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ANGPTL4 as Biomarker in Early Detection of Cardiovascular Complications in Diabetic Patients

KEYWORDS: ANGPTL4; Blood Glucose; Lipid Profile; CVD.

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ABSTRACT *Background: Angiopoietin-like 4 (ANGPTL4) is a multifunctional protein involved in lipid regulation, energy metabolism, angiogenesis, and inflammation. It is highly expressed in the liver. Diabetic patients have metabolic derangement that may linked to ANGPTL4. The aim of this study was to address the role of ANGPTL4 in cardiovascular diseases in type 2 diabetes and confirming its relation with lipid and glucose metabolism.*
Methods: Quantitative determination of serum ANGPTL4 in 29 patients with DM, (17 without CVD and 12 with CVD) and 12 non diabetic healthy controls was done.
Results: Fasting and postprandial ANGPTL4 was higher in complicated diabetic group. Total cholesterol, triglycerides, LDL-cholesterol, risk ratio1 and 2, FBG and PPBG were elevated in both complicated and non complicated groups, while HDL-cholesterol was lower in complicated group. Fasting ANGPTL4 was higher than postprandial ANGPTL4 in complicated patients. There was a positive correlation between ANGPTL4 and total cholesterol, LDL-cholesterol and triglycerides in CVD patients.
Conclusions: Elevated ANGPTL4 associated with elevation of cholesterol, triglycerides and LDL-cholesterol and lowering HDL-cholesterol could be reflects cardiovascular risk in diabetics or even normals.

Introduction
Angiopoietin-like proteins (ANGPTLs) are considered as a group of proteins which play an important role in energy metabolism and they have similarity in their tertiary structure with angiopoietins containing two domains, one N-terminal coiled-coil domain and one C-terminal fibrinogen-like domain (Kersten, 2005).

ANGPTL4 is a member of ANGPTLs and it is known as peroxisome proliferator-activated receptor (PPAR) containing two ligands, PPARα and PPARα (Kersten, 2000). ANGPTL4 is known as fasting-induced adipose factor (FIAF) where it is controlled by nutritional state, so it increased (especially in liver) by fasting and decreased by fatty meal feeding (Yoon, 2000); also it is identified as hepatic fibrinogen angiogenic-related protein (HFARP) (Kim, 2000). The expression of ANGPTL4 was in higher concentrations in liver, then in adipose tissue, followed by thyroid gland, brain and finally in intestine (Kersten, 2009). It is involved in the energy releasing during α-oxidation of fatty acids and also involved in the consumption of energy (Yu, 2005).

ANGPTL4 seems to be a hyperlipidemic agent because it makes an inhibition to lipoprotein lipase (LPL) by conversion of it from an active dimmer form to an inactive one producing monomers leading to decreasing lipase activity in adipose tissue (Yoshida, 2002 and Sukonina, 2006). On the other hand, the deficiency of ANGPTL4 makes lipid metabolism better and may avoid certain cardiovascular problems like atherosclerosis (Adachi, 2009). Also it improves glucose tolerance by decreasing blood glucose level (Xu, 2005). ANGPTL4 was found to be upregulated in the adipose tissue of women and men suffering from obesity which indicates and confirms the essential role of ANGPTL4 in the metabolism of glucose and lipids in obese women and men (Rodríguez-Acebes, 2010).

Type 2 diabetes mellitus (DM) is a chronic condition and considered as a major cause of mortality and morbidity for its micro-vascular (retinopathy, nephropathy and neuropathy) and macro-vascular (coronary heart disease, peripheral vascular disease and stroke) complications. It is a multi-factorial disease that in its development in addition to genetic predisposition, play role in a number of environmental factors such as poor nutrition, lack of physical activity and obesity. Among these factors, obesity has more potent relationship with type 2 diabetes which may link the ANGPTL4 to diabetic complications (Li, 2011).

CVD broadly defined as stroke, coronary artery disease, and peripheral vascular disease is the major cause of morbidity and mortality in persons with type 2 DM. CVD leads to 65% of deaths in diabetic patients. Diabetes creates alterations in function of endothelial cells, smooth muscle cells and platelets that lead to vascular dysfunction. Also insulin resistance, hyperglycemia and dyslipidemia can lead to arterial atherosclerosis in diabetic patients (Beckman, 2002).

This study has been conducted to evaluate fasting and postprandial serum ANGPTL4 levels and its relation to lipid profile and cardiovascular complications in type 2 diabetic patients aiming to address the role of ANGPTL4 as a predictor for CVD in type 2 DM.

Subjects and methods
Study Population: This study was conducted on 29 patients with DM as well as 12 non diabetic healthy volunteers to serve as control. All subjects were informed of the purpose of the study and their consent was obtained. DM is diagnosed and classified according to the American Diabetes Association criteria (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). A detailed history and complete clinical examination was done. All patients' demographic and clinical data were collected in the form of age, gender, weight and height. Body mass index (BMI) was calculated as an index of the weight (in kilograms) divided by the square of the height (in meters). Centers for Disease Control and Prevention (CDC) classify the normal range of BMI to be between 18.5–24.9 kg/m2, overweight BMI between

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25–29.9 kg/m², and the obese BMI > 30 kg/m2. Systolic and diastolic blood pressures were recorded as a mean of 3 successive measurements. Non diabetic volunteers were judged to be in good health according to their medical history and physical examination as well as their fasting and postprandial blood glucose.

Diabetic patients were classified to 2 groups according to the presence of CVD (hypertension, ischemic heart disease and cerebrovascular stroke); group D without CVD and group DC with CVD as well as group C (control). Diabetic patients with CVD were 68.2% hypertensive, 25.8% ischaemic heart diseases as well as 6% with cerebrovascular stroke.

Sampling: three blood samples were drawn from the patients. Overnight fasting sample for lipid profile determination. 6-8 hours fasting and 2 hours postprandial samples for other parameters. All blood samples were allowed to stand till clotting; the serum was aspirated and divided into aliquots for analysis of different parameters.

Laboratory investigations were performed including; serum ANGPTL4 which was measured using a human ANGPTL4 ELISA kit (Ray Biotech, Inc.) by the method of Robciuc et al., 2010 ; fasting and postprandial blood glucose (FBG and PPBG) levels were determined by colorimetric method of Pal et al., 2008; serum total cholesterol (TC) was measured using the method of Allain et al., 1974; serum triglycerides (TG) was measured using the method of Ter Welle et al., 1984; serum HDL-cholesterol (HDL-C) was measured using the method of Burstein et al., 1970 and serum LDL-cholesterol (LDL-C) was calculated using the Friedewald formula.

Risk ratio1 (RR1) is obtained by dividing of total cholesterol by HDL-cholesterol (TC: HDL-C) and risk ratio2 (RR2) is obtained by dividing of LDL-cholesterol by HDL-cholesterol (LDL-C: HDL-C), and these ratios are considered the greatest valuable for evaluation of CVD (National Cholesterol Education Program, 1994 and Grundy, 1989).

Statistical analysis

The collected data were tabulated and statistically analyzed using GraphPad InStat Version 3 for windows XP and GraphPad Prism 4 (GraphPad Software, Inc.). All data were expressed as mean ±SEM. The level of statistical significance was taken at P<0.05, comparison of variables among groups of the study was made by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test to judge the difference between various groups. Correlation between serum ANGPTL4 and other parameters of the subjects was determined by Pearson's Product correlation coefficient (r) to test the strength of association between serum ANGPTL4 and other variables. Differences were considered statistically significant at P<0.05 and highly significant at P<0.01.

Results

Table 1 summarizes the different characters of control and all diabetic groups. There was no significant difference between diabetic patients and control in age, weight, height and BMI. As regard systolic and diastolic blood pressure were of high significant difference between control and diabetic patients.

Table 1: Comparison between the control (C) group and all diabetic groups in all characters (Mean±SEM). P< 0.05 significant. a=high significance from control (C) group.

Characters	Group 2 and 3 n=29	Group 1 (C) n=12	P value	Significance
Age (year)	33 – 72	31 – 75	0.07	N.S.
Weight (Kg)	83.22±2.50	75.50±5.17	0.13	N.S.
Height (cm)	175.06±1.28	176.75±1.08	0.43	N.S.
BMI (Kg/m²)	27.11±0.70	24.22±1.78	0.07	N.S.
Systolic B. P. (mmHg)	135.55±1.70 [a]	121.25±1.75	< 0.0001	H.S.
Diastolic B. P. (mmHg)	89.82±1.20 [a]	82.50±0.75	0.0005	H.S.

Table 2 represents the lab investigations of the diabetic patients compared to the control. There were a highly significant increase in FBG, PPBG, fasting and postprandial ANGPTL4, TC, LDL-C, RR1 and RR2 in diabetic patients compared with control (P<0.01). TG show significant increase in diabetic patients compared with control (P<0.01). While HDL-C was not significantly differ between the 2 groups (P< 0.05).

Table 3 and figure 1 shows highly significant increase in both fasting and postprandial ANGPTL4 beside LDL-C, risk ratio1 and 2 in CVD complicated diabetic patients compared to non CVD complicated patients (P<0.01). Also TC was significantly increased in CVD diabetic patients compared to non CVD complicated patients (P<0.01). However FBG, PPBG, TG and HDL-C were not significantly differing between the 2 groups (P< 0.05).

Table 2: Comparison between the control (C) group and all diabetic groups in all parameters (Mean±SEM). P< 0.05 significant. a=significance from control (C) group.

Parameters	Group 2 and 3 n=29	Group 1 (C) n=12	P value	Significance
Fasting blood glucose (mg/dl)	176.03±11.29 [a]	85±2.05	< 0.0001	H.S
Postprandial blood glucose (mg/dl)	232.27±12.33 [a]	98.5±2.37	< 0.0001	H.S
Fasting ANGPTL4 (pg/ml)	855.48±159.22 [a]	445.41±49.04	< 0.0001	H.S
Postprandial ANGPTL4 (pg/ml)	735.86±136.44 [a]	477.25±81.25	0.0009	H.S
Total Cholesterol (mg/dl)	198.13±6.23 [a]	151.0±5.73	< 0.0001	H.S
Triglycerides (mg/dl)	129.79±7.84 [a]	82.00±7.55	0.04	S
HDL-Cholesterol (mg/dl)	49.68±2.60	56.16±4.43	0.33	N.S.
LDL-Cholesterol (mg/dl)	122.41±6.43 [a]	81.83±6.01	< 0.0001	H.S
Risk Ratio1	4.27±0.26 [a]	2.87±0.24	0.003	H.S
Risk Ratio2	2.70±0.23 [a]	1.59±0.19	0.005	H.S

Table 3: Comparison between the non complicated diabetic (D) group and complicated diabetic (DC) group in all parameters (Mean±SEM). P< 0.05 significant. a=significance from (D) group.

Parameters	Group 2 (D) n=17	Group 3 (DC) n=12	P value	Significance
Fasting blood glucose (mg/dl)	172.06±14.09	181.67±19.24	0.30	N.S
Postprandial blood glucose (mg/dl)	225.94±15.30	241.25±20.97	0.29	N.S
Fasting ANGPTL4 (pg/ml)	387.11±33.53	1519±292.1^{7} a	< 0.0001	H.S
Postprandial ANGPTL4 (pg/ml)	390.58±34.08	1225.0±273.9^{0} a	< 0.0001	H.S
Total Cholesterol (mg/dl)	187.17±6.52	213.66±10.68 [a]	0.03	S
Triglycerides (mg/dl)	127.53±12.20	133.0±8.33	0.73	N.S
HDL-Cholesterol (mg/dl)	53.64±3.56	44.08±3.28	0.07	N.S
LDL-Cholesterol (mg/dl)	107.94±6.59	142.91±10.01 [a]	0.005	H.S
Risk Ratio1	3.69±0.27	5.10±0.41 [a]	0.006	H.S
Risk Ratio2	2.17±0.22	3.46±0.37 [a]	0.003	H.S

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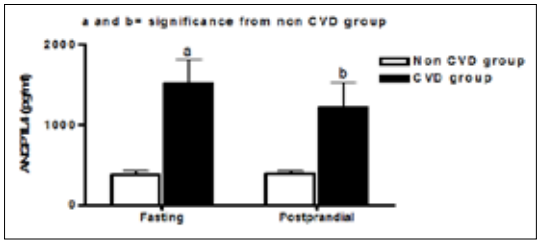

Figure 1: Fasting and postprandial serum ANGPTL4 in diabetic patients with and without CVD (Mean±SEM). P<0.05 significant.

Table 4: Correlation between fasting and postprandial serum ANGPTL4 and lab data in diabetic patients with and without CVD complication. Significant at P<0.05 and highly significant at P<0.01.

Variables	Fasting serum ANGPTL4			Postprandial serum ANGPTL4		
	P	R	Sig.	P	R	Sig.
Group D						
TC	0.03	0.50	S	0.04	0.49	S
LDL-C	0.01	0.58	S	0.04	0.49	S
HDL	0.51	-0.17	NS	0.36	0.23	NS
TG	0.47	0.18	NS	0.51	-0.16	NS
RR1	0.04	0.49	S	0.48	0.18	NS
RR2	0.03	0.52	S	0.24	0.29	NS
FBG	0.82	-0.05	NS	0.59	0.13	NS
PPBG	0.45	-0.19	NS	0.52	-0.16	NS
Group DC						
TC	0.009	0.71	HS	0.01	0.69	S
LDL-C	0.047	0.58	S	0.04	0.58	S
HDL	0.54	0.19	NS	0.41	0.25	NS
TG	0.007	0.72	HS	0.03	0.61	S
RR1	0.41	0.25	NS	0.89	0.04	NS
RR2	0.43	0.24	NS	0.94	0.02	NS
FBG	0.83	-0.06	NS	0.82	-0.07	NS
PPBG	0.64	-0.14	NS	0.77	-0.09	NS

Fasting ANGPTL4 was positively correlated with both TC and LDL-C in CVD complicated diabetic patients as well as non complicated (table 4 and figure 2). There was positive correlation with TG in CVD complicated diabetic patients only. No correlation was found between ANGPTL4 and HDL in both groups, but it positively correlated with both risk ratio1 and 2 in diabetic patients without CVD complication.

Also, postprandial ANGPTL4 was positively correlated with TC and LDL-C in diabetic patients with and without CVD complication (table 4 and figure 3). There was positive correlation with TG in CVD complicated diabetic patients only. There was no significance for fasting and postprandial ANGPTL4 with FBG and PPBG (figure 4).

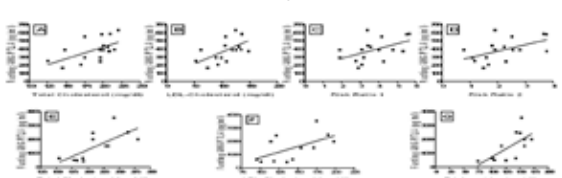

Figure 2: Correlation between fasting serum ANGPTL4 and other parameters in diabetic groups with and without CVD. P< 0.05 significant. The correlation was positive with A=TC, B=LDL-C, C=RR1, D=RR2 in D group and with E=TC, F=LDL-C, G=TG in DC group.

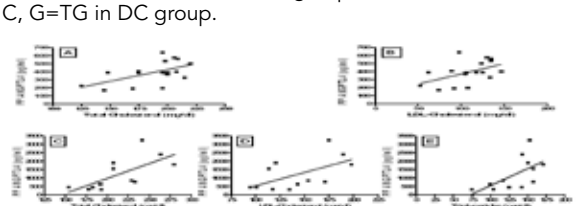

Figure 3: Correlation between postprandial serum ANGPTL4 and other parameters in diabetic groups with and without CVD. P< 0.05 significant. The correlation was positive with A=TC, B=LDL-C in D group and with C=TC, D=LDL-C, E=TG in DC group.

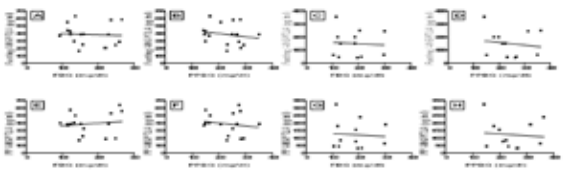

Figure 4: Correlation between fasting and postprandial serum ANGPTL4 and FBG and PPBG in diabetic groups with and without CVD. P< 0.05 significant. There was no correlation.

Discussion

Over the past decade research has shown, adipose tissue has an important endocrine function beside its role for the storage and release of fatty acids (Batra, 2012). In addition, ANGPTL4 has role in lipid metabolism as been confirmed by an increased plasma TG by intravenous injection of ANGPTL4 into the mice (Yoshida, 2002).

Our study measured serum ANGPTL4, one of the adepokines, to define its role in CVD in diabetic patients. The results show its highly significant increase in diabetic patients compared to control. ANGPTL4 inhibit lipoprotein metabolism via their ability to inhibit the activity of LPL. LPL activity in a given tissue is the rate limiting step for the uptake of triglyceride-derived fatty acids. Imbalances in the partitioning of fatty acids have major metabolic consequences as DM (Miida, 2010). Also our results revealed a highly significantly increase in ANGPTL4 in diabetic patients with CVD compared with diabetic without CVD.

Plasma TG concentrations are determined by the balance between production of the triglyceride-rich lipoproteins VLDL and chylomicrons in liver and intestine, and their LPL-mediated clearance in peripheral tissues. ANGPTL4 impair TG clearance by inhibiting LPL (Mattijssen, 2012). Our results are in line with Stejskal et al, 2008 which reported a positive correlation between TG levels and ANGPTL4 in a study of patients with metabolic syndrome. They also reported a negative correlation between HDL and ANGPTL4.

However these results contradict Smart-Halajko, 2010 and Robciuc, 2010 results which show that circulating ANGPTL4 levels did not correlate with plasma TG levels. And they explain the results by Nilsson et al., 2009 data which reported that the inactivation of LPL by ANGPTL4 was significantly weakened in the presence of TG-rich lipoproteins and concluded that circulating ANGPTL4 may have less of an effect on LPL activity than tissue-bound ANGPTL4.

The mean values of serum ANGPTL4 in diabetic CVD group in fasting were higher than those in postprandial which confirm the data of Li, 2006 that ANGPTL4 plays important roles in both fed and fasted states however its major role in fasting to regulate the tissue-specific delivery of lipoprotein-derived fatty acids. ANGPTL4 is thus an endocrine or autocrine/paracarine inhibitor of LPL depending on its sites of expression.

The present study also shows significant increase in TC and LDL in diabetic patients with CVD compared with those without CVD which confirm the role of screening as well as treatment of atherogenic dyslipidemia (Santoso, 2006). This data was in line with Lichtenstein et al., 2007 and such elevation is due to hepatic lipase inhibition by ANGPTL4 which encourage liver to increase its levels of cholesterol.

Also TG in complicated group show higher levels than other groups either by the inhibition of ANGPTL4 to LPL (Desai,

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2007). HDL-C values in complicated group are lower than those of non complicated and control group, in turn the low HDL-C and higher TG levels can be used as a good indicator for various CVD (Grundy, 2005 and Rana, 2007).

Conclusions

Serum ANGPTL4 was increased during prolonged fasting which associated with corresponding elevation of TC, TG, LDL-C, RR1 and RR2 in type 2 diabetic patients with and without CVD complications rather than normal persons. Briefly, measurement of fasting serum ANGPTL4 beside lipid profile may be used as good predictors for detection of early CVD in normal persons and type2 diabetic patients without CVD complications.

REFERENCE 1. Kersten S (2005) Regulation of lipid metabolism via angiopoietin-like proteins. Biochem. Soc. Trans. 33 (Pt 5): 1059 – 62. | 2. Kersten S, Mandard S, Tan NS, Escher P, Metzger D et al. (2000) Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. J. Biol. Chem., 275, 28488 – 28493. | 3. Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y et al. (2000) Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Mol. Cell. Biol.: 20:5343 – 5349. | 4. Kim I, Kim HG, Kim H, Kim HH, Park SK et al. (2000) Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. Biochem J.: 346 Pt 3: 603 – 10. | 5. Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks H et al. (2009) Caloric Restriction and Exercise Increase Plasma ANGPTL4 Levels in Humans via Elevated Free Fatty Acids. Arteriosclerosis, Thrombosis and Vascular Biology; 29: 969 – 974. | 6. Yu X, Burgess SC, Ge H, Wong KK, Nassem RH et al. (2005) Inhibition of cardiac lipoprotein utilization by transgenic overexpression of ANGPTL4 in the heart. Proc Natl Acad Sci U S A.; 102: 1767 – 1772. | 7. Yoshida K, Shimizugawa T, Ono M, Furukawa H (2002) Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. J Lipid Res.; 43: 1770 – 1772. | 8. Sukonina V, Lookene A, Olivecrona T, Olivecrona G (2006) Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. Proc Natl Acad Sci U S A.; 103: 17450 – 17455. | 9. Adachi H, Fujiwara Y, Kondo T, Nishikawa T, Ogawa R et al. (2009) Angptl4 deficiency improves lipid metabolism, suppresses foam cell formation and protects against atherosclerosis. BBRC 379(4):806 – 811. | 10. Xu A, Lam MC, Chan KW (2005) Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc. Natl. Acad. Sci. U.S.A. 102 (17): 6086 – 91. | 11. Rodríguez-Acebes S, Palacios N, Botella-Carretero J, Olea1 N, Crespo L et al. (2010) Gene expression profiling of subcutaneous adipose tissue in morbid obesity using a focused microarray: Distinct expression of cell-cycle- and differentiation-related genes. BMC Medical Genomics, 3:61. | 12. Li S, Zhao JH, Luan J, Langenberg C, Luben RN, Khaw KT, et al. (2011) Genetic predisposition to obesity leads to increased risk of type 2 diabetes. Diabetologia; 54: 776 – 82. | 13. Beckman JA, Creager MA, Libby P (2002) Diabetes and atherosclerosis. Epidemiology, pathophysiology and management. JAMA; 287: 2570 – 81. | 14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2003) Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care; 26 (Suppl.1): 5 – 20. New York, Springer-Verlag, pp 547 – 560. | 15 Robciuc MR, Tahvanainen E, Jauhiainen M, Ehnholm C (2010) Quantitation of serum angiopoietin-like proteins 3 and 4 in a Finnish population sample. J Lipid Res.; 51: 824 – 831. | 16. Pal S, Lim S, Egger G (2008) The effect of a low glycaemic index breakfast on blood glucose, insulin, lipid profiles, blood pressure, body weight, body composition and satiety in obese and overweight individuals: a Pilot Study. J. Am. Coll. Nutr. 27 (3), 387 – 393. | 17. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem; 20: 470 – 5. | 18. Ter Welle HF, Baartscheer T, Fiolet JWT (1984) Influence of free glycerol on enzymatic evaluation of triglycerides. Clin Chem; 30: 1102 – 3. | 19. Burstein M, Scholnick HR, Morfin R (1970) Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res; 11: 583 – 95. | 20. Friedewald, WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18: 499 – 502. | 21. National Cholesterol Education Program (1994) Second Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, Adults Treatment Panel II. Circulation; 89: 1329 – 1345. | 22. Grundy SM, Goodman D, Rifkind BM, Cleeman JI (1989) The place of high density lipoprotein in cholesterol management. A prospective from the National Cholesterol Education Program. Arch Intern Med; 149: 505 – 510. | 23. Batra A, Siegmund B (2012) The role of visceral fat. Dig Dis.; 30 (1): 70 – 4. | 24. Miida T, Hirayama S (2010) Impacts of angiopoietin-like proteins on lipoprotein metabolism and cardiovascular events. Curr Opin Lipidol.; 21(1): 70 – 5. | 25. Mattijssen F, Kersten S (2012) Regulation of triglyceride metabolism by Angiopoietin-like proteins. Biochim Biophys Acta.; 1821 (5): 782 – 9. | 26. Stejskal D, Karpisek M, Reutova H, Humenanska V, Petzel M et al., (2008). Angiopoietin-like protein 4: development, analytical characterization, and clinical testing of a new ELISA. Gen Physiol Biophys.; 27: 59 – 63. | 27. Smart-Halajko MC, Robciuc MR, Cooper JA, Jauhiainen M, Kumari M et al. (2010) The relationship between plasma angiopoietin-like protein 4 levels, angiopoietin-like protein 4 genotype, and coronary heart disease risk. Arterioscler Thromb Vasc Biol.; 30(11): 2277 – 82. | 28. Nilsson S, Larsson M, Lookene A, Heeren J, Olivecrona G (2009) Triacylglycerol-rich lipoproteins influence the inactivation of lipoprotein lipase by Angptl3 and ANGPTL4. XV International Symposium on Atherosclerosis, Boston, MA; June 14 – 18. | 29. Li C (2006) Genetics and regulation of angiopoietin-like proteins 3 and 4. Curr Opin Lipidol.; 17(2): 152 – 6. | 30. Santoso T (2006) Prevention of cardiovascular disease in diabetes mellitus: by stressing the CARDS study. Acta Med Indones; 38(2):97 – 102. | 31. Lichtenstein L, Berbee JF, van Dijk SJ, van Dijk KW, Bensadoun A et al. (2007) ANGPTL4 upregulates cholesterol synthesis in liver via inhibition of LPL- and HL-dependent hepatic cholesterol uptake. Arterioscler Thromb Vasc Biol.; 27: 2420 – 2427. | 32. Desai U, Lee EC, Chung K, Gao C, Gay J et al. (2007) Lipid-lowering effects of anti-angiopoietin-like 4 antibody recapitulate the lipid phenotype found in angiopoietin-like 4 knockout mice. Proc. Natl. Acad. Sci. USA 104:11766 – 11771. | 33. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH et al. (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientifi c Statement. Circulation. 112:2735 – 2752. | 34. Rana JS, Nieuwdorp M, Jukema JW, Kastelein JJ (2007) Cardiovascular metabolic syndrome - an interplay of, obesity, inflammation, diabetes and coronary heart disease. Diabetes Obes. Metab. 9: 218 – 232. |