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The effects of atorvastatin treatment on the mean platelet volume and red cell distribution width in patients with dyslipoproteinemia and comparison with plasma atherogenicity indicators—A pilot study

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ABSTRACT

Objectives: The mean platelet volume (MPV) and red cell distribution width (RDW) have recently arisen interest because of their association with an increased cardiovascular risk. The aim of our study was, therefore, to determine whether an association exists between MPV, RDW and lipoprotein sub-fractions, and to show the impact of statin therapy on these new possible biomarkers of atherosclerotic risk.

Design and methods: A cohort of 40 patients with hypercholesterolaemia (29 females, mean age 62.9 ± 9 years), without previous hypolipidaemic treatment were enrolled. The patients were treated with atorvastatin 40 mg/day for 12 weeks. Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density cholesterol (HDL-C), triglycerides (TG), LDL-C sub-fractions [large LDL-C 1–2 and small dense (sd)-LDL-C 3–7], apolipoproteins (apoA1, apoB), apoB/apoA1 ratio, atherogenic index of plasma (AIP), haematological parameters (including MPV, RDW) and safety parameters (renal, hepatic) were measured before and after 12 weeks of atorvastatin treatment.

Results: At baseline, a strong correlation between HDL-C, TG, sd-LDL-C, apoB, apoB/apoA1, and AIP with MPV ($r = -0.55$, $p < 0.001$; $r = 0.57$, $p < 0.001$; $r = 0.73$, $p < 0.001$; $r = 0.41$, $p < 0.05$; $r = 0.52$, $p < 0.001$; $r = 0.61$, $p < 0.001$, respectively) and RDW ($r = -0.49$, $p < 0.001$; $r = 0.62$, $p < 0.001$; $r = 0.67$, $p < 0.001$; $r = 0.41$, $p < 0.05$; $r = 0.43$, $p < 0.05$; $r = 0.65$, $p < 0.001$, respectively) was found. After 12 weeks of treatment with atorvastatin, MPV and RDW values underwent significant modification only in those patients displaying the strongest lipid-lowering effect.

Conclusions: Values of MPV and RDW seem to reflect a pro-atherogenic lipoprotein profile mainly represented by the presence of sd-LDL-C.

Introduction

Atherogenic dyslipidaemia is characterized by three lipid abnormalities: elevated triglycerides (TG), small LDL particles, and reduced HDL cholesterol and is a prominent risk factor for cardiovascular diseases [1,2]. Over the last two decades, it has been demonstrated that routine measurement of total cholesterol, LDL-C and HDL-C fails to distinguish all lipoprotein abnormalities associated with cardiovascular diseases [3]. By contrast, the analysis of lipoprotein sub-fractions appears more accurate in assessing the risk of cardiovascular complications [4]. In this regard, LDL-C and HDL-C lipoproteins exhibit a heterogenic distribution ranging from small, dense to large and lighter peculiar structure. Remarkably, these LDL sub-fractions display different atherogenic properties [5], with the small, dense LDL showing the highest atherogenic potential, since subjects carrying high concentration of these latter are at increased risk of developing coronary heart

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disease and other cardiovascular diseases [6]. Moreover, recent experimental and epidemiological reports have shown that small, dense LDL are crucial players in the pathophysiology of atherogenesis if compared to larger particles [6,7].

In the last years, the mean platelet volume (MPV), considered an easy marker of platelet activation, has arisen great interest in cardiovascular research. This is why platelets undergo a dramatic change in shape during the activation process, from quiescent discs to swollen spheres with an increased MPV. Large platelets, indeed, are more adhesive and prone to aggregate than smaller ones [8], and elevated MPV values have been reported in cardiovascular diseases [9].

Another haematological parameter which seems to play a role is the red cell distribution width (RDW) that measures the variability of red cell size. RDW has been reported to be a strong and independent predictor of adverse outcomes in the general population [10].

Recent clinical observations have demonstrated that the beneficial effects of statins are not limited to the LDL-C lowering effect [11], but they display additional favourable effects on platelet activation, endothelial function, inflammation, and coagulation cascade [12,13]. Therefore, the protective effect of atorvastatin in decreasing the cardiovascular risk could be also sustained by reduction of MPV levels [14]. The patients with low HDL-C, indeed, have high levels of MPV [15]. Likewise, a negative association between RDW and HDL-C values has been found in a large trial involving 4874 outpatients [16]. However, only scanty information is available on the relationship between MPV and RDW with low density lipoprotein levels.

The aim of the present study was, therefore, to elucidate the relationship between low density lipoprotein sub-fractions and these haematological parameters. The point of interest was to examine whether MPV and RDW have predictive potential for plasma levels and composition of pro- or anti-atherogenic lipoproteins. In addition, we have focused on the effects of a course of atorvastatin treatment on these haematological markers.

Materials and methods

Study design

A total of 40 participants (11 men, mean age 60.0 ± 8.9 years, and 29 women, mean age 63.6 ± 9.1 years) were volunteered and enrolled in the study. They were recruited from the outpatients of the 2nd Department of Internal Medicine, University Hospital Bratislava, Slovakia, from date to date. All participants signed an informed consent and underwent a screening protocol which included evaluation of their medical history, physical examination and testing for standard haematological and biochemical analysis.

Exclusion criteria were: fasting glycaemia > 7 mmol/L, history of diabetes mellitus (oral glucose tolerance test was performed to identify impaired glucose tolerance), glomerular filtration rate less than 60 mL/min (estimated glomerular filtration rate using MDRD equation [GFR] < 60 mL/min) and abnormal liver tests (AST, ALT) or history of hepatopathy, history of acute myocardial infarction or stroke, hypothyroidism or hyperthyroidism (abnormal TSH), cancer, history of pancreatitis, alcohol or drug abuse, smoking, systemic connective tissue diseases, history of anaemia or any haemoglobinopathy, abnormal blood count, red blood cell transfusion, supplementation of iron, folate or stimulation of erythropoiesis. Patients were eligible to take part in the study if they met the criteria of the National Cholesterol Education Program-Adult Treatment Panel 3 (NCEP-ATP III) [1]. Participants with dyslipoproteinaemia (isolated hypercholesterolaemia—21 patients, and combined hyperlipoproteinaemia—19 patients) without previous lipid-lowering therapy were treated with atorvastatin (40 mg/day) for 12 weeks as a primary and secondary prevention. Atorvastatin was well-tolerated during the study. Physical and laboratory examinations were carried out after 1st, 2nd and 3rd months of treatment. During this period, no serious adverse events were noted.

The study was approved by the local ethics committee of University Hospital Bratislava and was conducted in accordance with the Declaration of Helsinki.

Measurement of serum lipid, lipoprotein levels and atherogenic index of plasma

Blood samples were drawn in the morning after 12 hour fasting period from cubital vein; the samples remained for 30 min at room temperature. The examination included laboratory tests (liver and kidney tests, glucose, electrolytes, thyroid stimulating hormone), and determination of plasma atherogenity—apolipoprotein B (apoB), apolipoprotein A1 (apoA1), ratio apoB/apoA1 by the immune-turbidimetric method (Roche, Germany), and atherogenic index of plasma (AIP) using the formula: log (triglycerides [TG] / high density lipoprotein cholesterol [HDL-C]). Plasma levels of total cholesterol (TC) were determined by photometric enzyme method (cholesterol oxidase and 4-aminoantipyrine; Assel, Rome, Italy), HDL-C was determined by direct immune inhibitory determination, and LDL-C was calculated according to Friedewald equation and photometric enzyme TG-method (glycerol-3-phosphate oxidase and 4-aminoantipyrine).

Measurement of haematological parameters

Haematological variables (including MPV, RDW) before and after treatment were measured by cell analysers (SysmexHaematology Analyzer XP-2000i, Japan). The normal ranges of MPV (fL) and RDW (%) in our laboratory were 7.8 – 11 and 10.0 – 15.2 respectively. The blood samples were collected in tripotassium EDTA tubes and the time delay between sampling and data analysis was strictly controlled to be less than 2 h.

Measurement of LDL sub-fractions

Sample processing

The samples underwent centrifugation for 10 min at 3000 rpm. The supernatants were then collected and frozen at −80 °C until use for the analysis of lipoprotein fractions and sub-fractions. Samples were stored less than 30 days.

Biochemical methods

The concentrations of VLDL, IDL, LDL fractions and sub-fractions were determined by the linear electrophoresis in polyacrylamide gel (Quantimetrix Lipoprint LDL System and Quantimetrix, California, USA). This method uses electrophoresis in tubes filled with 3% polyacrylamide gel. Aliquots of 25 μl of serum were mixed with 200 μl of liquid gel solution and Sudan Black, and then added to the gel tube. Polymerization of the gel took 30 min at room temperature. Subsequently, electrophoresis was performed for 1 h (3 mA/1 tube). Densitometric reading and conversion to concentrations of lipoprotein fractions and sub-fractions were performed using Lipoware software (Quantimetrix, California, USA).

The QuantimetrixLipoprint LDL System divides lipoproteins based on electrophoretic mobility and determines the concentration of VLDL, large, medium and small IDL particles, large LDL particles (LDL 1), medium LDL particles (LDL 2), and small, dense LDL particles (sdLDL 3–7) [17].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 software for Windows. The D'Agostino Pearson test and Kolmogorov–Smirnov test were used to verify the normal distribution of parameters in the cohort. The normally distributed parameters were considered to be those, which have passed the verification in both tests. Continuous variables were expressed as mean ± SD or median and interquartile range. We used Spearman's and Pearson's correlation analyses to find

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