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LIPID INDICES, ELASTIN TURNOVER AND THE DEVELOPMENT OF MICROVASCULAR COMPLICATIONS – A STUDY IN DIABETIC PATIENTS WITH ARTERIAL HYPERTENSION

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Abstract. Background and Aims: *An important factor in the development of vascular wall lesions is the degradation of the elastic fiber major protein – elastin. Elastin peptides (EDP) derived from this degradation are present in the circulation and are a stimulus for the production of anti-elastin antibodies (AEAbs) IgM, IgG and IgA. The aim of this study was to investigate the possible association between AEAbs, lipid indices and the development of microvascular complications.* ***Material and Methods:*** *Sera of 93 patients with type 2 diabetes mellitus (T2DM) and arterial hypertension (AH) were investigated (mean age 61,4 ± 11,3 years, diabetes duration 9,88 ± 3,12 years; hypertension duration 9,28 ± 4,98). ELISA was used for determination of anti-elastin antibodies. These levels were compared to serum AEAbs in 42 age- and sex-matched controls. Diabetic patients were divided in two groups according to the presence – Group 1 (n = 67) or absence – Group 2 (n = 26) of microangiopathy. The lipid profile and lipid indices (log TG/HDL, LDL/HDL, TC/HDL and TG/HDL) were also studied.* ***Results:*** *Patients with T2DM and AH showed statistically significant higher levels of serum AEAbs IgA than healthy controls – 0,338 (0,133÷0,452) vs. 0,006 (0,052÷0,068) (KW = 19,54; P < 0.0001). Group 1 showed statistically significant higher levels of AEAbs IgA than patients without microangiopathy – 0,353 (0,173÷0,471) vs. 0,235 (0,098÷0,377) (KW = 3,36; p = 0.05) and healthy controls – 0,353 (0,173÷0,471) vs. 0,006 (0,052÷0,068) (KW = 20,37; p < 0,0001) (0.37 ± 0,03 vs. 0.06÷0.01) (p = 0.0001). Patients from Group 2 showed significantly higher levels of AEAbs IgA than controls 0,235 (0,098÷0,377) vs. 0,006 (0,052÷0,068) (KW = 8,54; P = 0.003). AEAbs IgA showed correlation with insulin dose (r = -0.35); (p = 0.01), SBP (r = 0.31); (p = 0.001), HbA1c (r = 0.21); (p = 0.04), BMI (r = 0.22); (p = 0.01). AEAbs IgA correlated with log TG/HDL (r = 0.28); (p = 0.001), LDL/HDL (r = 0.22); (p = 0.01) TC/HDL (r = 0.22); (p = 0.01) and with TG/HDL (r = 0.15); (p = 0.05).* ***Conclusion:*** *Our study proved a relationship between elevation of AEAb IgA, high lipid indices and the development of microvascular complications in patients with type 2 diabetes mellitus and arterial hypertension.*

Key words: *diabetes mellitus, arterial hypertension, anti-elastin antibodies (IgG, IgM, IgA), microangiopathy, lipid indices*

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INTRODUCTION

Total cholesterol (TC)/high-density lipoprotein cholesterol (HDL) TC/HDL and low- density lipoprotein cholesterol (LDL) LDL/HDL ratios are used to predict ischaemic heart disease risk. The atherogenic index (AI) can be estimated as log TC/HDL [1]. Non-HDL cholesterol (total cholesterol_HDL) includes all known and potential atherogenic lipid particles that predict further cardiovascular disease [2-4]. Total cholesterol/HDL ratio has been reported to be associated with metabolic abnormalities predictive of cardiovascular risk and related to insulin resistance [5, 8]. Triglyceride/HDL ratio has been reported as a marker of insulin resistance and small LDL [9, 10].

The extracellular matrix protein elastin is responsible for the major part of tissue elasticity and is an insoluble component of elastic fibers in the skin, lung and arteries [11, 12]. The detection of circulating elastin-derived peptides (EDP) in the serum of healthy subjects, shows that the elastin macromolecule is not fully degraded by elastases in vivo. These EDP may become a stimulus for immunocompetent cells and for the production of anti-elastin antibodies (AEAbs) [13, 14, 15, 16]. Alterations in elastin structure and function lead to some pathologies of the arteries and lung [17]. Several elastase-type proteases, which selectively hydrolyze elastin, are present in smooth muscle cells, platelets, endothelial cells and fibroblasts [18]. During pathological processes these enzymes can be released and the result is degradation of elastic tissue. The following findings support the thesis that soluble EDP are released and they appear in the circulation [19]. These EDP are immunogenic and induce specific antibody production [20, 21]. Therefore, in the present study we quantitavely studied basement membrane elastin in the sera of type 2 diabetic patients with AH. Patients were divided in two groups according to presence or absence of microangiopathy. We also tested the hypothesis that lipid indices might have an association with ECM (extracellular matrix) main protein elastin turnover and with the development of microvascular complications.

Patients with type 2 diabetes mellitus are at high risk for vascular disease. This risk is increased with existence of arterial hypertension. In diabetic patients morbidity and mortality are mainly related to the presence of late complications, namely macro- and microangiopathy.

To the best of our knowledge, a relationship between lipid indices and immunological aspects of elastin turnover in diabetics with arterial hypertension has not been reported yet. The aim of this study was to identify a possible association between lipid indices, AEAbs and development of microvascular complications in patients with type 2 diabetes mellitus and arterial hypertension. We present the results of the determination of anti-elastin antibodies of different immunoglobulin classes (IgG, IgM and IgA) and lipid indeces in subjects with type 2 diabetes mellitus and arterial hypertension.

MATERIAL AND METHODS

Subjects

The experimental group consisted of 93 patients (37 men, 56 women) with type 2 diabetes mellitus and arterial hypertension (mean age 61,4 ± 11,3 years, diabetes duration 9,88 ± 3,12 years; hypertension duration 9,28 ± 4,98). These values were compared to serum antibodies to elastin in 42 age and sex-matched controls with no family history of diabetes, atherosclerosis or emphysema. All patients signed an informed consent prior to the initiation of the study.

Diabetics were divided in two groups according to the presence – Group 1 (n = 67) or absence – Group 2 (n = 26) of microvascular complications (Table 1). Group 1 consisted of 39% men and 61% women. Fifty-five percent were smokers and 45% were non-smokers. Group 2 consisted of 42% men and 58% women. Fifty-eight percent were smokers and 42% non-smokers. Controls consisted of 45% men and 55% women. Twenty-seven percent were smokers and 73% non-smokers (Table 2). Microalbuminuria was defined as a persistent urinary albumin excretion rate (AER) in the range of 20 to 200 μg/min in sterile urine. None of the patients had a diagnosis of renal disease unrelated to diabetes during the follow-up period.

Procedures

I. ELISA

Anti-elastin antibodies (IgG, IgM and IgA) were measured by an enzyme-linked immunosorbent assay (ELISA). ELISA included the following steps:

- Coating of polystyrene plates with human aortic α-elastin, prepared as described by Baydanoff et al. [3], (1 μg of elastin in 100 μl of 0.05 M carbonate buffer, pH 9.6).
- Blocking of the remaining “active” centres of the polystyrene wells by polystyrene plate incubation for 24 h with 1% solution of bovine serum albumin (BSA) (SIGMA, USA) in phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 20.
- Incubation with test human sera diluted 1:10 with PBS for 1 h at 37°C.

Table 1. Clinical data of patients with T2DM and AH

CLINICAL DATA	Group 1	Group 2	Controls
Age	62,5 ± 12,58	60,4 ± 8,4	58,9 ± 7,56
Gender (M/F)	26/41	11/15	10/12
Mean diabetes duration	9,30 ± 5,36	9,16 ± 7,59	N/A
Mean hypertension duration	9,50 ± 7,63	8,68 ± 7,26	N/A
HbA1c	*7,63 ± 2,03	7,27 ± 1,63	N/A
SBP (mmHg)	142,83 ± 18,05	140,58 ± 20,51	114,29 ± 15,74
DBP (mmHg)	82,23 ± 11,52	81,35 ± 11,96	72,5 ± 10,4
BMI	29,62 ± 4,99	28,42 ± 3,96	22,61 ± 2,27
TCL (mmol/l)	*5,26 ± 1,40	5,18 ± 0,93	3,99 ± 0,65
HDL (mmol/l)	*0,88 ± 0,30	0,93 ± 0,30	0,96 ± 0,20
LDL (mmol/l)	3,18 ± 1,19	3,16 ± 1,09	2,43 ± 0,64
TGL (mmol/l)	2,91 ± 1,68	2,53 ± 1,49	1,31 ± 0,61
Insulin dose (U/kg/24 h)	2,57 ± 0,52	2,03 ± 0,93	N/A
MAU (μg/min)	*78,94 ± 52,87	8,53 ± 4,69	N/A
MAU	(n = 43)	-	
Retinopathy	(n = 20)	-	
Neuropathy	(n = 4)	-	
Smokers	37/67	15/26	16/42
Number	67	26	42

Group 1 – patients with microvascular complications (n = 67);
Group 2 – patients without microvascular complications (n = 26);
Controls (n = 42); All data are presented as mean values ± SD

Table 2. Percentage of smokers in the groups

Group 1	
Men	Smokers
39%	55%
Women	Non-smokers
61%	45%
Group 2	
Men	Smokers
42%	58%
Women	Non-smokers
58%	42%
Controls	
Men	Smokers
45%	27%
55%	73%

- Incubation with immunoconjugates (anti-human immunoglobulin peroxidase conjugates (SIGMA, USA) to heavy chain of IgG, IgM and IgA) for 1 h at 37°C. All immunoconjugates were diluted 1:10,000 with PBS containing 1% BSA and 0.05% Tween 20.
- Incubation with substrate solution (o-phenylenediamine, 4 mg/ml in 10 ml 0.05 M citrate buffer, pH 5.0 + 0.01% H_2O_2 for 1 h at room temperature in a dark chamber).
- The reaction was stopped by adding of 4M sulfuric acid to each polystyrene well. All the extinction values were read at 492 nm using a Microelisa Reader 210 (OrganonTeknika, Belgium).

The following controls of the reaction were used: 1) Substrate control. The elastin-coated wells were incubated directly with substrate solution; 2) Immunoconjugate control. The specificity of each immunoconjugate was tested once against polystyrene elastin-coated wells (without human samples) and then against wells coated with immunoglobulins of the remaining two classes, e.g. the immunoconjugate against IgG was tested against human IgM and IgA. All the samples were tested in triplicate and peripheral wells were not used to avoid so called "bordering" effect. Intra-assay variation was less than 6% and inter-assay variation was less than 10%.

II. Other tests performed

1. Ophthalmoscopy through dilated pupils was carried out in all diabetic patients by the same ophthalmologist.

2. Glycated haemoglobin was measured using high-pressure liquid chromatography (normal range of HbA1c – 4-6%).

3. Serum total cholesterol and triglyceride concentrations were measured by enzyme assay (Boehringer Mannheim, Mannheim, Germany).

4. Arterial blood pressure was measured using a standard mercury sphygmomanometer, to the nearest 2 mm Hg, in the dominant arm after at least 10 min rest in the supine position.

5. AER was determined by nephelometry using a commercial kit containing specific antibody (Behringwerke AG, Marburg, Germany).

Statistical analyses

All data are presented as mean ± SD. Statistical analyses were done using the computer programs EXCEL and STATGRAPHICS plus for WINDOWS. The Student t-test and ANOVA were used to assess differences between the groups. The correlation and regression analyses were used. The level of significance was determined as $p < 0.05$.

RESULTS

Patients with T2DM and AH showed significantly higher levels of serum AEAbs IgA than healthy controls 0,338 (0,133÷0,452) vs. 0,006 (0,052÷0,068) (KW = 19,54; P < 0.0001). Group 1 showed statistically significant higher levels of AEAbs IgA than patients without microangiopathy 0,353 (0,173÷0,471) vs. 0,235 (0,098÷0,377) (KW = 3,36; p = 0.05) and healthy controls 0,353 (0,173÷0,471) vs 0,006 (0,052÷0,068) (KW = 20,37; p < 0,0001) (0.37 ± 0,03 vs. 0.06÷0.01) (p = 0.0001). Patients from Group 2 showed significantly higher levels of AEAbs IgA than controls 0,235 (0,098÷0,377) vs. 0,006 (0,052÷0,068) (KW = 8,54; P = 0.003) (Table 3) (Fig. 1). AEAbs IgA showed correlation with insulin dose (r = -0.35); (p = 0.01), SBP (r = 0.31); (p = 0.001), HbA1c (r = 0.21); (p = 0.04), and BMI (r = 0.22); (p = 0.01). AEAbs IgA correlated with log TG/HDL (r = 0.28); (p = 0.001), LDL/HDL (r = 0.22); (p = 0.01) TC/HDL (r = 0.22); (p = 0.01) and with TG/HDL (r = 0.15); (p = 0.05). Serum levels of AEAb IgG and IgM in patients with T2DM and AH were lower compared to controls, but these differences are not statistically significant. Male patients with T2DM and AH showed statistically significant higher ratio of TG/HDL than female patients 3,3 (2,5÷5,2) vs. 2,0 (1,5÷2,7) (KW = 10,78; P < 0.001).

Male patients with T2DM and AH showed statistically significant higher ratio of TC/HDL than female patients 7,2 (5,3÷9,0) vs. 5,2 (3,9÷6,2) (KW = 8,07; P = 0.004).

Male patients with T2DM and AH showed statistically significant higher ratio of LDL/HDL than female patients 4,1 (3,1÷5,8) vs. 2,9 (2,0÷4,5) (KW = 5,85; P = 0.01).

Log TG/HDL values in men were higher than these in women but this differences are not statistically significant (Table 4) (Fig. 2).

DISCUSSION

The atherogenic index of plasma (AIP) (log TG/HDL) is calculated in an attempt to predict cardiovascular risk. In the scope of AIP, triglycerides and HDL refer to the relationship of atherogenic lipids to protective lipids. The AIP has been related to cardiovascular risk in clinical trials. Values less than 0.11 are classified as low cardiovascular risk, between 0.11 to

Table 3. Serum levels of AEAbs IgA in patients with T2DM and AH

Groups	AEAbs IgA (ng/ml)	Comparison with other groups		
	M÷(Q1-Q3)	Group 1	Group 2	All Diabetics
All Diabetics	0,338 (0,133÷0,452)	NS	NS	–
Group 1	0,353 (0,173÷0,471)	–	P = 0,05	NS
Group 2	0,235 (0,098÷0,377)	P = 0,05	–	NS
Controls	0,066 (0,052÷0,068)	P < 0.0001	P = 0.003	P < 0.0001

Abbr.: Q1 – first quartile; Q3 – third quartile; Q1-Q3 – interqurtile range

Table 4. Lipid indices in male and female patients with T2DM and AH

	Men with T2DM and AH	Women with T2DM and AH	Significance
Lipid indices			
TG/HDL	3,3 (2,5÷5,2)	2,0 (1,5÷2,7)	(P < 0.001)
TC/HDL	7,2 (5,3÷9,0)	5,2 (3,9÷6,2)	(P = 0.004)
LDL/HDL	4,1 (3,1÷5,8)	2,9 (2,0÷4,5)	(P = 0.01)
AIP (log TG/HDL)	0,14 (0,12÷0,16)	0,13 (0,11÷0,14)	NS

*NS – non-significant

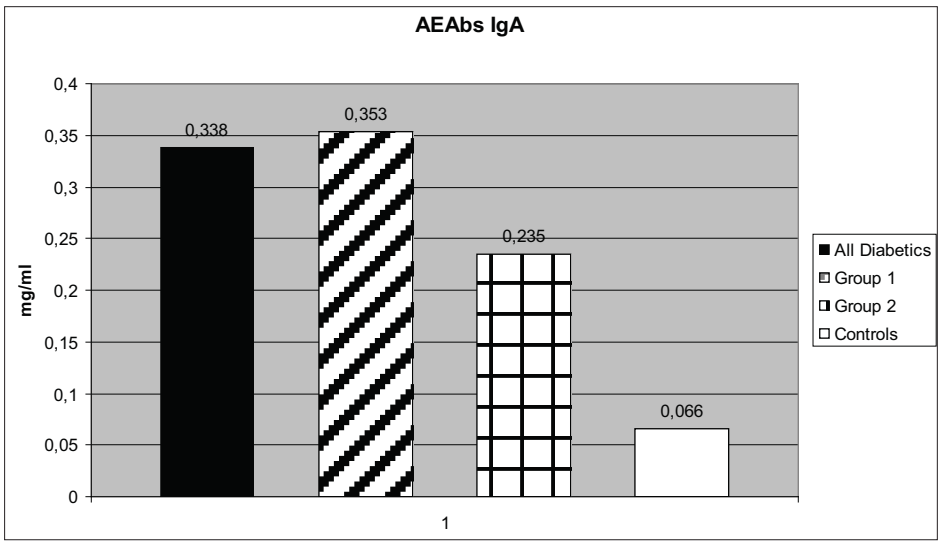

Fig. 1. Serum levels of AEAbs IgA in patients with T2DM and AH

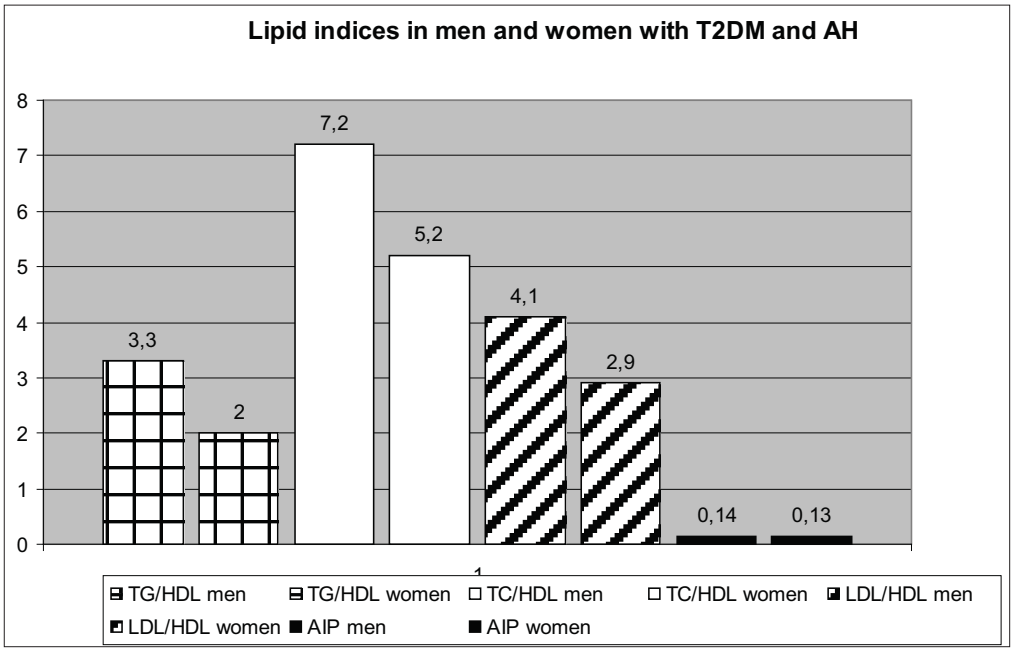

Fig. 2. Lipid indices in men and women with T2DM and AH

0.21 there is an intermediate cardiovascular risk, and greater than 0.21 there is increased risk.

The total cholesterol TC/HDL ratio is more indicative of cardiovascular disease than TC (total cholesterol), if HDL and LDL in the blood are added together. For men an acceptable ratio of TC/HDL is 4.5 or below, and for women is 4.0 or below. HDL levels have an inverse relationship with coronary heart disease. The ability of HDL to predict the development of coronary atherosclerosis has been estimated to be four times higher than LDL and eight times – than TC. For men an acceptable ratio of LDL/HDL is 3.6 or below, and for women – 3.2 or below. In adults, the triglyceride TG/HDL-"good" cholesterol ratio should be below 2. More precisely, the triglyceride/HDL ratio of 2 or less is considered ideal, 4 – high, and 6 – extremely high. People with the highest ratio of triglycerides to HDL had 16 times the risk of heart attack as those with the lowest ratio of triglycerides to HDL in the study of 340 heart attack patients and 340 of their healthy, same age counterparts. The ratio of triglycerides to HDL was the strongest predictor of a heart attack, even more accurate than the LDL/HDL ratio [22].

Epidemiological studies have shown a strong relationship between diabetes mellitus, arterial hypertension and cardiovascular disease. In diabetic patients morbidity and mortality are mainly related to the presence

of late complications, namely macro- and microangiopathy. Diabetes mellitus (both type 1 and type 2) is a major risk factor for cardiovascular disease [23]. In the Framingham Study, the risk of cardiovascular disease was doubled by the presence of diabetes [24].

Arterial hypertension and diabetic vascular complications are connected with an elevated degradation of elastic tissue. As a result soluble EDP are released in the circulated blood, which are a pathological stimulus for an increased production of AEAbs. Because it is very important to find characteristics of pathological activation of elastin turnover, we studied diabetic patients with arterial hypertension. Our data suggest an association between the activity of elastin turnover (especially increased serum levels of IgA AEAbs) and vascular complications. In a recent investigation, patients with microvascular complications showed statistically significant higher levels of AEAbs IgA than patients without micrangiopathy and healthy controls. AEAbs IgA showed correlation with systolic blood pressure, too. Serum levels of AEAb IgG and IgM in patients with T2DM and AH were lower than in controls, but these differences are not statistically significant.

The first immunoglobulin synthesised during the early phase of a pathologically activated immune response is IgM. The immune system then switches to production of IgG. The elevation of AEAbs of the IgM and IgG types is, therefore, the first indicator of the pathological turnover of elastin and the development of vascular complications in diabetic patients. That is a possible explanation of our results. The later rise in the level of IgA AEAbs and its association with diabetic microvascular complications may be explained as follows: IgA AEAb may be “spared” by the phagocytes, which mainly possess receptors for the Fc-fragments of IgG and IgM. Thus, the elimination of IgG bound to a specific antigen will be greater than that of IgA [25] during the later stages of the development of vascular complications.

Our data suggest an association between the activity of elastin turnover, high lipid indices and vascular complications. We found that the values of AEAbs IgA in patients with vascular complications are significantly higher than in patients without vascular damages and healthy controls. This means that the diabetics from Group 1 show pathologically high immune response to elastin. Although the data from Group 2 reveals levels of IgA AEAbs higher than those of the controls, these levels still remain lower than the measured in patients with vascular complications from Group 1. It is extremely important to monitor if the group of patients with high levels of IgA AEAbs will develop vascular lesions before the patients without such elevated levels of IgA AEAbs. We suppose that the high lipid indices indicate a higher probability of the development of vascular changes in these patients, particularly in those from group 1. According to our results, determination of serum AEAbs IgA seem to be a useful method for identifying a high atherosclerotic risk in diabetic patients with hypertension.

Extracellular matrix proteins – collagen type IV (CIV) and elastin – are uniquely present in basement membranes and represent their predominant structural element. Metabolic alteration of elastin occurs mainly in the microvascular basement membrane of diabetic patients. Arterial hypertension and diabetes mellitus are connected with an elevated degradation of elastic tissue, loss of elasticity, increasing rigidity of arterial wall, and abnormal increase in the collagen/elastin ratio. As a result soluble elastin (EDP) derived peptides are released in the circulated blood, which are a pathological stimulus for an increased production of anti-elastin (AEAbs) antibodies.

There was a correlation established between AEAbs IgA, log TG/HDL ($r = 0.28$); ($p = 0.001$), LDL/HDL ($r = 0.22$); ($p = 0.01$) TC/HDL ($r = 0.22$); ($p = 0.01$) and with TG/HDL ($r = 0.15$); ($p = 0.05$). Accumulating evidence points toward a role of elastic fibers in the process of lipid precipitation in arteries and our results support these findings.

Interestingly, AEAbs IgA did not show correlation with the serum total cholesterol and triglyceride levels but correlated with high lipid indices. That is why we suppose that lipid indices are more strongly associated with development of ECM dysfunction (because of correlation with elastin antibodies) and development of vascular lesions than widespread use of routine lipid markers. Probably lipid ratios and especially TC/HDL and TG/HDL ratio are more useful for identification of subjects who are more susceptible to microvascular complications than isolated determination of these variables. It is known that the elevated levels of serum triglycerides which contain multiple types of potential atherogenic lipoproteins enrich the picture of diabetic dyslipidemia. It is the most common deviation in poorly controlled diabetes. In patients with microvascular complications the attachment of triglycerides to the arterial wall can lead to the conversion of elastin into an immunogenic form [31, 32]. In the present study, the triglycerides were elevated over the normal range in patients with vascular complications which supports the idea about this feature.

Identification of biological markers would allow more aggressiveness in the achievement of tight glycaemic control and a better search for initial lesions in those patients who are predisposed to complications than in those who are not. The understanding of the relation between high lipid indices, the altered pattern of expression of various

antibodies and the dysregulated vascular remodeling at the level of the target tissue would allow design of therapeutic approach targeted to the individual tissue and to the relevant biochemical, functional and structural alterations. Our study shows, that determination of biological markers of ECM turnover and lipid indices can be useful in monitoring vascular injury.

In conclusion, our results suggest an association between the level of anti-elastin IgA antibodies, high lipid indices and the development of vascular wall lesions in patients with diabetes mellitus type two. Elevation of AEAbs IgA may indicate increased elastin degradation and development of microvascular complications. However, a larger study is necessary for clarification of these possibilities.

Conflict of interests

The authors have nothing to declare.

REFERENCES

1. Lemieux I., Total Cholesterol/HDL cholesterol ratio vs. LDL cholesterol/HDL cholesterol ratio as indices of ischaemic heart disease risk in men- The Quebec Cardiovascular Study, Arch Intern Med/Vol 161, Dec 10/24, 2001, 2685-1692.
2. Cui Y, Blumenthal RS, Flaws JA, et al. Non-highdensity lipoprotein cholesterol level as a predictor ofcardiovascular disease mortality. Arch Intern Med2001;161:1413-1419.
3. Bittner V, Hardison R, Kelsey SF et al. Non-high-density lipoprotein cholesterol levels predict five-year outcome in the Bypass Angioplasty Revascularization Investigation (BARI). Circulation 2002;106:2537-2542.
4. Ridker PM, Rifai N, Cook NR et al. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as riskfactors for cardiovascular disease in women. JAMA2005;294:326-333.
5. Jeppesen J, Facchini FS, Reaven GM. Individuals with high total cholesterol/HDL cholesterol ratios are insulinresistant. J Intern Med 1998;243:293-298.
6. Ovbiagele B, Saver JL, Fredieu A, et al. PROTECT: a coordinated stroke treatment program to prevent recurrent thromboembolic events. Neurology 2004;63:1217-1222.
7. Smaha LA. The American Heart Association Get withthe Guidelines program. Am Heart J 2004;148:S46-S48.
8. Lee LJ, Kidwell CS, Alger J et al. Impacton stroke subtype diagnosis of early diffusionweightedmagnetic resonance imaging and magnetic resonance angiography. Stroke 2000;31:1081-1089.
9. Bianchi C, Penno G, Malloggi L, et al. Non-traditionalmarkers of atherosclerosis potentiate the risk of coronaryheart disease in patients with type 2 diabetes andmetabolic syndrome [published online ahead of printApril 7, 2007]. Nutr Metab Cardiovasc Dis.
10. Sumner AE, Finley KB, Genovese DJ et al. Fasting triglyceride and the triglyceride-HDL cholesterolratio are not markers of insulin resistance in AfricanAmericans. Arch Intern Med 2005;165:1395-1400.
11. Mecham RP. Overview of extracellular matrix. Curr Protoc Cell Biol. 2012 Dec;Chapter 10:Unit 10.1. doi: 10.1002/0471143030.cb1001s57.
12. Steven R. Van Doren. Matrix metalloproteinase interactions with collagen and elastin, Matrix Biol. 2015, 44-46, 224-231.
13. Nicoloff G, Petrova Ch, Christova P, Nikolov A. Detection of free elastin-derived peptides among diabetic children. Atherosclerosis 2007, 192;342-347.
14. Baydanoff S, Nicoloff G, Alexiev Ch. Age-related changes in anti-elastin antibodies inserum from normal and atherosclerotic human subjects Atherosclerosis 1987;63:267-71.
15. Fulop T, Wei S, Robert L, Jacob MP. Determination of elastin peptides in normal andatherosclerotic human sera by ELISA Clin Physiol Biochem 1990;08:273-8.
16. Colburn K, Langga-Shariffi E, Kelly G, et al. Abnormalities of serum antielastinInfluence of elastin-derived peptides on metalloprotease production in endothelial cells. J. Investiq Med., 2003,51(2),104-9.
17. Siemianowicz K, Gminski J, Goss M et al. Influence of elastin-derived peptides on metalloprotease production in endothelial cells. Experimental and Therapeutic Medicine. 2010, 1(6):1057-1060.
18. Robert L, Molinari J, Ravelojaona V, Andrès E. Age- and passage-dependent upregulation of fibroblast elastase-type endopeptidase activity. Role of advanced glycation endproducts, inhibition by fucose, Archives of gerontology, 2010 – Elsevier.
19. Jimenez F, Mitts T, Hinek A. Elastin protective polyphenolics and methods of using the same – US Patent 8,114,829, 2012 – Google Patents.
20. Fulop T, Wei S, Robert L, Jacob MP. Determination of elastin peptides in normal andatherosclerotic human sera by ELISA. Clin Physiol Biochem 1990;08:273-8.
21. Lazarovici P, Pomerantz C, Chen X, Wei Y. Co-electrospun blends of PLGA, gelatin, and elastin as potential nonthrombogenic scaffolds for vascular tissue engineering, 2010 – ACS Publications.
22. Gaziano JM, Hennekens CH, O'Donnell CJ et al. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 1997;96:2520-2525.
23. Wakabayashi I, Associations of Blood Lipid-Related Indices with Blood Pressure and Pulse Pressure in Middle-Aged Men, Metab Syndr Relat Disord, 2015,31(1):22-8.
24. Kannel WB, Gordon T. Physiological and medical concomitants of obesity. The Framingham study. In: Bray G, ed. Obesity in America. Betesda, MD: US Department of Health, education and welfare, Public Health, Health service, National institutes of Health; 1979; 125-163.
25. Spiegelberg H. Immunoglobulins. In: Inflammation: Basic Principles and Clinical Correlates (Edited. by Gallin, J. and Goldstein, I.) Raven Press; New York, 1988,11-19.
26. Peterszegi G, Mandet C, Texier S et al. Lymphocytes in human atherosclerotic plaque exhibit the elastin-laminin receptor: potential role in atherogenesis. Atheroscl 1997a;135:103-107.
27. Peterszegi G, Texier S, Robert L. Human helper and memory lymphocytes exhibit an inducible elastin-laminin receptor. Intl Arch Immunol 1997b;114,:218-223.
28. Mestecky J, McGhee JR. Immunoglobulin A (IgA): molecular and cellular interactions involved in IgA byosynthesis and immune response. Adv Immunol 1987;40:153-245.
29. Mestecky J. Interaction of lymphoid cells with epithelial cells: selectivity of the expression of IgA in mucosal plasma cells and of the transport of polymeric IgA. In: Monographs in Allergy (Edited by Dukor, P, Shakib, F, Trunka, Z, Waksman, BH) S. Karger, Basel, 1988,83-90.
30. Podet EJ, Shaffer DR, Gianturco SH, et al. Interactionof low density lipoproteins with human aortic elastin. Atheroscler Thromb 1991;11:116-122. 25.
31. Taskinen MR. Diabetic dyslipidaemia: from basic researchto clinical practice. Diabetologia, 2003;46:733-749.
32. Kennedy L. Glycation of immunoglobulins and serumproteins. Diabetologia, 2003;46:733-749.27.
33. Singh R, Barden A, Mori T, Beilin L (2001)Advanced glycation end-products: a review. Diabetologia, 44: 129-146.