Human & Veterinary Medicine
International Journal of the Bioflux Society HVM

OPEN ACCESS
Research Article

Paraoxonase 1 activities and gene polymorphisms in non-alcoholic steatohepatitis – preliminary results of a pilot study

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Abstract. Introduction: Very few studies have evaluated the possible association between low Paraoxonase 1 (PON1) serum activities or certain expression of the *PON1* gene and non-alcoholic steatohepatitis (NASH). The aim of our study was to identify variations in PON1 activity and *PON1* gene polymorphisms in adult patients with NASH. Materials and methods: The study was performed on 17 patients with NASH, diagnosed by persistently elevated aminotransferase levels, ultrasonographic aspect of hepatic steatosis, negative markers for viral liver infection, no significant alcohol consumption and no other diseases that might influence the PON1 activity. We also studied an equal number of healthy subjects without liver disease or other pathology known to affect PON1 activity. Clinical data, routine laboratory data and serum activities of PON1 were assessed in all patients. By using the PCR-RFLP method, we evaluated the *PON1* gene polymorphisms L55M, Q192R, -108C>T, -909G>C and -162A>G. Results: We did not observe statistically significant differences between the NASH group and the control group regarding the serum activities of PON1. L55M polymorphisms presented the greatest variability between groups, the heterozygous variant being present in 41.2% of the NASH patients and in only 17.6% of the controls; the p-value was slightly above the statistical significance level (p=0.08), possibly due to the small size of the study groups. Conclusions: *PON1* genotype seems to influence the status of patients with NASH. Studies on large groups of patients and controls are required to confirm a possible role of *PON1* polymorphisms in the prediction and/or evolution of NASH.

Key Words: non-alcoholic steatohepatitis, paraoxonase-1, polymorphisms

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Introduction

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are a spectrum of diseases largely studied in the last decade, mainly due to the rapidly increasing incidence and the potential evolution towards cirrhosis and hepatocellular carcinoma (Ratziu 2015, Goh and McCullough 2016). Paraoxonase 1 (PON1) is an enzyme with antioxidant properties, capable of removing oxidised-lipidis, and, thus, to protect the human body against inflammation. The main PON1 activities are paraoxonase, arylesterase and lactonase, the others being promiscuous (Mackness and Mackness 2015). Its serum concentration and activities are modulated by enzyme polymorphisms, and it was proven that the Q192R polymorphism plays an important role in enzymatic activity, while L55M polymorphism affects the enzyme concentration and also the enzyme activity (Goswami et al 2009). There could be a relation between low serum levels and activities of PON1 and NAFLD/NASH, because it is shown that NASH is a disease induced by inflammation in the fatty liver (Farrell et al 2012).

Very few studies have evaluated the possible association between low levels of PON1 activity or certain expression of the PON1 gene and NASH (Baskol et al 2005, Baskol et al 2007). Considering on one side, the important inflammatory component of NASH and the antioxidant and anti-inflammatory properties of PON1, the aim of this study was to identify the variations in PON1 activities and *PON1* gene polymorphisms in patients with NASH, comparing them to a group of patients without NASH. To our knowledge, this is the first study to evaluate simultaneously and correlate the serum activities of PON1 and the most important *PON1* gene polymorphisms in patients with NASH.

Material and methods

The study was conducted during May-October 2014 in the Clinical CF Hospital from Cluj-Napoca. It was approved by a local Ethics Committee and conducted according to the Declaration of Helsinki. We studied a group of 17 patients with non-alcoholic steatohepatitis and 17 healthy controls, age and gender-matched, selected according to the following inclusion

Table 1. Characteristics of patients with and without NASH (* number of patients/percentage, ** median value/25;75 percentiles)

Variables	Patients with NASH (n=17)	Patients without NASH (n=17)	P
Age (years)	48 (34.5;63)	48 (35.5;62)	0.97
BMI (kg/m^2)	29.67 (23.15;33.20)	26.95 (22.07;31.14)	0.34
Waist circumference (cm)	106 (85;118.50)	94 (82.50;103.50)	0.15
Family history of cardiovascular diseases*	10 (58.8%)	5 (29.4%)	0.1
Hypertension*	6 (34.3%)	5 (29.4%)	0.34
Diabetes mellitus*	1 (5.9%)	1 (5.9%)	1
Impaired fasting glucose and/or impaired glucose tolerance (pre-diabetes)*	10 (58.82%)	3 (17.64%)	<0.05
Metabolic syndrome*	7 (41.2%)	5 (29.4%)	0.72
Glycemia (mg/dl)**	91 (79;102)	89 (84;100)	0.9
AST (UI/L)**	52 (47;72.5)	22 (18.5;29)	<0.001
ALT (UI/L)**	75 (65;85)	18 (16.5;25)	<0.001
ALP (U/L)**	158 (124.5;199.5)	145 (110.5;171.75)	0.42
GGT (U/L)**	28 (19.5;43)	24 (18;29.75)	0.34
Total Cholesterol (mg/dl)**	181 (165.5;213.5)	193 (162.5;235)	0.54
HDL-cholesterol (mg/dl)**	45 (37;51.5)	43 (40;50.5)	0.84
Triglycerides (mg/dl)**	142 (95.5;214)	129 (85;160)	0.73
Total Bilirubin (mg/dl)**	0.7 (0.45;0.85)	0.9 (0.65;1.3)	0.066
PLT (10^3/ul)**	256 (179.5;298)	251.5 (207.75;281.5)	0.9
Serum albumin (g/dl)**	4.33 (3.95;4.66)	4.25 (4.09;4.45)	0.83
hs-CRP (mg/dl)**	1.3 (0.35;2.91)	1.74 (0.62;3.85)	0.37

and exclusion criteria. Inclusion criteria: patients diagnosed with NASH, having ultrasonographic-proven hepatic steatosis (evaluated by the same experienced operator), persistently elevated aminotransferases, with negative markers for hepatic viral infection or other liver disorder (autoimmune hepatitis, primary biliary cirrhosis or cholangitis, hemochromatosis, Wilson disease etc.). Exclusion criteria: significant alcohol consumption (more than 20 g/day) and any other disease that might influence PON1 activity (autoimmune diseases, thyroid gland dysfunction, malignancies, psychiatric disorders). The control group consisted of 17 healthy controls with no ultrasonographic aspect of fatty liver and normal aminotransferases, without any of the "exclusion criteria".

We collected information about each patient: age, body-mass index (BMI), waist circumference, family history, comorbidities. A blood sample was obtained from each patient for routine measurements (glycemia, AST, ALT, ALP, GGT, platelets count, serum bilirubin, cholesterol, triglycerides, albumin and high-sensitivity C-reactive protein – hs-CRP) and for specific testing of PON1 activities and genotypes. The serum activities of PON1 (paraoxonase, arylesterase and lactonase) were evaluated using spectrophotometric assays (Ciumărnean et al 2015). Using the PCR-RFLP method as described in one previous study (Ciumărnean et al 2015), we determined the PON1 gene polymorphisms L55M, Q192R (situated in the coding part of the gene), -108C>T, -909G>C and -162A>G (situated in the regulatory part of the gene). Statistical analysis was performed using SPSS version 21.0.

Results

Relevant clinical data in our study groups are shown in Table 1. The criterion pre-diabetes was present more frequently in the NASH group ($p<0.05$). There was a significant difference ($p<0.001$) in the aminotransferase serum levels between the two groups. There were no other statistically significant differences between the two groups in terms of the other parameters, not even in the levels of hs-CRP, which was unexpectedly slightly lower in the NASH group.

The measured serum values of PON1 activities are recorded in Table 2. Paraoxonase and arylesterase median activities were lower in the NASH group, but the difference was not statistically significant. No statistically significant difference between the two groups was found on the lactonase activity of PON1. Also, in this table there are shown the variations of the 5 polymorphisms of *PON1* gene.

The L55M polymorphism presented the greatest variability between groups, the heterozygous variant *LM* being present in 41.2% of the patients in the NASH group and only in 17.6% of the patients in the control group. The difference was close to the statistical significance level ($p=0.08$).

Discussion

It is a difficult task to completely explain the pathophysiology of NAFLD and an even more difficult one to completely understand NASH. Both disorders have increased in prevalence in the last decades. Since the first description of Ludwig in 1980 (Ludwig et al 1980), NASH has emerged as a hot topic for researchers

Table 2. Paraoxonase 1 activities and gene polymorphisms (* median/25;75 percentiles, ** number of patients/percentage)

Variables		Patients with NASH (n=17)	Patients without NASH (n=17)	P
Paraoxonase activity (kU/L) *		193.3 (144.1;469.8)	315.3 (169.75;501.8)	0.34
Arylesterase activity (kU/L)*		64.55 (49.28;70.53)	66.89 (55.92;71.27)	0.67
Lactonase activity (kU/L)*		57.37 (47.57;65.87)	54.6 (44.9;63.77)	0.78
Q192R polymorphism [rs662]**	**QQ**	4 (22.2%)	3 (18.7%)	0.17
	QR	9 (50%)	5 (31.2%)	
	RR	5 (27.7%)	8 (50%)	
L55M polymorphism [rs854560]**	**LL**	6 (35.2%)	12 (70.5%)	0.08
	LM	7 (41.2%)	3 (17.6%)	
	MM	4 (23.5%)	2 (11.8%)	
-108C>T polymorphism [rs705379]**	**CC**	2 (11.8%)	2 (11.8%)	0.71
	CT	12 (70.6%)	10 (58.8%)	
	TT	3 (17.6%)	5 (29.4%)	
-909G>C polymorphism [rs854572]**	**GG**	4 (23.5%)	3 (17.6%)	0.7
	GC	10 (58.8%)	9 (52.9%)	
	CC	3 (17.6%)	5 (29.4%)	
-162A>G polymorphism [rs705381]**	**AA**	1 (5.9%)	1 (5.9%)	0.2
	AG	10 (58.8%)	5 (29.4%)	
	GG	6 (35.3%)	11 (64.7%)	

trying to fully understand the disease. Although it was intensively studied, it still has no targeted therapy (Ilan 2016).

A recent meta-analysis showed that all metabolic comorbidities have a high prevalence in patients with NASH (Younossi et al 2016). In our study, abdominal obesity, hypertension and metabolic syndrome were more frequent in the NASH group, but only the status of pre-diabetes attained a statistically significant difference ($p<0.05$). Impaired fasting glucose level alone is a helpful marker to identify patients with high insulin resistance, but only the HOMA-index (Homeostatic metabolic assessment) was shown to be an independent predictive factor for the presence of NASH (Fierbinteanu-Braticevici et al 2011). Other predictors mentioned by this study were CRP and albuminemia. In our patients, there were no statistically significant differences between the groups regarding these two parameters. In a previously published study, we found that CRP, IL-6 and TNF-α serum levels were higher in patients with NASH (Bocsan IC et al 2017). A concomitant evaluation of pro-inflammatory cytokines and a correlation with serum albumin and insulin could prove to be a better predictor for the presence of NASH.

Paraoxonase 1 is an enzyme that inhibits lipid oxidation and can hydrolize a large number of substrates, preventing atherosclerosis and reducing inflammation. It is already known that PON1 activities are lower in the serum of patients with various hepatic disorders, including alcoholic and non-alcoholic steatohepatitis (Marsillach et al 2007, Samy and Hassanian 2011, Baskol M et al 2005). In our study, although not statistically significant, paraoxonase and arylesterase activities of PON1 were lower in the NASH group compared to the control group, similar to the study of Baskol. The lactonase activity had no statistically significant difference in our groups. Interestingly, in a study conducted by Savu et al, it was found that lactonase activity of PON1 was increased in serum of patients with type 1 diabetes mellitus. They supposed that this lactonase activity increasing is an inefficient form of compensation for the level of chronic inflammation due to low antioxidant capacity (Savu et al 2014). To the best of our knowledge, no study described the genotype variations of PON1 in patients with NAFLD/NASH. The study of Ferré et al (2005), conducted on patients with chronic hepatitis C virus infection, showed that the RR isoform of the Q192R polymorphism had a higher frequency in chronic hepatitis group (Ferré et al 2005). In our study, the greatest variability between groups was observed for the L55M polymorphism, the heterozygous variant appearing more frequent in the NASH group ($p=0.08$). The three polymorphisms from the regulatory part of the gene (-108C>T, -909G>C and -162A>G) were not found to have a frequence with statistically significant difference between groups. It was previously shown that those three polymorphisms influence PON1 activities, regardless of the presence of the metabolic syndrome (Ciumărnean et al 2015). A study on a larger population of patients with NASH has been currently initiated by us in order to verify if there is indeed a subtype of patients with certain expression of PON1 gene who is going to develop NASH.

Conclusion

Paraoxonase 1 genotype seems to influence the status of patients with non-alcoholic steatohepatitis. The polymorphism that might be involved in the expression of PON1 gene in patients with NASH seems to be L55M, from the coding part of the gene. Studies on large groups of patients and controls are required to confirm a possible role of PON1 polymorphisms in the prediction and/or evolution of NASH.

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Citation	Milaciu MV, Ciumărnean L, Sâmpelean DP, Bocșan IC, Popa DE, Pașca S, Acalovschi M. Paraoxonase 1 activities and gene polymorphisms in non-alcoholic steatohepatitis – preliminary results of a pilot study. HVM Bioflux 2017;9(4):157-160.
Editor	Ştefan C. Vesa
Received	20 November 2017
Accepted	19 December 2017
Published Online	19 December 2017
Funding	None reported
Conflicts/ Competing Interests	None reported