Correlation between several extrinsic factors and Paraoxonase 1 activities

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Abstract. Objective: to investigate the PON1 activity (aryl esterase, paraoxonase and lactonase) as well as the possible influence of non-genetic factors in a representative cohort of patients with steatosis. Material and methods: We enrolled 39 subjects with steatosis that were hospitalised for multidisciplinary evaluation. The control group comprised 60 subjects without steatosis present at ultrasonography. Both groups were matched for age and sex. Each study participant answered a questionnaire regarding their lifestyle, comprising questions about diet, physical activity performed, use of medications, drugs or tobacco. We determined paraoxonase activities for all subjects. Results: There were no differences regarding paraoxonase activities between patients with steatosis as compared with healthy subjects. We did not get any statistical significance in the analysis of all studied extrinsic factor in relation to paraoxonase activities. Conclusion: steatosis and diet did not influence paraoxonase activities.

Key Words: steatosis, paraoxonase activities, extrinsic factors.

Introduction

Non-alcoholic fatty liver disease represents a large spectrum of conditions which ranges from steatosis, that generally has a benign, nonprogressive evolution, to non-alcoholic steato-sis, a form which can develop into cirrhosis and hepatic cancer (Adams et al 2011; Samy et al 2011; Lee 2011; Younossi et al 2012; Li et al 2012). The cause that leads to fatty non-alcoholic liver resides in the accumulation of triglycerides in the liver, (Zimmermann et al 2011; Samy et al 2011; Ndumele et al 2011; Grđic Rajkovic et al 2011), that represents the source of oxidative stress and promote the inflammatory process. Human plasmatic Paraoxonase 1 (PON1), which is encoded by a gene found on the long arm of chromosome 7 q21.3 (La Du 1996; She et al 2012), is a esterase/lactase which is associated with high density lipoproteins (HDL) which contain apolipoprotein J (clusterin) and apolipoprotein A (Beltowski et al 2003; Audikovszky et al 2007; Çakatay et al 2008). PON1 is associated to HDL hydrolyseparaoxon, organophosphorus compounds, unsaturated aliphatic esters, carboxyl aromatic esters (Costa et al 2003; Aviram et al 2004; Soran et al 2009; Kedage et al 2010; James et al 2010). Reduced values of PON1 activity were associated to diseases that have oxidative lesions and lipid peroxidation (Costa et al 2005; Bhattacharyya et al 2008; Camps et al 2009) and also, at an individual level show large variations of enzymatic activity, genetic factors explaining only in part these variations.

After recent studies demonstrated that the native PON1 activity is that of a lactonase (Khersonsky et al 2005; Aharoni et al 2005; Khersonsky et al 2006) while the previously intensive studied arylesterase and paraoxonase activities, are unexpected, a correct evaluation of PON1 activity implies the evaluation of all three activities: arylesterase, paraoxonase and lactonase. Although many studies focused on it, the results are controversial with regards to the effect of hypolipidemic drugs on PON1: some have demonstrated an increase in PON1 activity, while others could only demonstrate an improvement of the lipid profile (Costa et al 2005). Similar to the oral antidiabetics (She et al 2012), aspirin seems to increase the activity of PON1 (Costa et al 2005), but other studies although claim an amelioration of the enzymatic activity, underline the fact this happens only to coronary patients (She et al 2012).

While the data concerning alcohol consumption and PON1 are inconsistent and in the same time dose dependant (van der Gaag et al 1999; Sierksma et al 2002; Rao et al 2003; Sarandol et al 2003), cigarette smoke reduces irreversibly the plasmatic levels of the enzyme (Nishio et al 1997; She et al 2012).

The pomegranate juice amplifies PON1 activity (Costa et al 2005, She et al 2012). Regarding the intake of vitamin C, the available data is inconsistent (Costa et al 2005; She et al 2012). Also a diet rich in triolein, olive oil or oleic acid increases the activity of plasmatic PON1, while olineic acid reduces it (Costa et al 2012).
It is accepted unanimously that constant physical exercises increases the activity of PON1 (Costa et al 2005; She et al 2012). The objective of the current study was to investigate the PON1 activity (aryl esterase, paraoxonase and lactonase) as well as the possible influence of non-genetic factors in a representative cohort of patients with steatosis.

Materials and methods

Participants

We enrolled 39 subjects with steatosis that were hospitalised for multidisciplinary evaluation. The control group comprised 60 subjects without steatosis present at ultrasonography. Both groups were balanced regarding age: 55.38±12.59 years in the steatosis group, respectively 55.11±12.64 years (p=0.9) in the control group, and sex (35.5% men with steatosis, 38.2% healthy men, p=0.8).

The diagnosis of steatosis was established based on ultrasound examination. Prior to the enrolment in the study, all the participants were explained the stages and objectives of this study. Written consent was obtained from each participant and the study was approved by the Ethics Committee. The consent and the research protocols were in agreement with the Helsinki Declaration of World Medical Association.

We excluded from our study patients that presented medical evidences of any pathology that may influence PON1 activity: liver damage (acute or chronic viral hepatitis), declared alcohol intake (≥20g/day – men, >10g/day – women) in the past 6 months, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, alpha-1 antitrypsin deficiency, hemochromatosis, porphyria, Budd-Chiari syndrome, hepatic cirrhosis, inflammatory diseases like infections, psychiatric disorders which influence the values of PON1, malignancies, renal diseases.

Anthropometric measurements and clinical examination

For each participant we recorded general data (age, sex, area of origin), clinical and laboratory data. Information about the presence of comorbidities: hypertension, heart failure, type 2 diabetes, cancer, stroke, coronary heart disease or angina were collected from an medical history and clinical examination. To exclude the influence of demographic data on the enzyme activity, the participants in this study belonged to the same geographical areas. All had their height, weight and waist circumference measured.

Also, each study participant answered a questionnaire regarding their lifestyle, comprising questions about diet, physical activity performed, use of medications, drugs or tobacco.

To measure the height, the subjects were seated in the upright position, without shoes and using a measuring rod. There were three determinations carried out and the arithmetic mean of these measurements in meters was recorded.

Also in the standing position, the abdominal circumference of each subject was determined using a flexible strip chart recorder. There have been three consecutive measurements with patients in maximum exhale, midway between the umbilicus and xiphoid appendix. The mean value of these determinations, expressed in centimetres, has been reported (O’Riordan et al 2009).

We calculated body mass index (BMI, kg/m²) by applying the equation: weight (in kilograms) divided by the height (in square meters). To assess the weight, study participants were lightly dressed (they had no shoes and wore only light clothing) in this way the maximum margin of error was of +/-50g. We assessed body mass index (BMI, kg/m²) as follows: normal≤25 kg/m²; overweight - BMI between 25 and 30 kg/m²; obesity grade I - BMI between 30.1 and 34.9 kg/m²; Grade II - BMI between 35 and 40 kg/m²; grade III - BMI> 40 kg/m² (WHO 2000).

Blood and serum samples

All subjects included in the study followed a 12-hour fasted overnight and the next morning blood samples were collected from the ulnar vein using vacutainers without anticoagulant. These samples were immediately refrigerated at 4°C. We then prepared samples for serum enzyme determinations, which we obtained by centrifuging blood (3 min at 3000 rpm). Subsequently they were frozen at -80°C and kept under these conditions until they were analysed.

Paraoxonase activities

According to Eckerson et al (1983), with minor modifications, we determined the PON1 activities (paraoxonase, aryl esterase and lactonase) by spectrophotometric methods in the heparinized plasma. To determine the paraoxonase activity paraoxon was used (O, O-diethyl -O-p-nitrophenylphosphate, Sigma Chemical co. Seelze, Germany). The sample contained a basic mixture made up of 1.0 mM of paraoxon, 2MNaCl and 1 mM CaCl₂ in 50 mM glycine - NaOH buffer (pH 10.5). By adding the plasma samples we initiated the reaction, and absorption was monitored for 90 sec at 405 nm. Using phenylacetate (Sigma Chemical Co., Steinheim, Germany) 1 mM in 20 mM Tris-HCl (pH 7.5) that contained 1 mM CaCl₂, we measured the aryl esterase and lactonase activity of PON1. The rate of phenylacetate hydrolysis at 25°C was measured together with the increase in absorbance at 270 nm for 90 seconds. At the same time, a control sample, which had the same content but without the plasma, was run simultaneously for some corrections for spontaneous decomposition of the substrate activity for both activity determinations. All samples were run in duplicate. Obtained data was expressed in U/l for paraoxonase activity of PON1 (paraoxonase nanomoles hydrolyzed per minute) and kU/l for arylesterase activity of PON1 (phenylacetate micromoles hydrolyzed per minute).

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics version 20. Nominal variables were described using frequencies and continuous variables and were described by mean and standard deviation, or by median and percentiles (25-75%). Kolmogorov-Smirnov test for normality of distribution was performed for all continuous variables. Chi-square test was used in order to compare the frequencies of nominal variables. Differences between groups regarding continuous variables were examined using the t test and Mann-Whitney test. To evaluate differences between groups with more than 2 categories concerning continuous variables we used ANOVA and Kruskal-Wallis. The correlation between two continuous variables was assessed using Pearson correlation or Spearman’s rho. All tests were chosen taking into account the normality of the distribution. The level of statistical significance was set at p<0.05.
Results

Clinical and demographic characteristics and laboratory findings of patients in the two groups are presented in Table 1.

We determined the existence of a weak inverse correlation between waist circumference values and A-ase (r=-0.2, p=0.009). HDL-C and TC variables had a strong, and respectively medium, influence on A-ase levels (r=0.5, p <0.001, r=0.34, p=0.001). We have established a medium correlation between the values of HDL-C and L-ase (r=0.3, p=0.002) and between CT values and P-ase (r=0.31, p=0.002). We did not find a correlation between age and P-ase, A-ase or L-ase (r=-0.1, p=0.2, r=-0.09, p=0.3, r=-0.09, p=0.3). The patients sex had no significant influence on P-ase, A-ase or L-ase (p=0.9, p=0.3 , respectively p=0.3). We found no correlation between BMI, glucose, TG and values of these three enzymes.

Smoking had a significant influence on the values of A-ase (p=0.04) and alcohol on P-ase values (p=0.002).

We obtained a value close to the threshold of 0.05, when analysing the relationship between effort, sausage consumption and P-ase values (p=0.08, p=0.06 respectively). We found a similar situation when assessing the relationship between meat consumption and L-ase values (p=0.07).

We did not get any statistical significance in the analysis of all the other variables in relation to A-ase, L-ase and P-ase.

Discussions and conclusions

Non-alcoholic fatty liver disease, the most common liver abnormality in the developed world, described recently as part...
of the metabolic syndrome (Marchesini et al 2001; Farrel et al 2006; Bajaj et al 2009; Oh et al 2011; Ndumele et al 2011; Younossi et al 2012), is one of the main causes of chronic liver disease. Along with type 2 diabetes, obesity or overweight, its prevalence has increased substantially (Aslan et al 2008) and it is increasing in Western countries, affecting approximately 20-30 % of the population (McCullough et al 2005; Farrell et al 2006; Yilmaz et al 2012; Younossi et al 2012).

The mechanism of liver injury is explained by the release of free fatty acids (FFA) from adipose tissue deposits into the portal circulation (Rodríguez-Hernández et al 2011) and an increased concentration of unsaturated FFA in the liver may lead to steatosis, considered the first blow to the liver (Samy et al 2011). Currently no common imaging technique (ultrasound) is able to detect minimal histological changes of inflammation and ballooning, so that hepatic steatosis, non-alcoholic steatosis or other alcohol-induced chronic liver diseases can be differentiated, because of the similar histological appearance (Samy et al 2011; Younossi et al 2012).

Although liver biopsy, and proton magnetic resonance spectroscopy (H-MRS) are considered the golden standard methods for quantification of fat deposits in the liver, recent studies indicate the effectiveness of ultrasound in the assessment of hepatic steatosis (Martín-Rodríguez et al 2013). Recently it has been suggested that PON1 activities associated with HDL are implicated in pathogenesis of metabolic syndrome by demonstrating the existence of reduced PON 1 activities in this syndrome (Aviram et al 1998; Ferretti et al 2005; Kotani et al 2009). The influence of different factors such as food, lifestyle, dietary habits or certain drugs, on PON1 activity have also been reported. In this study we aimed to investigate the PON1 activities (arylesterase, paraoxonase and lactonase) and the possible influence of lifestyle in a representative cohort of subjects with hepatic steatosis.

This was the first study from Romania to investigate the PON1 activity in patients with hepatic steatosis and to evaluate a possible association of some lifestyle factors on enzyme activity. In this study, we observed statistically significant differences between the 2 groups for triglycerides, glucose, BMI, waist circumference, diabetes, hypertension and lipid-lowering medication, the data obtained are consistent with the results of other studies (Ndumele et al 2011). In disagreement with our expectations, we did not obtain significant differences between the two groups of participants for total cholesterol and HDL, which could be explained by statistically significant differences in the use of lipid-lowering medication, which may be responsible for these results.

We obtained no difference between groups in terms of PON1 enzyme activities similar with other researchers (Tabur et al 2010), although other reports have shown low values of their pathologies involving oxidative stress (Kotani et al 2009). Hepatic steatosis is the first hepatic change and it was speculated that this context lipotoxicity would be on the forefront, to an extent more important than insulin resistance (Bajaj et al 2009). In these circumstances the changes can only be in the RNA enzyme and because of the longer half-life of PON1 they cannot be detected at this stage of liver damage. The alterations of enzymatic activities will appear later (Feingold et al 1998). We observed an inverse correlation between the arylesterase activity, namely the enzyme bioavailability values, and the waist circumference, and a direct correlation with HDL-C and TC, in agreement with data from other studies (Tabur et al 2010).

Lactonase activity was positively correlated and we observed a weak association with HDL-C, similar to studies that have shown association only with certain subtraction of HDL (Grdic Rajkovic et al 2011) while, contrary to our expectations, P-ase activity was weakly associated only with CT values. These results could be explained by the small number of participants in the study or because of PON1 catalytic inactivity (assessed by P-ase activity) present at this stage of liver disease.

Like other studies, we observed no influence of sex (Costa et al 2005) on enzyme activities. We found no correlation between PON1 activity and blood glucose, triglycerides, BMI, the data so far being controversial (She et al 2012). These discrepancies are explained by the genetic variability of different groups investigated.

Among lifestyle factors, smoking had a significant influence on the values of A-ase, as in other reports (Costa et al 2005; She et al 2012), and this is probably linked to reactive aldehyde. P-ase activity was influenced by alcohol intake in agreement with other publications (Costa et al 2005, She et al 2012). There are no genetic polymorphisms of the PON1 that modulate the activity in relation to the dose of alcohol consumption, but changes in enzyme activity, that have an effect on protein-kinase C. This kinase is important for the phosphorylation of an active site (Sp1) in the promoter region of the PON1 gene (Costa et al 2005).

Close values to statistical significance threshold were obtained when analysing the relationship between exercise, eating sausage and P-ase values, as in other studies (Costa et al 2005; She et al 2012).

Similarly, when analyzing the relationship between meat consumption and L-ase values, the data obtained was close to statistical significance probably because of the small number of subjects that participated in the study. Meat consumption probably validates PON1 antioxidant function, assessed by L-ase values. In other cases, the lack of statistical significance may be due to the small number of participants in the study. On the other hand, it should be considered that the data obtained was based on the honesty of the participants in the study and therefore the accuracy of reported data could not be assessed.

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