CLINICAL IMPACT OF *HFE* MUTATIONS IN PORTUGUESE PATIENTS WITH CHRONIC HEPATITIS C

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Introduction

Chronic hepatitis C (CHC) is often associated with alterations in iron and lipid metabolisms, which may affect the long-term prognosis and the response to antiviral treatment (1,2). Some studies have suggested that the occurrence of *HFE* mutations may contribute to modulate these metabolisms in CHC (3,4). In this study, the prevalence of two common *HFE* mutations (C282Y and H63D) was determined in a group of Portuguese CHC patients and the findings were correlated with their clinical, histological and virological features.

Methods

- Clinical parameters were measured by standard techniques: AST, ALT, GGT, lipid profile (LDL, HDL, total cholesterol and triglycerides), iron, ferritin, transferrin and saturation, insulin, glucose, HOMA- IR and peptide-C .
- 82 patients were treated with SOC (Pegynterferon + Ribavirin).
- HCV-RNA was determined by PCR and genotype by Inno-Lipa.
- Liver steatosis, fibrosis stage and degree of inflammation (grading) were assessed by liver biopsy (Peter Scheuer score).
- *HFE* polymorphisms, H63D and C282Y, were analyzed by PCR-RFLP.
- Antioxidant potencial (tGSH/GSSG Ratio) was evaluated by spectrofluorimetry.
- Statistical analysis was performed by SPSS 19.0 (level of significance $p<0.05$). Clinical data results were corrected for age and BMI using a General Linear Model – Univariate.

Population

- One hundred and eighty three CHC patients were enrolled in this study.
- Population clinical parameters, liver histology, virus genotype and type of anti-viral response are described in Tables 1-4.
- Patients exclusion criteria: other chronic liver diseases, alcohol ingestion >40g/day, HIV infection, metabolic and autoimmune diseases.

Table 1: Population Clinical Parameters

Clinical parameters *(normal distribution)*	Mean	Std Deviation	Clinical parameters *(non normal distribution)*	Median	Min.	Max.
Age (years)	45.84	11.46	HCV-RNA (UI/mL)	267857	0	$1.5E^6$
BMI (Kg/m^2)	25.45	3.96	Triglycerides (mmol/L)	1.1	0.3	13.6
Total Cholesterol (mmol/L)	4.43	1.10	Alcaline Phosphatase (µg/dL)	66	27	395
HDL (mmol/L)/ LDL (mmol/L)	1.34/ 2.49	0.53/ 0.97	AST / ALT (UI/L)	47/ 75	16/ 16	654/ 505
Transferrin (µg/dL)/ Sat (%)	303.50/ 44.41	74.91/ 20.66	Gama GT (UI/L)	45	10	1139
Haptoglobin (mg/dL)	92.63	39.48	Ferritin (ng/mL)	178	9.4	2479
Ceruloplasmin (mg/dL)	37.44	12.15	Insulin (µU/mL)	9.9	1.6	81.2
tGSH (µg/mL)	21.34	10.49	Glycemia (mg/dL)	87	61	203
GSSG(µg/mL)	3.73	1.82	HOMA (µU/mL xmg/dL)	2.1	0.3	19.6
tGSH/GSSG Ratio	6.24	2.31	Iron (µg/dL)	117.5	30	349

Table 2. Liver Histology

Parameter	Stage	N (%)	Total (N)
Fibrosis (*staging*)	F1/2	90 (76.9)	117
	F3/4	27 (23.1)	
Steatosis	With	72 (75.0)	96
	Without	24 (25.0)	
Steatosis Grade	Mild (1+2)	52 (72.2)	72
	Moderate or severe (3+4)	20 (27.8)	
Inflammation (*grading*)	Mild (1-3)	25 (22.3)	112
	Moderate (4-6)	87 (77.7)	
	Severe (>6)	(0)	

Table 3. Virus Genotype

Type	Subtype	N (%)
1	1a	47 (27.5)
	1b	64 (37.4)
2	2,2a, 2c, 2a+2c	4 (2.3)
3	3a	39 (22.8)
4	4, 4c, 4d, 4a+4c+4d, 4c+4d	16 (9.4)
other	1a+3a	1 (0.6)
Total		171

Table 4: Type of Antiviral Response (n= 82)

Non Responders (NR) /Relapsers (RR)	21 (25.6%) / 8 (9.8%)
Sustained viral Response (SVR)	53 (64.6%)

Results

✓ ***HFE* polymorphisms frequency**
*HFE*_H63D and C282Y genotype frequency in the CHC studied population is summarized in Table 5.

Table 5. *HFE*_H63D and C282Y genotype frequency in the CHC studied population (N= 183)

HFE_H63D	N (%)	HFE_C282Y	N (%)
HH	121 (66.1)	CC	173 (94.5)
HD	54 (29.5)	CY	10 (5.5)
DD	8 (4.4)	YY	0

✓ ***HFE* polymorphisms and the type response to antiviral therapy**
No significant difference was found comparing *HFE* polymorphisms and the type of antiviral response.

✓ ***HFE* polymorphisms and clinical or histological data:**

Table 6. Association between *HFE*_H63D and Inflammation

Parameter	Stage	H63D (HH) N (%)	H63D (HD+DD) N (%)	*p* Value
Inflammation (*grading*)	Mild (1-3)	10 (13.5)	15 (39.5)	0.004
	Moderate (4-6)	64 (86.5)	23 (60.5)	

- ***HFE*_H63D:** regarding all the clinical and histological data, we observed a decrease in the degree of inflammation (Table 6) and in tGSH/GSSG ratio, and an increase in total cholesterol (Table7; Fig. 1 and 2) in CHC patients presenting the H63D mutant allele (HD+DD) comparing to HH individuals.

Table 7. Association of *HFE*_H63D with Total Cholesterol and tGSH/GSSG Ratio

Clinical parameters *(normal distribution)*	*HFE*_H63D	Mean	Std Deviation	N	*p* Value
Total Cholesterol (mmol/L)	HH	4.289	0.984	108	0.042
	HD+DD	4.689	1.255	57	
tGSH/GSSG Ratio	HH	6.87	2.15	31	0.006
	HD+DD	4.81	2.08	14	

Fig. 1

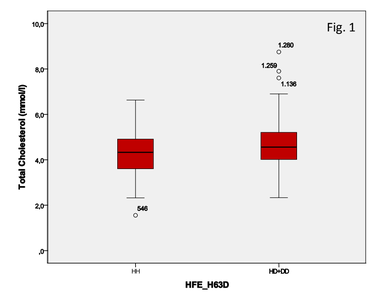
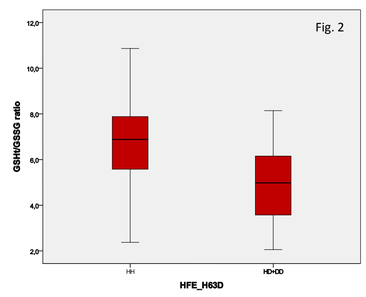


Fig. 2



- ***HFE*_C282Y:** Our study revealed that heterozygous C282Y had lower Total Cholesterol ($p<0.0001$) and higher serum Iron and Transferrin Saturation levels ($p<0.0001$ and 0.006, respectively); (Table 8; Fig. 3-5).

Table 8: Association of *HFE*_C282Y with Total Cholesterol, Transferrin saturation and serum Iron level

Clinical parameters *(normal distribution)*	HFE_C282Y	Mean	Std Deviation	N	*p* Value
Total Cholesterol (mmol/L)	CC	4.48	1.11	156	<0.0001
	CY	3.72	0.38	9	
Transferrin saturation (%)	CC	43.32	20.50	86	0.006
	CY	63.18	14.35	5	
Clinical parameters *(non normal distribution)*	**HFE_C282Y**	**Median**	**[min-max]**	**N**	***p* Value**
Iron (µg/dL)	CC	115	[30-349]	111	0.038
	CY	161	[99-220)	7	

Fig. 3

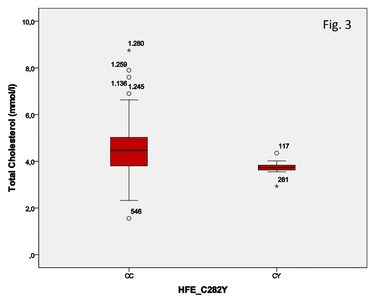


Fig. 4

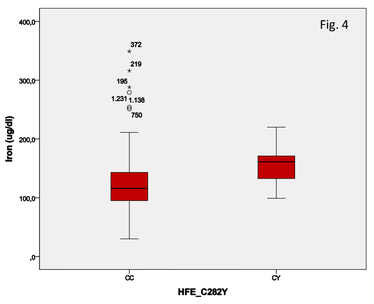
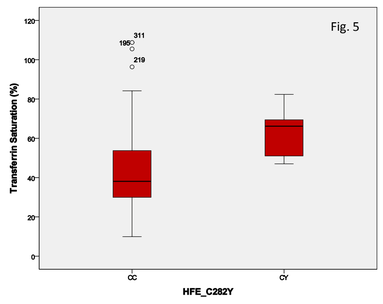


Fig. 5



Conclusions

✓ In this CHC population, the C282Y polymorphism was associated to higher serum iron levels. This biochemical phenotype was in turn observed in patients with higher fibrosis stages.
✓ C282Y was also found associated with lower total cholesterol, which in turn was observed in patients with more severe liver inflammatory and steatosis grade.
✓ On the other hand, the H63D polymorphism was found associated with higher total cholesterol levels and less necroinflammation. In addition, it was also associated with a decreased antioxidant potential (tGSH/GSSG ratio).
✓ These data suggest a relevant role of *HFE*_H63D and C282Y polymorphisms in CHC progression (liver fibrosis, inflammation and steatosis).
✓ We did not find association between these two *HFE* polymorphisms and the type of response to the anti-viral therapy (Pegynterferon + Ribavirin).

✓ **Association between Total Cholesterol, Iron and Transferrin Saturation and histological data or type of response to antiviral therapy** (Table 9)

- Total Cholesterol was found to be increased in patients with less necroinflammation and steatosis ($p=0.023$ and $p=0.046$), respectively.
- Higher serum iron levels are observed in patients with higher fibrosis stages (moderate and intense); ($p=0.042$).

Table 9. Association between Total Cholesterol and Iron and Histological data

Clinical parameters *(normal distribution)*	Histological data		Mean	Std Deviation	N	*p* Value
Total Cholesterol (mmol/L)	Inflamation (*grading*)	Mild (1-3)	4.92	1.16	23	0.023
		Moderate (4-6)	4.36	0.98	77	
	Steatosis Grade	Mild (1+2)	4.55	0.97	47	0.046
		Moderate and severe(3+4)	3.70	0.89	18	
Clinical parameters *(non normal distribution)*	**Histological data**		**Median**	**[min-max]**	**N**	***p* Value**
Iron (µg/dL)	Fibrosis (*staging*)	F1/2	114	[38-211]	52	0.042
		F3/4	139	[30-316]	17	

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