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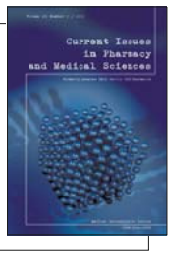

Concentration of isoprostanes as a marker reflecting oxidative status in moderate dyslipidemia

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ABSTRACT

Dyslipidemia and oxidative stress are considered one of the major causes which lead to many diseases, especially atherosclerosis development, cardiovascular disorders and aggravate risk for cancers, aging and others. Lipid hydroperoxides are the initial products when lipids are damaged by free radicals and in plasma HDL is their major carrier. F_2-IsoP are stable compounds and specific products of lipid peroxidation only in phospholipids *in vivo* through nonenzymatic free-radical mechanisms. Also, they are found in detectable quantities esterified in all biological tissues and in free form in all normal biological fluids. Quantifying of them is considered as accurate way to measure oxidative stress in vivo and could be used as an indicator of lipid peroxidation which process is a risk factor for atherosclerosis and other systemic diseases. Our study was performed in the group of young people (n=10, age 19-30) with moderate dyslipidemia and the reference group. In both groups we have investigated serum lipids (TG, TC, LDL-C, HDL-C, nonHDL-C), lipoproteins (apoAI, apoB) and lipid (TC/HDL-C, TG/HDL-C, LDL-C/HDL-C) and lipoprotein (apoA, apoB, HDL-C/apoAI) ratios and oxidative status by measuring free and total (free and esterified) F_2-isoprostanes (F2-IsoP). According to the available data reports and taking into account results obtained in our study we concluded that even mild lipid and lipoprotein abnormalities which are often present in population might by connected with elevated oxidative status which is sensitively reflected by F_2-IsoP concentration. Thus measurement of F_2-IsoP seems to be very helpful in recognition of early stage of oxidative stress however further studies are required.

Keywords: dyslipidemia, HDL, oxidative stress, isoprostanes (F_2-IsoP)

INTRODUCTION

Dyslipidemia and oxidative stress are considered one of the major causes leading to many diseases, especially atherosclerosis development, cardiovascular disorders and aggravate risk for cancers, aging and others. Serum lipoproteins which are carriers for cholesterol, are known to contain a variable amounts of lipid peroxides [1,17]. Non-enzymatic, free radical-induced lipid peroxidation is a very rapid process that leads to disarrangement in biochemical complexes as well as individual compound such as lipids which in effect gives a number of pathologies such as inflammation and imbalance in the antioxidants-oxidants status [2,10]. Lipid hydroperoxides are the initial products when lipids are damaged by free radicals and in plasma HDL is their major carrier. HDL, whose elevated concentration is inversely correlated with atherosclerosis and CVD might play a major role in the transport and metabolism of lipid hydroperoxides *in vivo,* which contribute to its cardioprotective role [6,7,12,16].

Isoprostanes (F_2-IsoP) are prostaglandin isomers formed by oxidation of arachidonic acid esterified only in phospholipids *in vivo* through nonenzymatic free-radical mechanisms. There are a number of favorable attributes that imply that measurement of F_2-isoprostanes may provide a reliable marker of lipid peroxidation *in vivo* [2,10]. F_2-IsoP*s* are stable compounds and specific products of free radical-induced lipid peroxidation. In addition, they are found in detectable quantities esterified in all biological tissues and in free form in all normal biological fluids. Their level substantially rises in even mild oxidative stress and the concentration does not depend on lipid content in diet, which was reported on animal models. Concentration of F_2-IsoP in plasma can provide a useful index of total endogenous production of isoprostanes. The basal level of free F_2-IsoP in healthy humans has been reported to be 25-28 pg/ml (range 10-80) and 30-40 times higher in urine in healthy subjects. However, the variation among individual is very wide and may depend on the used method. There have been no systematical studies performed in plasma till now [2,10].

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The majority of the studies have measured free F_2-IsoP and only few of them investigated total F_2-IsoP in plasma (i.e., free and esterified) and it is worth mentioning that most of the studies did not report the type of F_2-IsoP measured (free, esterified, total) [4,11]. The potential drawback in assessing the F_2-IsoP in serum is the formation of prostaglandin-like compound *ex vivo* if the samples are not handled correctly. Thus, the biological material should be kept in very cold conditions (-70°C). Plasma samples measured by EIA give excellent correlation to GC/MS if purified by SPE prior to analysis [2,10].

The aim of the study was to investigate lipids, lipoproteins and lipid and lipoprotein ratios and oxidative status by measuring free and total (free and esterified) F_2-isoprostanes in young people with moderated dyslipidemia and control group.

MATERIAL AND METHODS

The studied group consisted of 10 persons (men and women) at the age of 19-30 who were without active inflammatory disease, liver disease, malignancy, hypertension or diabetes mellitus, and they were not smokers, but they present abnormal lipid profile characterized by moderate dyslipidemia. The control group (n=10) was chosen among apparently normolipidemic individuals who were symptom free and had no evidence of previous cardiac, hypertensive, active inflammatory disease, obesity or diabetes and were at comparable age to the studied group. The study was conducted in accordance with the guidelines of the Ethics Committee, Medical University of Lublin, Poland.

Lipids, lipoproteins, and routine laboratory parameters were obtained in serum after a fourteen-hour overnight fasting. Blood was taken from veins into commercial tubes. Serum was immediately separated, divided and stored in aliquots at –80°C until use; samples prepared for F_2-IsoP were added 0.005% BHT. Lipids and lipoproteins were determined in fresh serum on Hitachi 902 analyzer. In the serum, we measured: total cholesterol (TC) with enzymatic colorimetric method; triglycerides (TG) using standard enzymatic technique; HDL-C was assayed by the enzymatic colorimetric method without precipitation. ApoA and apoB were determined on the Dade Behring nephelometer BN™ II System Siemens Healthcare Diagnostics Inc. Germany with using immunonephelometric method. Non-HDL cholesterol was obtained as TC minus HDL-C; LDL-C was calculated according to the Friedewald formula; TC/HDL-C/apoA-I, LDL-C/HDL-C, TG/HDL-C, and apoA-I/apoB were also calculated.

Total (free and esterified) and free 8-iso Prostaglandin $F_{2\acute{a}}$ (F_2-IsoP) were determined with using EIA kit Cayman and 8-Isoprostane Affinity Column for the purification. Before the preparation, all samples were centrifuged 1,500 rpm for 10 minutes to remove precipitated proteins.

To the samples prepared for total F_2-IsoP assay an equal volume of 15% KOH was added and the solution was incubated at 40°C for 60minutes to cleave any esterified isoprostanes. Next the samples were neutralized to pH 7.0-7.4 with 1M potassium phosphate buffer and 1M HCl.

In the second step samples for both total and for free F_2-isoP analysis were diluted with eicosanoid affinity column buffer (Co. 400220) and then applied to the column (Cayman, USA 8-IsoAffinity column Co. 10010366) for the purification. The samples were passed through the packing material, which had been activated by rinsing with column buffer, then water. After the sample has passed through the column, it was rinsed again by elution solution (96% ethanol, Co. 400230) and the eluent was collected. The column was regenerated by washing with water and column buffer. In the collected samples, the solvent (elution solution) was evaporated to dryness under a stream of dry nitrogen and the samples were reconstituted with assay buffer (Co. 400060). A quantity of free and total F_2-IsoP was analyzed with an 8-isoprostane kit (Cayman Chemical, Ann Arbor, MI, USA). By using a plate reader, absorbance at 405 nm was measured and the raw data were corrected for recovery. The assay was validated by adding a series of known amount of pure 8-iso$PGF_{2\acute{a}}$ standard to an equal volume of purified plasma samples. The concentrations of total and free isoprostanes were determined by using enzyme immunoassay (EIA). The correlation between the known amounts of added pure 8-iso-$PGF_{2\alpha}$ and the concentration obtained by EIA was very high. The antiserum of the 8-Isoprostane EIA had 100% cross-reactivity with 8-Isoprostane; and with PGF 1_α, $PGF_{3\alpha}$, PGF E_1, PGF D_2 and PGF $F_{2\alpha}$ from 0.71 to 0.14%. The detection limit of for F_2-isoP was 2.7 pg/ml.

Statistical analysis was performed using one-way analysis of the Student t-test for comparison of people with moderate dyslipidemia and the reference group. Continuous variables were tested for normal distribution and logarithmically transformed if they were not normally distributed. However, the values in the tables are presented as nontransformed data. All results are expressed as means ±SD. A p value of less than 0.05 was considered statistically significant. For statistical analysis of obtained results, Statistica program (StatSoft., Krakow, Poland) was used.

RESULTS

Concentrations of serum lipids: triglycerides, total cholesterol, LDL-C, HDL-C, nonHDL-C and lipoproteins: apoAI, apoB as well as total (free and esterified) and free isoprostanes (F_2-IsoP) are presented in Table 1. The studied subjects presented mild changes in lipid and lipoprotein profile, which were characterized by significant elevation in all lipid and lipoprotein profile

parameters in comparison to the controls. Only total cholesterol concentration was not statistically significant in the studied groups. Subjects with moderate dyslipidemia presented significantly higher concentration of total (free and esterified) F_2-IsoP than the controls, which confirms higher oxidative status in this group. However, neither of studied groups showed significant differences in the concentration of free F_2-IsoP. Table 2 shows that all lipid (TC/HDL-C, TG/HDL-C and LDL-C/HDL-C) and lipoprotein (apoAI/apoB and HDL-C/apoA-I) ratios were significantly higher in the moderate dyslipidemic subjects with higher total F_2-IsoP, which points that their lipid and lipoprotein profiles were more atherogenic than the controls accompanied by aggravation in oxidative status. Also in this group we have observed a decreased lipoprotein ratio HDL-C/apoA-I which suggests that the distribution in HDL particles family were disturbed with the tendency to smaller size particles [6,7].

Table 1. Concentration of serum lipids, lipoproteins and isoprostanes in the moderated dyslipidemia subjects and reference group

Parameter	Subjects with moderate dyslipidemia X±SD	Reference group X±SD	Level p<0.05
TG (mg/dl)	138±12	70±29	0.001
TC (mg/dl)	181±21	179±20	ns
LDL-C (mg/dl)	114±25	79±23	0.01
HDL-C (mg/dl)	39±7.02	66,8±7,4	0.001
nHDL-C (mg/dl)	142±20	110±25	0.01
apoAI (mg/dl)	150±29	173±31	0.01
apoB (mg/dl)	105±19	70±17	0.001
8-F_2-ISO (pg/ml)	562±139	277±68	0.001
Free 8-F_2-ISO (pg/ml)	175±136	145±66	ns

8-F_2-ISO – total F_2-IsoP (free and esterified), Free 8-F_2-ISO – free F_2-IsoP

Table 2. Lipid and lipoprotein ratios in subjects with moderate dyslipidemia and reference group

Parameter	Subjects with moderate dyslipidemia X±SD	Reference group X±SD	Level p<0,05
TC/HDL-C	4.65±0.65	2.71±0.50	0.001
TG/HDL-C	3.54±0.62	1.03±0.55	0.001
LDL-C/HDL-C	3.50±0.55	1.12±0.42	0.001
HDL-C/apoAI	0.26±0.05	0.37±0.04	0.001
apoAI/apoB	1.41±0.33	2.47±0.30	0.001

DISCUSSION

Lipid peroxidation generates products of advanced oxidation such as alkanes, aldehydes and isoprostanes. F_2-IsoP displays a potent bioactivity and its elevation is seen in a number of syndromes associated with oxidant injury such as atherosclerosis, hypercholesterolemia, habitual smoking, alcohol consumption, diabetes, pulmonary and liver diseases, rheumatic diseases, neurodegradation and vascular reperfusion [2,9,10]. Both esterified to tissue phospholipids and free in the circulation increase substantially in serum and urine in animal models of oxidant injury [18,19]. Quantifying of F_2-isoprostanes is thought as accurate way to measure oxidative stress in vivo and could be used as an indicator of lipid peroxidation the process of which is a risk factor for atherosclerosis and other systemic diseases [2,10,16].

Proudfoot et al. [12] detected F_2-IsoP in isolated lipoproteins and found that HDL in the major lipoprotein carrier of isoprostanes in human plasma. Levels of F_2-IsoP were approximately twice higher in HDL than LDL and 50% higher than in VLDL (normalized to cholesterol). Level of F_2-isoprostanes were respectively 2 and 3-fold higher in HDL2 and HDL3 than in total LDL, while the content of arachidonic acid (isoprostanes precursor) was significantly higher in LDL than in HDL. This observation indicates that HDL is a major carrier for both early and late products of lipid oxidation [3,11,12].

F_2-isoprostanes in HDL particles could increase because of removal of oxidized lipids from cell membranes, from macrophages in atherosclerotic lesions or after extraction of oxidized lipids from LDL by HDL. The higher concentration of antioxidants in LDL may also result in HDL being a preferential target for oxidation, acting as a "sink" for oxidized lipids, as it is a major carrier of lipid hydroperoxides in plasma. Thus, HDL is more susceptible to oxidation than LDL, which was reported in *in vitro* free radical oxidation [12,13].

It is possible that platelet-activating factor acetylhydrolase (PAF-AH) and paraoxonase-1 (PON1) activities are important determinants of F_2-IsoP distribution in LDL and HDL. Platelet-activating factor acetylhydrolase is responsible for the degradation of F_2-IsoP. Reduced F_2-IsoP in LDL may reflect the higher PAF-AH activity associated with LDL (88%) while HDL2 accounts for only 12% and HDL3 – no activity [5,8,10,12]. There is a debate whether PAF-AH is pro– or antiatherogenic. Plasma levels of PAH-AH are positively associated with CVD. However, we still do not know if activity of this factor affects atherosclerosis or whether the levels rise because of the inflammatory environment [5,10,12].

Another enzyme carried by HDL is PON1 the activity of which is associated mainly with HDL2 particle – what could be the cause of lower level of F_2-IsoP in HDL2 than HDL3 fraction [12]. Reduced F_2-isoprostanes in HDL2 compared with HDL3 may be related to the elevated paraoxonase-1 activity in HDL2 [12]. However, PON1 had no effect on lipid peroxides or F_2-IsoP during free radical *in vitro* HDL oxidation, which indicates that the reported antiatherogenic effects of HDL are not due to antiatherogenic effects of PON-1 but are likely due to other mechanisms such as protection of LDL from oxidation [8,12].

Presented data might suggest that the higher content of peroxides in HDL may impair the main HDL function, which is reverse cholesterol transport, and marker, which provides a reliable approach to assess oxidative stress in vivo, seems to be F2-IsoP.

In our study in moderate dyslipidemic group higher level of total and free isoprostanes accompanied lowered HDL-C and apoA-I (decreased HDL-C/ApoAI) concentration which points to more aggravated oxidative status and disarrangement in HDL particles family in those subjects in comparison to control group [6,7,17].

The direct biological action of free and esterified F_2-IsoP are not well known. F_2-isoprostanes are not simply markers of oxidative stress. During interpretation of isoprostanes the dual role of F_2.IsoP should be considered; thus they are a marker of oxidative stress and the mediators of vital biological effects representing regulators of the crucial response and adaptation [10,11]. On the one hand a number of free F_2-IsoP have been found to exert a variety of pro-atherogenic actions particularly in vasculature, on the other hand, inhibition of macrophage inflammatory response was reported which may suggest that they are negative feedback regulators of inflammation. It is also worth mentioning that esterified F_2-IsoP exert important effects in the regulation of atherogenesis, such as induction of endothelial synthesis of IL-8 monocyte-chemotactic protein-1 [10,19,20]. These findings are in agreement with findings of PAF acetylhydrolase deficient subjects who presented decreased capacity to release free isoprostanes from esterified precursors and have higher risk of developing coronary artery disease [19].

Esterified F_2-IsoP may be more long-lasting markers of lipid peroxidation than free F_2-IsoP and may enable the site of endogenous oxidative modification to be identified although measurement of free F_2-IsoP may better reflect systemic changes in lipid peroxidation. However, metabolism of free isoprostanes is more rapid than the esterified F_2-IsoP so measurement of this form is plasma seems to be more suitable [10,11].

In our study the concentration of total (esterified and free) F_2-IsoP was significant in moderate dyslipidemic subjects while the free form didn't show significant differences, which seems to be in agreement with the quoted data. Concentration of esterified F_2-IsoP seems to be more stable also because free F_2-IsoP are much affected by PLA2 (phospholipase A_2 enzymes) activity which rises e.g. during exercises and not by reactive species generation [1]. Esterified F_2-IsoP are about 5-fold higher than free ones, which we also observed in our study (moderate dyslipidemic patients) [15].

CONCLUSIONS

According to the available literature reports and taking into account results obtained in our study, we concluded that even mild lipid and lipoprotein abnormalities, which are often present in population, might be connected with elevated oxidative status, which is sensitively reflected by F_2-IsoP concentration. Thus measurement of F_2-IsoP seems to be very helpful in recognition of early stage of oxidative stress however further studies are required.

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