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Research paper

Thrombophilic genetic factors PAI-1 4G-4G and MTHFR 677TT as risk factors of alcohol, cryptogenic liver cirrhosis and portal vein thrombosis, in a Caucasian population

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ABSTRACT

The thrombophilic genetic factors (THRGFs), PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q and Prothrombin 20210A, were studied as risk factors in 865 Caucasian patients with liver cirrhosis, consecutively enrolled from June 2008 to January 2014. A total of 582 HCV, 80 HBV, 94 alcohol, (82 with more than one etiologic factor) and 191 cryptogenic patients with liver cirrhosis had been consecutively enrolled; 243 patients showed portal vein thrombosis (PVT). At least one of the above THRGFs was present in 339/865 patients (39.2%). PAI-1 4G-4G and MTHFR 677TT were the most frequent THRGFs, statistically significant in patients with alcohol, cryptogenic liver cirrhosis, and PVT: respectively 24 and 28, 50 and 73, and 65 and 83 (all chi-square tests > 3.84, and p values < 0.05). Two logistic regression analysis, using PAI-1 4G-4G and MTHFR 677TT, as dependent variable, confirmed the independent significant relationship of these THRGFs with alcohol, cryptogenic liver cirrhosis and PVT. PAI 1 and MTHFR 677 genotypes, deviated from those expected in populations in Hardy–Weinberg equilibrium (all p values < 0.05), in the subgroups of patients with alcohol, cryptogenic liver cirrhosis and presence of PVT. Our study shows the pivotal role of PAI-1 4G-4G and MTHFR 677TT in patients with alcohol, cryptogenic liver cirrhosis, and PVT, in a Caucasian population. In conclusion, thrombo and fibro-genetic mechanisms of PAI-1 4G-4G and MTHFR 677TT, could have a role in the development of liver cirrhosis, mainly in patients without HCV and HBV, and PVT.

1. Introduction

The thrombophilic genetic factors (THRGFs), PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q and Prothrombin 20210A, have been studied as risk factors in patients with liver cirrhosis, hepatocellular carcinoma (HCC) and portal vein thrombosis (PVT).

We have published three studies on the proportion of THRGFs in liver cirrhosis and HCC: MTHFR 677TT was found as significant risk factor of complications, after OLT for cryptogenic cirrhosis, in the first study (Pasta et al., 2006a); in the second, the same thing occurred in patients with liver cirrhosis and PVT (Pasta et al., 2006b), but PAI-1 was not analyzed in these first two studies. In the third study (D'Amico et al., 2009), MTHFR 677TT, PAI-1 4G-4G and Prothrombin 20210A were found as significant risk factors in HCC patients, mainly in the presence of PVT.

Many studies were published on the role of these THRGFs in the development the PVT (Janssen et al., 2000; Balta et al., 2002; Primignani et al., 2005; Primignani and Mannucci, 2008; Tsantes et al., 2008; Parikh et al., 2010) and liver fibrosis of various etiology (Adinolfi et al., 2005; Toniutto et al., 2008; Assy et al., 2005; Anstee et al., 2011; Perez-Pujol et al., 2012; Maharshak et al., 2011), but never to date, to our knowledge, in the same study, in a consecutive series of patients with liver cirrhosis.

Abbreviations: THRGFs, thrombophilic genetic factors; HCC, hepatocellular carcinoma; PVT, portal vein thrombosis.

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We planned this prospective study in order to study PAI-1, MTHFR 677TT, V Leiden 506Q and Prothrombin 20210A, in patients with liver cirrhosis of various etiologies with or without PVT.

2. Material and methods

2.1. Patients

Inclusion criteria: all Caucasian patients with diagnosis of liver cirrhosis, consecutively observed in the Medicine and Hepatology Department of Cervello Hospital of Palermo, from June 2008 to January 2014, were included.

Exclusion criteria: non-Caucasian patients, or with biliary cirrhosis, autoimmune cirrhosis, celiac disease, HCC, and other neoplasms were excluded.

The presence of PVT and the extension of the thrombosis to mesenteric or splenic vein were registered, and accepted when unambiguous diagnostic evidence was detected by proper imaging techniques.

All patients underwent endoscopy and the size of esophageal varices was recorded as large–medium/small-absent. All patients were asked if they had had episodes of gastrointestinal bleeding in their history.

The Local Human Research Committee has approved this study protocol.

2.2. Thrombophilic genetic factors and definition of thrombophilia

To evaluate the role of PAI-1, MTHFR 677, V Leiden 506Q and Prothrombin 20210A mutations, genotyping of these polymorphisms was performed by PCR-RFLP according with Patnaik et al. (2004), in heterozygous and homozygous status. We have defined genetic thrombophilia as the presence of at least one of the following THRGFs: PAI-1 4G-4G, MTHFR 677TT, V Leiden Q506, Prothrombin 20210A, as in our previous studies (Pasta et al., 2006b; D'Amico et al., 2009).

All patients signed an informed consent and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.3. Statistical analysis

Effect size and sample size calculations: we have assumed a relative risk increase of thrombophilia near 60%, from 25.5%, as found in the 94 controls of one of our studies (D'Amico et al., 2009), to 40% in patients with liver cirrhosis, with an enrollment ratio of 1:9, alpha error of 0.05 and beta error of 0.20; as a consequence, it would be necessary to include 837 patients, according to an interactive sample size calculator (Sample Size Calculator, 2014).

First, we looked at the differences between the proportions of each THRGF, associated to the clinical variables, using the contingency tables (2-way Contingency Table). We considered only statistically significant differences, if the chi square test was >3.84, and p value < 0.05.

Then we performed the logistic regression analysis, using an interactive web program (Logistic Regression, 2015), in which a dichotomous outcome was predicted by one or more variables. Logistic Regression Model fit values were calculated according to Hosmer–Lemeshow test (Hosmer and Lemeshow, 1989).

Any variable, whose chi square test was >3.84 and p-value < 0.05, as above described, was chosen as a candidate for inclusion in the multivariate analysis, as independent variable, where the single THRGF was considered as the dependent variable.

Moreover, we compared the observed frequencies of the THRGF genotypes, with those predicted in a population by Hardy–Weinberg equilibrium, using a web interactive calculator (Rodriguez et al., 2008).

3. Results

The whole group consisted of 865 patients: 582 HCV, 80 HBV, 94 alcohol (>80 ml/die for at least 5 years), and 191 cryptogenic; more than one etiologic factor was present in 82 patients. A total of 243 patients showed PVT; mesenteric and/or splenic vein thrombosis was present in 37 patients; large–medium, small and absent esophageal varices were present in 343, 310 and 212 patients respectively.

A total of 277 patients had had one or more bleeding episodes, and 4 patients showed thrombosis in other sites. Table 1 shows the main demographic and clinical characteristics and the frequency of the studied THRGFs in 865 patients with cirrhosis of various etiologies. A total of 339 patients (39.2%) showed thrombophilia (of whom 65 with more than 1 THRGF: 63 patients with 2 THRGFs, and 2 with 3 THRGFs): 173 PAI-1 4G-4G, 139 MTHFR 677TT, 61 V Leiden Factor, 33 Prothrombin G20210 A.

No one V with Leiden 506Q or Prothrombin 20210A homozygosis was present. The proportion of PAI-1 polymorphisms 4G-5G and 5G-5G was respectively 387 and 305. The proportion of MTHFR polymorphisms C677T and CC677 was, respectively, 422 and 303.

All clinical variables were tested, with 2-way contingency table analysis, versus the presence of the whole thrombophilia, or the single THRGF. The presence of thrombophilia was statistically significant in the patients with cryptogenic liver cirrhosis and PVT. Analyzing the relation of the single THRGF with the clinical variables, MTHFR 677TT and PAI-1 4G-4G was statistically more frequent in patients with alcoholic, cryptogenic liver cirrhosis, and PVT: respectively 24 and 28, 50 and 73, 65 and 83: all chi-square >3.85 and $p < 0.05$. Table 2 shows the corresponding odd ratios with 95% confidence intervals (OR, with 95% CI), of these three categories of patients.

Two logistic regression analysis, using PAI-1 4G-4G and MTHFR C677TT, as dependent variables, to evaluate the independent relationship with alcohol, cryptogenic liver cirrhosis and PVT, (whose chi-square tests were >3.84, and p value < 0.05, analyzed with 2-way contingency table), were performed. In this way, the alcohol, cryptogenic liver cirrhosis, and PVT were confirmed as clinical variables independently associated with PAI-1 4G-4G and MTHFR 677TT (Table 3). Logistic

Table 1
Thrombophilic genetic factors V Leiden, prothrombin G20210A, MTHFR C677TT and PAI-1 4G-4G, in relation to main demographic and clinical characteristics of 865 patients with cirrhosis of various etiology: HCV, HBV, alcohol (>80 ml/die for at least 5 years), and cryptogenic (i.e., without any of the above etiologic factors).

	Patients	At least 1 THRGF	V Leiden Factor	Prothrombin G20210A	MTHFR 677TT	PAI-1 4G-4G
Total	865 (100)	339 (100)	61 (100)	33 (100)	139 (100)	173 (100)
Male sex	477 (55.1)	195 (57.5)	34 (55.7)	19 (57.6)	82 (58.9)	107 (61.8)
HCV	582 (67.3)	169 (50.0)	35 (57.4)	22 (66.6)	72 (51.7)	72 (41.6)
HBV	80 (9.2)	36 (10.6)	8 (13.1)	(6) (18.1)	16 (11.5)	10 (5.7)
Alcohol	94 (10.1)	44 (13.0)	8 (13.1)	4 (12.1)	*24 (17.2)	*28 (15.3)
Cryptogenic	191 (22.1)	*130 (38.3)	18 (29.5)	11 (33.3)	*50 (36.0)	*73 (42.2)
Portal vein thrombosis	243 (28.1)	*139 (41.0)	23 (37.7)	13 (39.5)	*65 (46.7)	*83 (48.0)
Large–medium esophageal varices	343 (39.7)	145 (42.8)	24 (39.3)	16 (48.5)	64 (46.0)	71 (41.0)
Patients with bleeding episodes	277 (32.0)	75 (22.1)	20 (32.8)	15 (45.5)	45 (32.4)	51 (29.5)
Age: median (range)	59 (19–83)	58 (19–79)	59 (27–69)	58 (24–69)	56 (19–79)	56 (23–76)
PLT: median (range)	76 (23–255)	78 (32–255)	67 (42–134)	78 (34–110)	68 (32–234)	78 (34–255)

* Significant differences in patients with vs patients without thrombophilia or single THRGF: $\chi 2 > 3.85$ and $p < 0.05$, analyzed with 2-way contingency table analysis.