± SD (%)	57 ± 18	46 ± 17	0.05
Cirrhosis (metavir F3/F4), n (%)	12 (75)	21 (75)	1.0
EOT virological response, n (%)	4 (25)	18 (64)	0.03
Sustained virological response, n (%)	0	11 (39)	0.003

SD: standard deviation; HCV: hepatitis C virus; ALT: alanine aminotransferase; TS: transferrin saturation; EOT: end of treatment

studies that included real life cohorts. Indeed, a retrospective analysis of 173 patients with genotype 2 or 3 treated with conventional interferon alpha and ribavirin in the Public Health System of the State of Rio Grande do Sul, Brazil, showed an overall SVR rate of only 39%<sup>(1)</sup>.

The lower SVR found in the present study could also be related to some characteristics of our cohort, namely the fact that 75% of the patients presented with advanced fibrosis and that only those with elevated serum ferritin at baseline were included. The reason for including only patients with serum ferritin levels above 500 ng/dL was to select genotypes 2 and 3 patients with really difficult to treat disease. Since transferrin saturation was not available for all patients, serum ferritin level above 500 ng/dL was also the best available marker for iron overload, despite the fact that, in this situation, less than 10% of patients are expected to have a genetic iron overload disorder<sup>(20)</sup>. Individuals with treatment compliance outside the 80/80/80 rule were excluded to avoid introducing confounding factors such as dose reduction or treatment interruption, which could bias any potential relationship between SVR and HFE gene polymorphisms.

There was significantly higher serum transferrin saturation among individuals with HFE polymorphism, despite no difference in serum ferritin levels. However, the absence of SVR observed in the group of individuals with this genetic marker was probably not directly related to iron overload, since most studies, so far, failed to report a significant difference in hepatic iron concentration between HCV patients with or without SVR after IFN/RBV treatment<sup>(23, 25, 29, 33)</sup>. Moreover, therapeutic phlebotomies generally did not increase SVR, indicating that reduction of body iron stores was not associated with treatment outcome<sup>(11, 21)</sup>.

It is possible that ferritin, as an acute phase reactant, behave as a marker of more active and advanced liver disease, instead of representing body iron content. In this regard, it has been reported that patients with chronic hepatitis C and high serum ferritin have significantly more liver inflammation and fibrosis compared to those with normal serum ferritin values<sup>(2, 3, 35)</sup>. This finding could explain the fact that the majority of our patients were cirrhotic. The recent finding of more rapid fibrosis progression among patients with chronic hepatitis C

infected with HCV genotype 3 could also explain the high percentage of those with advanced disease in our cohort, since almost 90% of our patients had this genotype<sup>(9)</sup>. There was no patient with hemochromatosis in the present study, further supporting the fact that the mere presence of high serum ferritin in HCV infected patients does not necessarily correlates with condition.

Data on the possible relationship between carriage of the HFE gene mutations and response to IFN based therapy have been controversial<sup>(5, 22, 24, 32, 35, 37)</sup>. Previous studies showed an association between H63D HFE polymorphism and higher SVR rates in patients with chronic hepatitis C<sup>(6)</sup>. This relationship could be due to the fact that the HFE gene is located on the short arm of chromosome 6, close to the major histocompatibility complex (MHC). In this regard, it is well known that MHC genes encode the human leukocyte antigens, which are important in antigen presentation and regulation of CD8 + and CD4 + T cells<sup>(18, 34)</sup>. Previous studies have shown an impact of MHC gene variants and response to therapy in chronic HCV infection<sup>(17, 28, 31, 36)</sup>.

Our finding contradicts the previously described association between H63D mutation and higher SVR<sup>(6)</sup>. The most seemingly explanation for the negative effect of HFE on SVR seen in the present study might be related to the unique characteristics of our cohort, comprised exclusively of individuals infected with genotype non-1 with elevated serum ferritin levels. It is also possible that HFE mutations, when associated with markers of iron overload such as serum ferritin and/or transferrin saturation, behave differently in respect to its gene expression profile.

If our results prove to be correct in future studies with larger samples and a prospective design, it could have a major impact on current Brazilian Guidelines, which still indicates IFN/RBV as first line therapy for HCV patients infected with genotypes 2 or 3. Indeed, PEG-IFN/RBV is only offered as initial therapy to HCV genotype 1 carriers and as second line therapy in HCV non-1 genotypes after failure of IFN/RBV. The finding of HFE polymorphism as a strong predictor of non-response in our sample should lead to further studies in order to better clarify if the negative impact of HFE on SVR of patients with genotypes 2 or

3 and high serum ferritin could be neutralized using more potent therapy.

The limitations of the present study have to be addressed, and are mainly related to the relative small sample size, retrospective design and absence of other predictors of response to therapy, such as viral load and IL-28B. Nevertheless, it is the only study conducted so far in this

patient population chronically infected with HCV genotypes 2 and 3 with high serum ferritin. Moreover, both viral load and IL-28B do not seem to have a major impact on SVR in the majority of non-1 HCV genotypes<sup>(26)</sup>. Finally, we believe the relationship between HFE mutations and absence of SVR in this cohort merits future research to clarify this seemingly important association.

Coelho-Borges S, Cheinquer H, Wolff FH, Cheinquer N, Krug L, Ashton-Prolla P. Efeito do polimorfismo do gene HFE sobre a resposta viral sustentada em portadores de hepatite crônica C com ferritina sérica elevada. *Arq Gastroenterol.* 2011;49(1):9-13.

**RESUMO – Contexto** - Níveis séricos anormais de ferritina são encontrados em aproximadamente 20%-30% dos pacientes com hepatite crônica C e estão associadas a uma baixa taxa de resposta à terapia com interferon. **Objetivo** - Avaliar a associação entre a presença de mutações do gene HFE e a taxa de resposta virológica sustentada ao interferon em pacientes portadores de hepatite crônica C com ferritina sérica elevada. **Métodos** - Um total de 44 pacientes, virgem de tratamento, infectado pelo vírus da hepatite C de genótipos não-1 (38 genótipo 3; 6 genótipo 2) e ferritina sérica acima de 500 ng/mL foi tratado com interferon (3 MU, três vezes por semana) e ribavirina (1000 mg/dia) por 24 semanas. Resposta virológica sustentada foi definida como HCV-RNA indetectável 24 semanas após o fim do tratamento. Foi utilizado técnica de reação em cadeia da polimerase em tempo-real com limite de detecção de 200 UI/mL. **Resultados** - Mutações do gene HFE foram detectadas por “restriction-enzyme digestion” com *RsaI* (análise de mutação C282Y) e *BclI* (análise de mutação H63D) em 16 pacientes (37%), todos heterozigotos (11 H63D, 2 C282Y e 3 ambos). Resposta virológica sustentada foi alcançada em 0 de 16 pacientes com mutações do gene HFE e 11 (41%) dos 27 pacientes sem mutações do gene HFE ( $P = 0,002$ ; teste exato de Fisher). **Conclusão** - A heterozigose para os genes H63D e/ou C282Y HFE está associada à redução significativa da taxa de resposta virológica sustentada ao tratamento com interferon e ribavirina em pacientes com hepatite crônica C, genótipo não-1 e com níveis séricos de ferritina acima de 500 ng/mL.

**DESCRITORES** – Polimorfismo genético. Hepatite C crônica. Ferritinas. Interferons.

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