

# Diabetic Microangiopathy – Etiopathogenesis, New Possibilities in Diagnostics and Management

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## 1. Introduction

Diabetic microangiopathy is characterized as a disorder of small vessels. It is one of the major chronic diabetic complications involving diabetic neuropathy, retinopathy and nephropathy (Adeghate et al., 2006). Its prevalence has rising tendency even in children population and is positively associated with duration of diabetes mellitus.

Diabetic neuropathy affects peripheral nerves (sensory, motor, autonomic) so all organ systems can be affected. In childhood, subclinical forms are typical when no clinical symptoms are evident, however sensitive diagnostic methods can detect them. Later, autonomic and sensory-motor neuropathy is very common (see the classification in table 1). Some forms of diabetic neuropathy are presented in 40 - 90% of patients with diabetes duration ten years and more and even in 5% of patients after one year of diabetes diagnostics.

Subclinical neuropathy				
Clinical neuropathy	Symetric	Distal symetric neuropathy	sensoric motoric mixed	
		Autonomic neuropathy		
		Proximal symetric neuropathy		
		Acute painful neuropathy		
	Asymetric	Cranial neuropathy		
		Peripheral mononeuropathy		
		Radiculopathy		
		Asymetric proximal motoric neuropathy		
	Mixed			

Table 1. Classification of diabetic neuropathy (Rybka, 2007)

Diabetic retinopathy is the most frequent cause of new cases of blindness among adults aged 20 - 74. It progresses from mild nonproliferative abnormalities characterized by increased vascular permeability to moderate and severe nonproliferative retinopathy

characterized by vascular closure to proliferative retinopathy with typical new blood vessels growth (table 2). The prevalence of retinopathy is noticed in 2 - 7% of diabetic patients after two years, in 50% of patients after ten years and in 75% of patients after twenty and more years of diabetes duration.

Diabetic nephropathy is characterized by glomerular, tubular and mesangial damage accompanied by basement membrane thickening, mesangial expansion and hyalinisation of glomerular intercapillary connective tissue. In clinical practice, progressive kidney disease with proteinuria, hypertension and gradual decrease in renal functions is typical (table 3). Manifest nephropathy is presented in 30 - 35% of patients with diabetes duration over fifteen years.

	Eye background
<b>Non-proliferative DR</b>	
Mild	Microaneurysmas, microhemorrhages, intraretinal hemorrhage, venous abnormalities
Moderate	Vessel changes at macula area, hard exudates, „cotton“ exudates
Severe	Intraretinal microvascular abnormalities (IRMA), retinal ischemia
<b>Proliferative DR</b>	
Beginning	Retinal or papilar neovascularisation
High risk	Traction amotio of retina, intravital hemorrhage
<b>Diabetic maculopathy</b>	Macular edema

Table 2. Clinical stages of diabetic retinopathy (DR) (Rybka, 2007)

	characteristics	Urinary findings
<b>1. Latent stage (hyperfiltration-hypertrophic)</b>	increase GF about 10 - 40%, ultrasound hypertrophic of kidneys, slightly enlargement of basal membrane	transitory microalbuminuria 30 - 100 mg/day, resp. 20 - 70 µg/min
<b>2. Incipient diabetic nephropathy</b>	decrease GF, common hypertension (mainly diastolic), progression in enlargement of basal membrane	permanent microalbuminuria 30 - 300 mg/day, resp. 20 - 200 µg/min
<b>3. Manifest diabetic nephropathy</b>	Next decrease GF, hypertension progression, sclerotisation of many glomerules	proteinuria >0,5 g/day
<b>4. Chronic renal insufficiency even kidney failure</b>	Terminal phase, uremia, dialysis necessary	proteinuria >0,5 g/day, serum creatinine > 200 µmol/l

Table 3. Clinical stages of diabetic nephropathy (Rybka, 2007)

## 2. Etiopathogenesis

The etiopathogenesis of diabetic microangiopathy is complex (Figure 1) and since now not completely understood. Long lasting hyperglycemia triggers the variety of pathways - non-enzymatic glycation of proteins, oxidative stress, polyole pathway and sorbitol production, activation of proteinkinase C production, decrease of vasodilatation products (nitric oxide, prostaglandines), decrease of myoinositol origin, change in Na<sup>+</sup>K<sup>+</sup>ATP-ase activity causing the endothelial damage and so the microangiopathy with disorder in many organ systems (Brownlee, 2005). However, according to the clinical experience, some patients with short duration and adequate compensation of diabetes mellitus (DM) have symptoms of microangiopathic complications, and some despite long duration and insufficient compensation do not suffer from any complications. That is why other factors have been considered as important in pathogenesis - genetic, epigenetic or immunologic factors (Villeneuve & Natarajan, 2010). According to Diabetes Control and Complication Trial (DCCT Research Group, 1993), important risk factors for microvascular complications are cigarette smoking and genetic susceptibility to hypertension at early stages of diabetes and poorer glycemic control, higher blood pressure and unfavourable lipid profile at later stages.

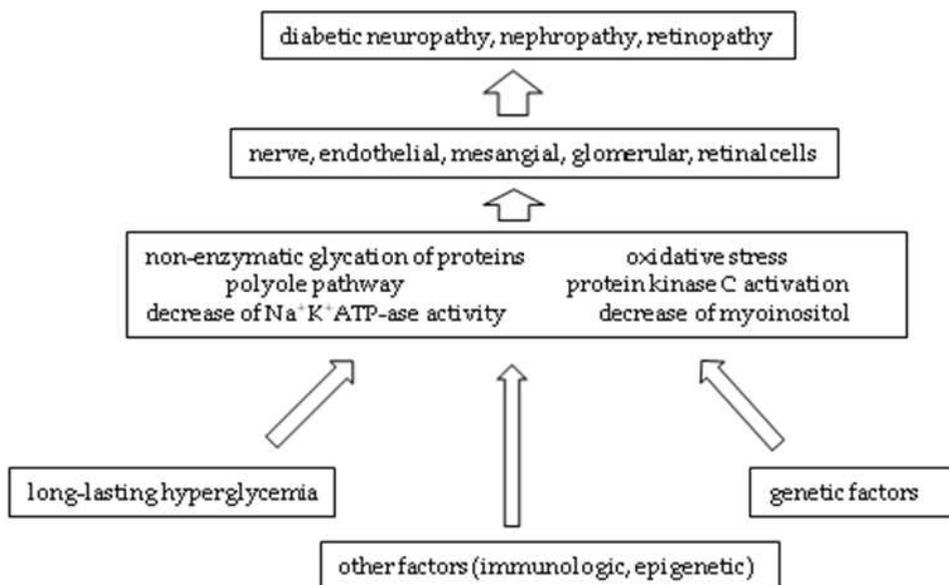


Fig. 1. Etiopathogenesis of diabetic microvascular complications

### 2.1 Advanced glycation end products

Advanced glycation end products (AGEP's) are heterogeneous group of modified proteins, lipids and nucleic acids arisen within intracellular hyperglycemia by non-enzymatic Maillard reaction. The initial product of this reaction is called a Schiff base, which spontaneously rearranges itself into an Amadori product. These initial reactions are reversible depending on the concentration of glucose and reactants. Series of subsequent reactions (dehydration, oxidation, reduction, other arrangements) lead to the formation of

AGEP's. As precursors AGEP's have typical ability for covalent crosslink formation between proteins, modified products have altered their structure and biological function (Peppas et al., 2003). Except endogenously formed AGEP's, another source is exogenous like tobacco smoke and certain food. Nearly 10% of AGEP's is absorbed through intestinal mucosa from food like meat with high content of fat, meals prepared at high temperature (grilled, fried) (Klenoviscová et al., 2008). Contrary, fruit, vegetable and boiled, braised or steamed meals have noticeably lower content of AGEP's.

AGEP's increase vascular permeability and production of growth factors and cytokines. They can modify extracellular matrix by changing its structure, solubility and charge leading to accumulation of collagen, fibronectin or laminin. Abnormalities in glomerular extracellular matrix lead to higher proliferation of mesangial cells and to increase in glomerular permeability which correlates with microalbuminuria (Cvetkovic et al., 2009). Glycated collagen can change the filtration abilities of glomerular basement membrane and can increase the binding of circulating plasmatic proteins.

Receptors for AGEP's (RAGE) were found on vascular endothelial cells, macrophages, mesangial cells or smooth muscle cells. Interaction between AGEP's and RAGE causes increase of oxidative stress, activation of transcriptional factor NFκB and expression of inflammatory genes for cytokines or growth factor inducing NO synthetase. Binding AGEP's on neural receptors RAGE causes the induction of oxidative stress, protein kinase C and NFκB responsible for inflammatory processes and apoptosis of nerve cells. As vasa nervorum supply nerve cells, diabetic neuropathy is caused also by changes of endothelial cells.

## 2.2 Polyole pathway

Polyole pathway has physiologically role in reduction of toxic aldehydes arisen due to reactive oxygen species (ROS) into inactive alcohols. An initial and rate limiting enzyme of this pathway is aldose reductase which uses reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Raising intracellular concentration of glucose increases the activity of this enzyme by which glucose is reduced into sorbitol and next into fructose. Elevated activity of aldose reductase leads to decrease of concentration of NADPH (needed also for glutathione reductase activity), by which intracellular oxidative stress is raising. Accumulation of osmotic active sorbitol and fructose causes the reduction of myoinositol and taurine in nerve fibers and decrement in membrane Na<sup>+</sup>K<sup>+</sup>ATP-ase activity. These processes lead to slow of axonal transport. Also oxidative stress and protein kinase C induce the sorbitol origin, apoptosis of nerve cells and microvascular dysfunction.

## 2.3 Oxidative stress

Biochemical and metabolic changes in diabetes duration cause the increase of reactive oxygen species (ROS) origin and also decrease of antioxidant systems activity. ROS, especially superoxid anion cause damage of endothelial cells leading to diabetic microangiopathy. In hyperglycemia condition endothelial cells are exposed to major glucose turnover, from glycolysis through pyruvate decarboxylasis into Krebs cycle with consequence of higher transport of electrons through mitochondrial enzymes. Electron-overloaded mitochondria produces significant amount of superoxid anions that lead into

nitric oxid decrease, DNA damage, AGEP's formation, protein kinase C activation and even polyole pathway activation.

Oxidative stress induces mRNA expression of tissue growth factor  $\beta 1$  (TGF-  $\beta 1$ ) and fibronectin playing important role in renal damage as they increase the extracellular matrix production. Overproduction of ROS alters the mesangial cells – the most important in diabetic nephropathy development – and supports the cell processes leading to apoptosis (Ha et al., 2008). ROS increase protein kinase C (PKC) activity in mesangial and glomerular cells and PKC stimulates the hyperproduction of ROS. These changes together with non-enzymatic glycation of proteins lead to kidney hyperperfusion, hyperfiltration, extracellular matrix cummulation, renal vessels vasoconstriction, reconstruction of renal structure and even nephrosclerosis.

Oxidative stress can directly damage neurocyte myelin, macromolecules and membranes of nerve cells and induces their apoptosis. Considering the blood supply of nerves through vasa nervorum, also dysfunction of endothelial cells contributes to diabetic neuropathy.

Microvasculature of retina consists of two types of cells – endothelial and pericytes. Early signs of diabetic retinopathy are characterized by dysfunction of pericytes and consequently by dysfunction of endothelial cells. Oxidative stress together with AGEP's induce nuclear factor NF $\kappa$ B and apoptosis of pericytes and endothelial cells. They contribute to thickening of retinal basement membrane, formation of acellular capillaries or microaneurysmas. Pigment epithelium derived factor (PEDF), glycoprotein of protease inhibitors family, is extracellular part of retina and was found in vitreous and intraocular fluid. PEDF can inhibit the growth and migration of endothelial cells, has antiangiogenic activity, cytoprotective and also antioxidant effect. Oxidative stress causes the decrease in PEDF leading to dysfunction and apoptosis of pericytes. The stability of vascular integrity is provided by angiopoietin 1 (ang-1), while angiopoietin 2 (ang-2) is its natural antagonist which break the connection between pericytes and endothelium (Patel et al., 2005). Oxidative stress and ROS increase the ratio of ang-2 / ang-1 in pericytes whereby impair the stability of retinal cellular structure.

## 2.4 Protein kinase C

Protein kinase C (PKC) belongs to the family of kinases responsible for intracellular signalisation in the cardiovascular, immunologic or other systems. Till now, twelve isoforms of PKC have been characterized according to the structure and cofactor requirements. The activator of the most of PKC isoforms is diacylglycerol. De novo formation of diacylglycerol is enhanced in higher concentration of intracellular glucose with the consequence of increase activity of isoforms PKC- $\beta 1$  and PKC- $\delta$ . PKC can be activated also by some growth factors, superoxid (induced by hyperglycemia) and by AGEP's. PKC mediated phosphorylation of substrate proteins triggers a cascade of pathophysiological responses. Isoforms PKC- $\beta 1$  and 2 deteriorate the blood flow through retina and kidneys, increase the capillary leak, induce the production of extracellular matrix and activate many inflammatory cytokines what contribute to microvascular damage.

Formation of vascular endothelial growth factor (VEGF), a cytokine with major role in retinal leakage, angiogenic activity and neovascularisation, is at least partly PKC dependent. PKC activates the release of adhesion molecules, tissue growth factor  $\beta$ ,

endothelin-1 and fibronectin. PKC also increases leucocyte adhesion through upregulation of intracellular adhesion molecules leading to capillary leakage, occlusion and microthrombosis. PKC activation mediates inhibition of  $\text{Na}^+\text{K}^+$  and  $\text{Ca}^{2+}$  ATP-ase activity contributing to reduction in membrane transporter activity and thus changes in nerve conduction, microcirculatory flow and capillary pressure (Casellini et al, 2007).

Described pathways (Ido et al., 1996) act not individually but in connected interactions (Figure 2).

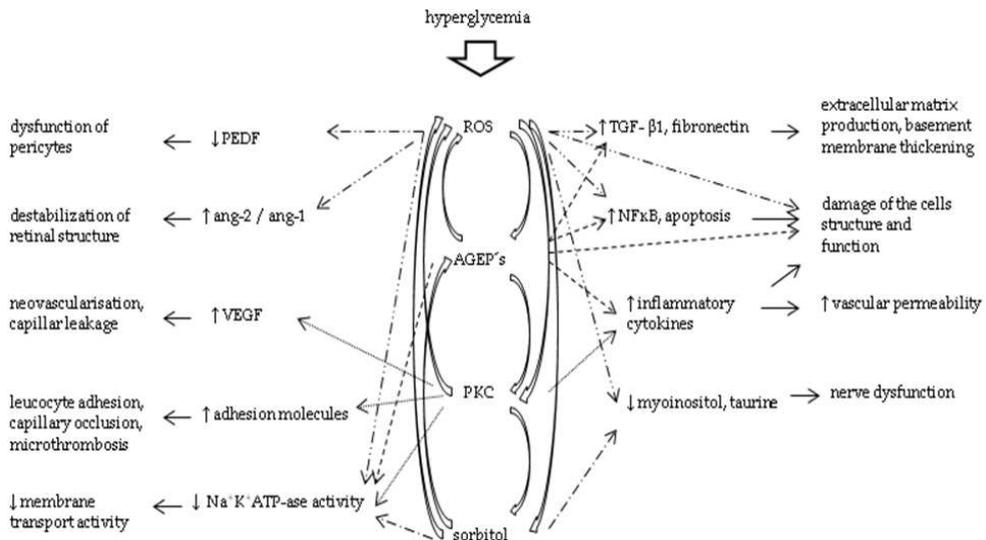


Fig. 2. Interaction between oxidative stress, non-enzymatic glycation, protein kinase C and polyole pathway and their impact on target structures (ROS=reactive oxygen species, AGEP's=advanced glycation end products, PKC=protein kinase C, PEDF=pigment epithelium derived factor, ang=angiotensin, VEGF=vascular endothelial growth factor, TGF=tissue growth factor, NFκB=nuclear factor κB)

## 2.5 Low C-peptide concentration

Disregarding hyperglycemia, the risk factor for microangiopathy development is decrease of C-peptide concentration in type 1 diabetes mellitus (Ekberg et al., 2007). C-peptide is produced by beta-cells of pancreas together with insulin and is used as a marker of endogenous insulin secretion as it is just minimally metabolized by the liver. The claims that it is biologically inactive molecule are now overcome. The binding of C-peptide to till now non-identified cell receptor leads to activation of G-proteins, increase of intracellular calcium concentration, increase of protein kinase C activity, MAP-kinase activity, increase of many transcriptional factors (NFκB, c-Fos), nuclear receptor PPARγ or antiapoptotic protein Bcl2. On the cellular level, C-peptide improves nerve function and erythrocyte deformability by increase of  $\text{Na}^+\text{K}^+$ ATP-ase activity, improves the endothelial function and protects from microangiopathy by nitric oxide synthetase induction and has antithrombic effect by inhibition of adhesive molecules expression (P-selectine, ICAM1). It can inhibit the

apoptosis by increasing the Bc12 concentration and can diminish the glomerular filtration by not completely understood mechanisms.

## 2.6 Angiotensin-converting enzyme

Angiotensin-converting enzyme (ACE) is circulating enzyme, exopeptidase, which participates in the renin-angiotensin-aldosterone system (RAAS) regulating the extracellular fluids volume and arterial vasoconstriction. ACE is secreted by endothelial cells of lungs and kidneys and catalyses the conversion of decapeptide angiotensin I into octapeptide angiotensin II (AngII) which acts like vasoconstrictor. ACE degrades vasodilator bradykinin and also other vasoactive peptides. Beside this, RAAS has a function in immune system – it regulates the extravasation and chemotactic migration of leucocytes, increases the expression of adhesive molecules, chemokines and chemokine receptors. T lymphocytes, macrophages and dendritic cells contribute to increase of renin and Ang II releasing through production of TNF and IL-1. According to the recent information, RAAS and Ang II have influence on origin of autoimmune diseases (Tippisetty et al., 2011). Ang II acts also like growth factor regulating the cell growth and tissue expansion what can lead to cell hypertrophy and fibrotic changes of tissues.

## 2.7 Risk factors

An important role of diabetes *compensation* in development of chronic diabetic complications was proved by multicentric randomized study Diabetes Control and Complication Trial (DCCT) between 1983 and 1993 (DCCT Research Group, 1993). 1441 patients with DM type 1 (diabetes duration 1 – 15 years) were enrolled to the study, divided into two groups according to the treatment by either conventional or intensified insulin regimen. Children younger than 13 years did not participate but 195 adolescents were enrolled. The prevalence of micro- and also macrovascular complications was significantly less frequent in the group treated intensively compared to the conventionally treated group. The most of the patients consequently continued in the next study Epidemiology of Diabetes Interventions and Complications (EDIC) where all of the subjects underwent intensified insulin treatment. After 4 years of EDIC study, the prevalence of chronic complications was still higher in the group initially treated by conventional regimen despite actual good compensation. In the subgroup of adolescents, the intensified therapy led to decrease of the risk of retinopathy by 53%, neuropathy by 60% and microalbuminuria by 60%. In the next following of subjects in EDIC study, these differences were enhanced – patients treated from the beginning by intensified regimen had lower prevalence of retinopathy by 74% and of microalbuminuria by 48% compared to the patients treated conventionally in the first phase. This study showed “the effect of metabolic memory” where each period of worsened compensation can negatively influence the prognosis of the diabetic patient. Some studies with lower number of patients did not confirm the association between diabetes compensation and chronic complications (M. Javorka et al., 2005).

Diabetes *duration* is also suggested as an important risk factor for development of chronic complications. The significant correlation between diabetes duration and autonomic neuropathy (based on at least one pathologic result in two cardiovascular tests – heart rate variability, systolic blood pressure decrease in orthostasis) was confirmed in the biggest

study focusing on chronic diabetic complications EURODIAB IDDM Complication Study (EURODIAB IDDM Complication study group, 1994) where 3250 patients with DM type 1 at the age 15 - 60 years took part. Similar correlation was not confirmed in some other studies (Scaramuzza et al., 1998) probably caused by different methods and lower amount of subjects.

Other risk factors were identified - increase in diastolic blood pressure  $\geq 90$  mmHg, increased triacylglycerides  $> 1,7$  mmol/l, decrease of serum HDL cholesterol  $< 1,0$  mmol/l, microalbuminuria  $> 20$   $\mu\text{g}/\text{min}$ , higher body mass index, presence of retinopathy, smoking and the period of puberty (Donaghue et al., 2009). The diabetes compensation in prepubertal period has lower impact on complication development compared to the period after gonadarche (Maguire et al., 2005). Many adolescents with DM type 1 have frequently worsened compensation due to endocrine changes in puberty leading to increase of insulin resistance (insulin like growth factor IGF-1, sexual hormones) (Court et al., 2008) but also due to excessive food intake, lack of physical activity, neglect of insulin treatment and hazardous behavior (alcohol, smoking, drugs, contraceptives).

Despite long lasting hyperglycemia, another important risk factor is *genetic predisposition* of the subject due to gene polymorphisms of enzymes involved in the pathogenesis of chronic complications origin and development (Donaghue et al., 2005). Probably other factors exist - immunologic, epigenetic, etc., which are not clear at present. This fact would clarify the clinical experience that some patients despite long diabetes duration and poor compensation do not have any signs of complications and on the other hand, some patients with adequate compensation and short diabetes duration suffer from some forms of complications.

### 3. Diagnostics

Diagnostic range of diabetic microangiopathic complications extends as it responds to ascending demands. Classic investigations (neurological examination, eye background investigation, microalbuminuria) could verify clinical manifesting forms of chronic complications. Nowadays, huge impact is put on the early diagnostics of clinically silent forms that has led to development of many new possibilities. New approach in diagnostics is investigation of gene polymorphisms of enzymes involved in the pathogenesis of microangiopathy that enables the individual approach to the patient and perhaps the "customized" therapy in the future.

#### 3.1 Patient's history and physical examination

Subclinical form of diabetic neuropathy is characterized as the absence of clinical manifestations of neuropathy, but specific tests can reveal the presence of some abnormalities. Clinical form can affect sensory-motor or autonomic nerve fibers. In **sensory-motor** form, the patient complains of sensory disturbance - paresthesia, dysesthesia, impaired vibration and / or thermal sensitivity, eventually of pain (neuropathic pain), and motor disturbances - decrease in muscle strength. Decreased tendon reflexes can be determined. These disorders can be expressed symmetrically or asymmetrically (Shy, 2007).

In **autonomic** form symptoms of disorder of various organ systems can be expressed:

- **cardiovascular** system: rest tachycardia, orthostatic hypotension, increased arrhythmogenesis of myocardium, cardiomyopathy, circulation instability, decreased tolerance of physical activity and heat, edema of lower limbs
- **gastrointestinal** system (Kycina et al., 2011): dysphagia, odynophagia, gastroparesis – nausea, diarrhea, constipation, fecal incontinence
- **urogenital** system: atonia of vesica urinaria, urine retention, common urinary tract infection, erectile dysfunction, painless while pressing testes
- **respiratory** system: decreased lung functions, prone to infections (Ďurdík et al., 2010), decreased cough reflex sensitivity
- **sudomotoric** system: feet anhidrosis, increased sweating of upper part of the body, sweating after food and at the night
- **endocrine** system: asymptomatic hypoglycemia, hormonal contraregulation disorder
- **other**: pupillary reflex disorder.

We note the patient's general condition, status and trophic skin and skin appendages – temperature, color, sweating, hair, skin appearance (the presence of dry, peeling skin), nail condition, pressure sores, infections. It is also necessary to assess trophic, tone and strength of selected muscle groups, loss of muscle mass, the state of the vascular system (pulsation of peripheral arteries) and condition of ligament structures of feet (shaped arch of the foot). Angiologic investigation – duplex Doppler ultrasound, transcutaneous measurement of oxygen saturation and blood pressure in lower limbs – is useful to detect the peripheral circulatory disorders.

Oriental neurologic instrumentary investigation involves (Consensus statement, 1988):

- **tactile sensitivity** investigated by 10 g Semmes-Weinsteins monofilaments which is attached to the top of the toe, 1<sup>st</sup> metatarsophalangeal joint, 5<sup>th</sup> metatarsophalangeal joint and the heel, bilaterally. Sensitivity is violated if a patient does not feel three or more touches of eight.
- **vibration sensitivity** investigated by graduated tuner (128 Hz). The tuner is attached to the thumb nail bed on the both legs. The tuner is graduated to 8 degrees, for damaged vibration sensitivity is considered the value of 3 or less at the age less than 50 years (the value 5 or less at the age over 50 years).
- **pain sensitivity** investigated by non-spiky sharp device on the tibia and feet
- **thermal sensitivity** investigated by glasses filled by cold and hot water
- **tendon reflexes** (Achillary tendon, pattelary reflex) investigated by neurological hammer

According to the American Diabetes Association (ADA), the screening of diabetic neuropathy in adults is recommended to be done once a year by careful physical investigation involving examination of tactile, thermal, pain and vibration sensitivity and the examination of ankle reflexes. The combination of at least two tests reflects more than 87% sensitivity for neuropathy detection (Boulton et al., 2005).

**Blood pressure.** Measuring the blood pressure belongs to the basic investigations which can also reflect the diabetic nephropathy or cardiovascular disorders. It should be measured in calm, at least twice. Values more than 140/90 or more than the 90<sup>th</sup> percentile for certain age in children are regarded as hypertension and adequate therapy is set.

**Eye examination** by ophthalmologist including vision examination and eye background examination (color fundus photography or ophthalmoscopy) can detect manifesting forms of diabetic retinopathy. Slit lamp examination can investigate the spatial relations between retina and vitreous.

The recommendation of complication screening is presented in table 4.

	<b>Recommendation of screening frequency</b>	<b>Methods</b>	<b>Therapy</b>
Retinopathy	Once a year in children at the age > 11 years with DM duration > 2 years or at the age 9 years in children with DM duration $\geq$ 5 years	Ophthalmoscopy in mydriasis or photography of fundus	Improvement of DM compensation laser therapy
Nephropathy	Once a year in children at the age > 11 years with DM duration > 2 years or at the age 9 years in children with DM duration $\geq$ 5 years	Albumin concentration in the first morning urine portion or albumin / creatinin ratio	Improvement of DM compensation therapy by ACE inhibitors, decrease of BP
Neuropathy	Consensus was not achieved	History, physical examination	Improvement of DM compensation
Macrovascular complications	At the age 12 years	Serum lipids every 5 years, blood pressure once a year	Improvement of DM compensation, decrease of BP

Table 4. Recommendation for microangiopathy complications screening

### 3.2 Laboratory examinations

Advanced glycation end products (AGEP's) are valuable markers for assessment of long-lasting hyperglycemia. In common practice **glycosylated hemoglobin** is used. Its concentration closely correlates with glycemia within last three months and it is influenced by many factors like recurrent hypoglycemia and processes influencing the life of erythrocytes (hemoglobinopathy, hyperbilirubinemia, uremia, hypertriacylglycerolemia and other).

Other AGEP's - **N-carboxymethyl lysine**, N-carboxyalkyl lysine, pentosidine, methylglyoxal, imidasol - are used for evaluation of microangiopathy (Peppia et al., 2003). The parameter reflecting the glycemia during shorter period than glycosylated hemoglobin (because of its biological halftime) is **modified albumin** - carbonyled albumin, carboxymethyl lysine albumin or glycosylated albumin. It is a marker of oxidative stress however further studies are necessary to confirm its importance as a predictor of chronic complications.

Other possibility for evaluation of diabetic complications are plasma markers reflecting the endothel dysfunction as they are released into the blood stream in relation to the endothelial damage (Adeghate et al., 2006), i.e. von Willebrandt factor, thrombomodulin, P-selectine, E-selectine, ICAM-1 (intracellular adhesive molecule), VCAM-1 (vascular cell adhesive molecule).

Diagnostics tests for diabetic nephropathy include urine sediment, protein urine test, **microalbuminuria**, urine and blood creatinine, blood urea and nitrogen. Microalbuminuria is usually set from 12-hour night urine sample while good glycemia compensation, normal blood pressure and without excessive physical activity. The result is considered as positive when at least two from three urine samples investigated at the period 3 – 6 months with the one month distance interval are positive. Investigation of metalloproteinase-9 in blood can detect earlier cases of kidney damage. Possible indicator of tubulopathy is **N-acetyl-beta-D-glucosaminidase** (NAG). Its positivity in urine precedes the positivity of microalbuminuria in diabetic nephropathy. In the early stage, filtered albumin does not pass into the urine due to increased reabsorption in the proximal tubule and only NAG is present. Next, the reabsorption capacity of tubules is exceeded and microalbuminuria together with NAG is positive, in the advanced stages of nephropathy the positivity of NAG is caused by glomerular basement membrane damage as well as by tubular cells destruction. NAG is set as a ratio to creatinine in the urine (upper border is 0,25 U/mmol of creatinine).

### 3.3 Examination of autonomic nervous system

Cardiovascular system can be investigated by **heart rate variability** (HRV) by spectral and frequency analysis using the Ewing's battery of cardiovascular tests (deep breathing test, orthostatic test, Valsalva maneuver) (K. Javorka et al., 2008; Havlíčková & Jurko, 2005). The frequency of heart rate is not absolutely regular but it oscillates about the average value. The result is heart rate variability which is influenced by autonomic nervous system – parasympathetic and sympathetic system together with endocrine system and other mechanisms.

In physiological condition HRV is significant as a consequence of cooperation of sympathetic and parasympathetic nerve fibers. In subclinical form of microangiopathy this balance is impaired and HRV is decreased. The first damaged non-myelinated nerve fibers in diabetic neuropathy is nervus vagus so rest tachycardia is a consequence of functional dominance of sympathicus (Maser & Lenhard, 2005). HRV is sensitive marker of heart rate regulation and activity of autonomic nervous system in adults, children and newborns (Havlíčková et al., 2009). Heart rate variability is technically provided by computer system (VariaPulse TF4, Dimea Group, Olomouc, Czech republic) with telemetrically scanning of R-R intervals by special software. The investigation is done in dark room, before noon, with avoiding of alcohol and smoking. Patient has loaded the monitoring belt around the chest to monitor the heart frequency which is telemetrically transferred into the computer. The subject is instructed (to lie, to stand up, again to lie, to make four deep breaths within 20 seconds) according to the actual test. The result is the protocol with parameters of high, low and very low frequency. The results are compared to reference values for certain age and sex (Tonhajzerova et al., 2002). To avoid false positive results is not available to investigate the patients with known disorders of cardiovascular and nervous system.

Other valuable method for early determination of microvascular complications is **cough reflex sensitivity** (CRS) (Čiljaková et al., 2009). Cough reflex is one of the defense mechanisms of respiratory system. The decreased cough reflex sensitivity is due to impaired nervus vagus function (as a part of reflex circle) and is detectable also in the first stages of microangiopathic complications (Behera et al., 1995). CRS is provided by inhalation of tussigenous matter in gradually increasing concentrations and is defined as the lowest

concentration of tussigenous aerosol which provokes the cough. Two parameters are set: C2 as the lowest concentration of tussigen able to provoke the two coughs and C5 as the lowest tussigen concentration which provokes five and more coughs. As the tussigen capsaicin aerosol (hot pepper extract) is used, active in micromolar and izoosmolar concentration at pH 7.4. Capsaicin is diluted ex tempore in gradually increasing concentrations (0,61 – 1250  $\mu\text{mol/l}$ ). Aerosol is prepared by the nebuliser which is a part of Koko Pulmonary Function Testing. The subjects should breath through the mouth (with the clipped nose). The nebulisation is directed by the computer system and the aerosol penetrates into the respiratory system within 400 ms. The higher capsaicin concentration is needed for two or five coughs the more damaged is nervus vagus and the more developed is diabetic neuropathy. Each patient has done the spirometry before CRS. The investigation is contraindicated in patients who suffered from acute respiratory infection in last 4 weeks, in patients with allergy on capsaicin and relatively contraindicated in subjects with chronic respiratory diseases (bronchial asthma, cystic fibrosis). These conditions damage the defense mechanisms of respiratory system (cilliar epithel, mucocilliar transport), cause the higher sensitivity of respiratory tract to tussigen and so cause the higher cough reflex sensitivity. Mentioned conditions would cause the false negative results in the target to diagnose diabetic neuropathy. Four weeks are necessary to repair the respiratory defense functions after acute respiratory infection (Varechová et al., 2007).

**Electrodermal activity (EDA)** is simple non-invasive electrophysiologic method allowing to detect the changes in the ability of the skin to conduct the electric signals (Papanas et al., 2007). This skin ability depends on eccrine sweat glands activity which is regulated by cholinergic sympathetic nerve fibers and endocrine system through adrenalin concentration in the circulation. If sweat glands produce enough salt sweat, EDA examination detects lower electrical resistance and better electrical conductance. Subjects with diabetic neuropathy can have damaged also sympathetic nerve fibers leading to lower secretion of sweat. The decrease amount of sweat results in lower electrical conductance or higher resistance of the skin. EDA examination represents the sensitive method which can show the disorder of sweat gland acitivity and so is one of the possible method for diagnosis of neuropathy.

### 3.4 Examination of peripheral nervous system

Electrophysiologic examinations represent sensitive, exact and well reproducible methods for the diagnosis of neuropathy. The principle of **electromyographic** examination (EMG) is detection of action potentials arisen in muscle and nerve fibers. Commonly used is needle EMG and conductory examination of nerve fibers (Shabo et al., 2007). The most frequently examined are n.fibularis (motoric nerve), n. suralis (sensitive nerve), n.plantaris medialis and n.tibialis on lower extremities and n.medianus, n.ulnaris and n.radialis on upper extremities. **EMG** shows the muscle response to electric signal mediated by nerve fibers after injection of needle electrodes. Various action potentials, their shape, duration, number of phases and amplitude are evaluated while muscle contraction. The principle of **conductory** studies is irritation of peripheral nerve or nerve stem by electric impulses where the quality of nerve conduction (latention of response, velocity and amplitude) is evaluated. The dysfunction of nervous system results in decrease or even block of nervous conduction and decrease in the amplitude. The main advantage of EMG is the possibility to diagnose the subclinical form of diabetic neuropathy (Karsidag et al., 2005).

**Quantitative examination of sensitive function** represents the subjective diagnostic test based on patient's indication. Unlike the clinical investigation of sensitivity, quantitatively exactly defined stimuli are applied. The vibration sensitivity is evaluated by biotensimeter and by exactly defined vibration stimulus (Kincaid et al., 2007). Device CASE (computer assisted sensory examination) enables to determine the threshold of vibration, pain and thermal sensitivity. Standard test algorithms are used and the results are compared to the reference values considering the localisation and kind of stimulus, sex, age and anthropologic parameters of the patient. The conductivity threshold for three types of sensitive fibers (A beta, A delta and C) and pain threshold by superficial electric stimulation with 5, 250 and 2000 Hz frequency can be detected by new device Neurometer. The computerized system of randomly generated stimuli enables to define the accuracy of patient's response.

### 3.5 Organ specific examinations

Several methods help to diagnose the diabetic gastrointestinal autonomic neuropathy, like esophagogastrosopy or gastrointestinal passage. Special endoscopic device enables to combine manometry and EMG signal registration in each parts of gastrointestinal system. New method is **electrogastrography** measuring the gastric myoelectrical activity from electrodes placed on the surface of epigastrium. In diabetic gastropathy, the normal electrical rhythm (3 cycles per minute) is replaced with bradygastria, tachygastria, mixed or nonspecific dysrhythmia (Koch, 2001). Gastric motility can be examined by <sup>13</sup>C octanoic acid breath test (Choi et al., 1997).

Diagnostic of diabetic urogenital neuropathy is possible by urodynamic examination, cytoscopy or ultrasound of urinal residuum. For the diagnosis of sudomotoric neuropathy colour skin tests with changing colours depending on sweat amount are available. QSART test (quantitative sudomotor axon reflex test) and TST test (thermoregulatory sweat test) are not available in common practice.

Respiratory complications (Brndiarová et al., 2011) of DM can be detected by lung function tests like spirometry, diffuse lung capacity for carbone monoxide or body plethysmography.

Examination of pupillary reflex latency is possible by classic investigation and also by **infrared reflex pupillography**. New non-invasive examination for early microangiopathy detection is **corneal confocal microscopy** which quantifies the pathology of small nerve fibers in cornea (Hossain et al., 2005). Cornea is the most dense innervated part of human body, contains A delta and C nerve fibers. Corneal confocal microscopy enables to analyse the density of corneal nerves, their morphology or branching.

Possible methods for diagnostics of diabetic retinopathy are **fluorescein angiography** showing retinal circulation, Amsler grid identifying what parts of visual field are damaged or eye ultrasonography used in the cases of vitreous hemorrhage or cataract. **Fluorophotometry** enables to measure posterior vitreous penetration ratio as the parameter reflecting the blood-retinal barrier permeability. New diagnostic tool for diabetic retinopathy and macular degeneration using LED technology is in development.

### 3.6 Gene polymorphisms detection

The new approach in diabetic microangiopathy diagnosis is establishment of gene polymorphisms for single enzymes which enables the individual approach to the patient.

**Aldose reductase**, rate-limiting enzyme of polyole pathway, converts glucose into sorbitol in NADPH-depending reaction. Consequently, sorbitol is converted into fructose by enzyme sorbitol dehydrogenase using  $\text{NAD}^+$  as a cofactor. In hyperglycemia condition glucose conversion by this way is increased leading to many metabolic and vascular abnormalities. Gene AKR1B1 for aldose reductase can appear in several variations which are associated with development of diabetic neuropathy, retinopathy and nephropathy. According to Donaghue et al. (Donaghue et al., 2005) genotype Z-2/Z-2 represented the predisposition to earlier development of neuropathy. Contrary, Z+2 allele was regarded as protective in relation with neuropathy development (Z=138bp in (CA)<sub>n</sub> repetitive sequence). Establishment of aldose reductase gene polymorphisms in diabetic population could be important as aldose reductase inhibitors (epalrestat, fidarestat) have successfully been used in some studies. This is the first step to the "individually tailored therapy" when aldose reductase inhibitors could be taken in patients carrying genotype predisposing to diabetic neuropathy.

**Uncoupling proteins** (UCP1, 2, 3) are proteins in the inner mitochondrial membrane which are important in oxidative phosphorylation process, thermogenesis and also in protection against reactive oxygen and nitrogen species. UCP proteins reduce inner membrane potential through dispersion of protein gradient over mitochondrial membrane and so mitochondrial production of reactive oxygen species is decreased. G-866A polymorphisms in UCP 2 gene and C-55T polymorphism in UCP3 gene were associated with diminished risk of diabetic neuropathy development in diabetic patients (Rudolfsky et al., 2006).

Decreased activity of membrane pump  $\text{Na}^+\text{K}^+$  **ATPase** plays significant role in microvascular, mainly in neuropathy pathogenesis. It is coded by several genes. ATP1 A1 is predominantly expressed in peripheral nerves and erythrocytes. Patients with type 1 diabetes who were the carriers of ATP1 A1 allele were more frequently affected by diabetic neuropathy (Vague et al., 1997). Identification of this risk factor can contribute to the prevention of neuropathy in the future.

Antioxidant enzymes are crucial in prevention against oxidative stress. **Glutathione-S-transferase** (GST) represents huge family of GST isoenzymes which catalyze the conjugation of glutathione with electrophilic substrates resulting in less reactive and easily eliminated compounds. Substrates for this reaction are many carcinogens, drugs and also reactive oxygen species arisen in oxidative stress. The most intensively studied polymorphisms are genes of GST T1, GST M1 and P1. Null genotype of GST T1 and also null genotype of GST M1 significantly correlated with risk of coronal atherosclerosis (Manfredi et al., 2009). Deficit of GST as the result of null polymorphism of GST M1 and T1 was significantly related to the decreased heart rate variability which is a consequence of oxidative stress influence on autonomic nerve system (Probst-Hensch et al., 2008). Association between GST T1 and M1 polymorphisms with DM is not completely clear and differ according to the authors, region and population. Some studies claimed higher risk of DM 2 in GST T1 null and T1 null / M1 null genotype (Hori et al., 2007), according to another study, in contrary, the presence of GST M1 allele was risk factor for development of DM 1

and M1 null allele was regarded as a protective (Bekris et al., 2005). Some authors did not find any significant relation between GST polymorphisms and diabetic neuropathy (Zotova et al., 2004). GST T1 null genotype was associated with chronic kidney disease in diabetic as well as non-diabetic subjects disregarding GST M1 genotype (Datta et al., 2010). Similarly, GST M1 null genotype seemed to have no influence on end stage renal disease while GST T1 null genotype increased this risk in diabetic patients (Y. Yang et al., 2004). In young adults (average age 27) with DM1 was shown that GST M1 wild genotype represented a risk factor for diabetic retinopathy but not nephropathy. GST M1 null/T1 null combination did not increase the risk of microvascular complications (Hovnik et al., 2009).

**Superoxide dismutase (SOD)** catalyzes the conversion of superoxide into oxygen and hydrogen peroxide, which is split by catalase into water and oxygen. According to (El Masry et al., 2005), frequency of Ala/Ala genotype of Mn-SOD2 was significantly lower in patients with diabetic neuropathy and the genotype Val/Val was significantly higher in patients without neuropathy.

Hydrogen peroxide is harmful side product of many metabolic processes, so as the prevention from damage it has to be converted into less harmful substance. **Catalase** is enzyme localized in peroxisomes, functionally able to degrade the hydrogen peroxide into water and oxygen. In the study of C1167T marker of catalase gene has been shown, that C allele prevalence was more and T allele prevalence was less frequent in patients with diabetic neuropathy compared to the patients without neuropathy (Strokov et al., 2003). According to another study (Christiakov et al., 2006), 262 TT genotype of catalase gene was associated with higher activity of this enzyme in erythrocytes compared to the 262CC genotype. These results supposed protective effect of 262 TT allele against rapid neuropathy development.

**Paraoxonase** is a group of enzymes associated with HDL – cholesterol which plays role in hydrolysis of organophosphates and has also antioxidant potential. Three genotype forms of paraoxonases (PON) have been described till now. PON1 is synthesized in the liver and transported together with HDL into plasma. Its function is to prevent oxidation of LDL cholesterol. Inflammatory changes and LDL cholesterol serum level influence plasma concentration of PON1. PON2 is ubiquitously expressed enzyme which protects cells against oxidative damage. PON3 has different substrate specificity as PON1. Its concentration is not influenced by inflammatory factors and oxidized lipids. As PONs act in prevention of oxidized LDL formation and so in prevention of atherogenous plaques they reduce the risk of atherosclerosis and ischemic heart disease. 192 Gln/Arg polymorphism of PON1 presented the risk of myocardial infarction in young patients till 45 years while in 311 Ser/Cys PON2 polymorphism was not proved similar relation (Gluba et al., 2010). Association between DM and its complications with PON polymorphisms has not been found till now however decreased activity of this enzyme was described in patients with DM 1 as well as in patients with diabetic retinopathy compared to the healthy subjects (Ikeda et al., 1998).

The first and rate-limiting enzyme in myoinositol pathway is **myo-inositol oxygenase (MIOX)**. Increased level of MIOX directly depends on increased glycemia and can be the cause of myo-inositol depletion in patients with diabetic complications. English authors studied single nucleotide polymorphisms (SNP) in patients with DM type 1. These patients

had decreased frequency of genotype combination CC (rs761745), GG (rs2232873) and GC (rs1055271) and less common haplotypes T/G/C and T/G/G compared to the healthy subjects (B. Yang, 2010).

I (insertion) / D (deletion) polymorphisms of **angiotensin-converting enzyme** (ACE) is characterized by presence (I) or deletion (D) of repeating sequence (287 bp) in introne 16. According to the combinations of alleles subjects with I/I, I/D and D/D ACE genotypes exist. Homozygotes D/D have nearly twice higher concentration of ACE compared to I/I homozygotes. Regarding ACE influence on capillary diameter, organ vascularisation and also inflammatory and autoimmune processes, ACE polymorphisms can effect also development of DM and its complications. Turkish authors claimed significantly more frequent prevalence of D/D ACE polymorphism in patients with DM type 2 compared to healthy subjects (Arzu Ergen et al., 2004). D/D genotype of ACE has been suggested to be in association with diabetic nephropathy in DM2 patients (Naresh et al., 2009). According to another study frequency of I allele was significantly higher in diabetic patients with polyneuropathy compared to diabetic patients without neuropathy. D allele was regarded as protective against neuropathy (Ito et al., 2002).

Determination of gene polymorphisms extends diagnostics of diabetic complications, enables individual approach to the therapy and gives the base for the “individually tailored therapy” in the future.

## 4. Management

Regarding the complex ethiopathogenesis, the management approach is also large. Till now, the only proved method how to slow the development of microangiopathic complications is maintain the optimal glycemia, even it is known that hyperglycemia is not the only one factor triggering the pathogenesis pathways. Arrangements contributing to euglycemia are also physical activity and adequate diet. As a supporting therapy, antioxidant alpha-lipoic acid is used although its effect was clearly proved just in intravenous application. L-carnitine and vitamins B have neuroprotective effect, vitamin C and E dispose of just poor antioxidant activity. Common possibility, especially in adult patients, is symptomatic therapy like tricyclic antidepressants or pregabalin in diabetic neuropathy, ACE inhibitors in diabetic nephropathy, laser therapy in retinopathy. Another therapeutic options are in experimental line mostly proved in animal models, till now.

### 4.1 Euglycemia maintenance

Despite of various experimental and clinical studies, the therapy of microangiopathy remains insufficient. The only possible therapeutic approach which is concurrently clinically proved, is sufficient compensation of diabetes mellitus (Mokán et al., 2009). The biggest study aimed at chronic diabetic complications, EURODIAB IDDM Complication Study, confirmed the significant correlation between glycosylation hemoglobin level and cardiovascular neuropathy. Even some authors denied this relation (M. Javorka et al., 2005), the optimal glycemia maintenance is strongly recommended as a prevention of diabetic complication origin. Children diabetic patients are treated by intensified insulin regimen. Patients with poor compensation are fully indicated to insulin pump therapy which best imitates the natural pancreatic secretion of insulin.

## 4.2 Dietary adjustment

Adequate physical activity and dietary style also attitude to euglycemia maintenance. Diabetic patients should avoid food with high content of sugar, food with higher concentration of fats and meals prepared by high temperature (fried, grilled) as these have excessive amount of AGEP's. Contrary, fruit, vegetable and boiled or steamed meals content lower concentration of these products. Prevention and treatment of diabetic nephropathy require also reducing salt intake at least less than 5-6 g per day. Advanced cases need restriction of phosphorus and potassium intake, as well. With advancing renal disease, protein compound should represent at the most of 20% of whole energy intake. Protein restriction of as much as 0.6 - 0.8 g/kg/day may retard the progression of nephropathy.

## 4.3 Symptomatic therapy

According to ADA recommendation (Boulton et al., 2005), painful form of diabetic neuropathy can be influenced by tricyclic antidepressives (e.g. amitriptylin 25 - 100 mg). However, the using of these drugs is limited in many patients because of anticholinergic and central side effects. From the group of anticonvulsives, gabapentin (1,8 g per day) and pregabalin are used (Rybka, 2007). Non-steroid antiphlogistics (ibuprofen, naproxen, indomethacin) are recommended only for short time due to their gastric side effects. The symptoms of diabetic autonomic neuropathy can be relieved by beta-blockers or ACE inhibitors. Other symptomatic therapy is presented in table 5.

Symptoms	Therapy
Pain	Gabapentin, lamotrigin, pregabalin, temporary NSAID, magnetotherapy
Rest tachycardia	Cardioselective beta - blockers
Ortostatic hypotension	Sufficient amount of fluids, bandage of lower extremities
Excessive sweating	Oxybutinin, glycolpyrolate
Gastroparesis, constipation	Prokinetics (metoclopramid, domperidon)
Diarrhoea	Probiotics, diet
Constipation	Lactulose, prokinetics
Urine residuum	Betanechol, doxazosin

Table 5. Symptomatic therapy of diabetic neuropathy

In the case of dyslipidemia, hypolipidemic drugs are used however we try to avoid them in the childhood. Every urinary tract infection should be treated and the prevention maintained.

**Inhibitors of angiotensin-converting enzyme** (ACE inhibitors) are drugs primarily used for treatment of hypertension and congestive heart failure. ACE inhibitors block the conversion of angiotensin I to angiotensin II. Therefore, they lower arteriolar resistance, increase venous capacity and decrease blood pressure, they increase cardiac output, lower renovascular resistance and lead to natriuresis. As the angiotensin II contributes also to ventricular remodeling and heart hypertrophy, ACE inhibitors prevent this effect. They cause the central enhancement of parasympathehic activity (Adigun et al., 2001) by which cardiac arrhythmia and sudden death are prevented. Angiotensin II induces several fibrogenic

chemokines, namely transforming growth factor and monocyte chemoattractant protein-1 (MCP-1) which induces monocyte immigration and differentiation to macrophages augmenting extracellular matrix production and tubulointerstitial fibrosis (Amann et al., 2003). Thus ACE inhibitors can slow the progression of diabetic nephropathy. ACE inhibitors are superior to beta-blockers, diuretics, and calcium channel blockers in reducing urinary albumin excretion in normotensive and hypertensive type 1 and type 2 DM patients. Another possibility is represented by **angiotension II receptor blockers** which do not inhibit the breakdown of bradykinin and are thus just rarely associated with side effects like dry cough or angioedema. Although, hyperkalemia remains as serious adverse effect so potassium monitoring is necessary during the therapy.

Proliferative stages of diabetic retinopathy and some severe nonproliferative forms require **prompt surgical treatment**. **Focal laser treatment** (photocoagulation) can stop or slow the leakage of blood and fluid in the eye by laser burns of abnormal blood vessels. **Scatter laser treatment** (panretinal photocoagulation) can shrink and scar the abnormal blood vessels of the retina away from macula. **Cryocoagulation** represents additional operation to photocoagulation if this could not stop the development of proliferative retinopathy. The treatment of macula edema involves focal laser treatment and intravitreal application of steroids (25mg triamcinolon). **Vitrectomy** can be used to remove blood from the vitreous or to remove scar tissue tugging on the retina. Surgery often slows or stops the progression of diabetic retinopathy but retinal damage and vision loss is possible.

#### 4.4 Supporting therapy

Drugs with content of **alpha-lipoic acid** are used in diabetic patients with diabetic neuropathy (Ziegler, 2004). It is an antioxidant acting as a coenzyme of oxidative decarboxylation of alpha-ketoacids. It is easily transformed from oxidative form into reduced dihydroform what claims about its antioxidant potential. Metaanalysis of 1258 patients showed the improvement of neurological symptoms of diabetic neuropathy after intravenous treatment by alpha-lipoic acid (600 mg i.v. per day) (Ziegler et al., 2006). Unlike the intravenous therapy, the improvement of symptoms after peroral cure is not so clear. In 40 adolescents with DM, no significant decline of quantitative markers of oxidative stress, changes in glycosylated hemoglobin concentration nor in microalbuminuria was found after three months of peroral treatment by alpha-lipoic acid (Huang & Gitelman, 2008).

**L-carnitine** is common available nutritional supplement with function to transport fatty acids from cytoplasm into mitochondria thus help to utilise them. Its other role is antioxidant - to prevent the lipoxidation of fatty acids. In 34 of 51 children patients with DM type 1 (average age 12 years) was found diabetic neuropathy by nerve conductivity examination. After two-months of treatment by L-carnitin (2 g / m<sup>2</sup> per day) the improvement of nerve conduction by 44% was claimed in subjects with early stage of diabetic neuropathy (Uzun et al., 2005).

Vitamins B are also used as an additional therapy. **Vitamin B6** (pyridoxamin) acts like carbonyl groups scavenger thereby diminishes the origin of AGEP's. Benfothiamin is a derivate of thiamin (**vitamin B1**) soluble in fats with higher biological availability (Haupt et al., 2005). Thiamin and benfothiamin are cofactors of transketolase, an important enzyme of pentose-phosphate pathway, in which some products of glycolysis are degraded and so

activation of metabolic pathways (AGEP's formation, protein kinase C formation) with tissue damage is prevented. Medicaments with content of **gamma linoleic acid** (omega 6 unsaturated essential fatty acid), like evening primrose oil or borage oil, are appropriate as this acid is an important compound of membrane phospholipids and has anti-inflammatory, anti-thrombotic and anti-atherogenic effect (Scott & King, 2004). **Vitamin C and E** as antioxidants act primarily non-enzymatic, can scavenge only part of oxidation stress end products, what explain their poor effect to prevent diabetic complications.

**Vitamin D** has an important role not only in calcium-phosphate metabolism but also in immune system and in renin inhibition (Agarwal, 2009). Patients with diabetes mellitus and especially with more advanced stages of diabetic nephropathy usually have lower levels of vitamin D and so are recommended to have supplementation of this vitamin. Vitamin D supplementation may reduce proteinuria in patients with diabetic nephropathy (deZeeuw et al., 2010).

**Sulodexide** is glycosaminoglycan with antithrombotic activity containing two compounds – 80% of low molecular weight heparin and 20% of dermatan sulphate. Daily dose of 60mg (600 U) injected intramuscularly during three weeks resulted in significant decrease of albuminuria and this effect lasted 3 – 6 weeks after drug withdrawal (Skrha et al., 1997). In the multicenter study, 237 diabetic patients with micro- or macroalbuminuria were treated by sulodexide 50mg per day for 6 months. Significant reduction of albuminuria was found similarly in type 1 and type 2 diabetes and was slightly greater in macroalbuminuric than in microalbuminuric patients (Blouza et al., 2010).

**Calcium dobesilate** is a venotonic drug with influence on membrane proteoglycan, blood viscosity, aggregability of erythrocytes and thrombocytes and with inhibitory function on PAF (Platelet Activating Factor). Some studies claimed that peroral treatment by calcium dobesilate (2 g daily for 2 years) improved the blood-retinal barrier permeability (Ribeiro et al., 2006), however randomised, double-blind, placebo-controlled, multicentre trial involving 635 patients with type 2 diabetes and mild-to-moderate non-proliferative retinopathy showed that treatment with calcium dobesilate 1500 mg daily within five years did not reduce the risk of development of clinically significant macular edema (Haritoglou et al., 2009).

#### 4.5 Experimental possibilities

Other therapeutic possibilities are in the experimental line, till now. Many of medicaments effective in animal models have not been applied in humans so their beneficial effect cannot be clearly proved. Huge negative of mentioned studies is lack of clinical verifying in children population.

**Aminoguanidin** is hydrazin derivative with its ability to bind reactive carbonyl compounds and so to prevent the formation of AGEP's (Thornalley, 2003). It is available on American trade (75 mg tablets) as a nutritional supplement against aging and diabetic complications origin. According to double blind randomized study (Bolton et al., 2004) with 690 patients with diabetes type 1, aminoguanidin at the dose 150 – 300mg during 2 – 4 years had protective effect to diabetic nephropathy. Contrary, some studies do not claim its therapeutic activity (Birrell et al., 2000).

**Alagebrium chlorid** (ALT-711) splits kovalent bindings between proteins and glucose thereby helps to AGEP's elimination (Little et al., 2005). Administration of alagebrium at the dose 210 mg per os daily in 62 patients with arterial hypertension led to the significantly increase in arterial flexibility compared to the group of patients treated by placebo (Kass et al., 2001). Peppia et al. (Peppia et al., 2006) gave alagebrium 1mg/kg daily to diabetic mice what had consequence in decrease the serum advanced glycation end products and microalbuminury. Also effect on diabetic nephropathy is assumed but further research is necessary to confirm it.

Recent studies showed beneficial effect of **C-peptide** substitution (Ekberg & Johansson, 2008). 46 adult subjects with diabetes mellitus type 1 and early stage of diabetic neuropathy enrolled the double-blind placebo-controlled study. Three months therapy by C-peptide (1,8mg per day) together with insulin substitution improved the functions of peripheral nervous system.

**Inhibitors of aldose-reductase** interfere directly into patophysiological process of sorbitol and fructose formation by polyole pathway. Epalrestat, ranirestat or fidarestat significantly improved the peripheral neuropathy in diabetic patients (Bril & Buchanan, 2006; Drel et al., 2008). According to the clinical study (Hotta et al., 2006) where 289 adult diabetics (type 1 and 2) were treated by epalrestat (50 mg 3-times daily per os) within 3 years, the symptoms of diabetic neuropathy were significantly diminished compared to the 305 patients treated by placebo. The medicament is not available on common trade.

**Ruboxistaurin**, selective PKC-  $\beta$  inhibitor, can improve circulatory parameters of retina, can decrease macular edema, reduces microalbuminury and improves the symptoms of simpler form of diabetic neuropathy (Aiello et al., 2006). 20 adult patients with DM type 1 and 2 treated by ruboxistaurin (32mg per day) within 6 months significantly improved in symptoms of neuropathy compared to the 20 patients treated by placebo (Cassellini et al., 2007). In Japan, ruboxistaurin is farmaceutically produced for treatment of diabetic neuropathy, retinopathy and macular edema. In America and Europe it is used in the clinical studies, till now.

Apfel et al. (Apfel et al., 2000) found in their study that subcutaneous administration of recombinant human **neural growth factor** (NGF) during 48 weeks did not improve the nervous system function in diabetic patients. Hernander-Pedro et al. (Hernandez-Pedro et al., 2008) in the experimental conditions confirmed positive result after treatment by **all-trans retinoic acid** which stimulates the origin of NGF. An interesting study is also treatment by monosialic gangliosid GM1 which decreases the proinflammatory cytokines and increases the NGF in pancreatic cells by what extends their survival (Vieira et al., 2008).

**Erythropoetin**, initially identified as a hemopoetic factor, is also expressed in the nervous system and probably has neuroprotective effect. Chattopadhyay et al. (Chattopadhyay et al., 2005) deal with vectors gene transpher based on HSV (herpes simplex virus) which are originally replied in nervous fibers. They found that mice with induced diabetes had significantly enhanced the function of nervous system after HSV-mediated transpher of erythropoetin.

The using of angiotensin growth factor is controversial. Intramuscular gene transpher of VEGF (vascular endothelial growth factor) to the rats with induced diabetes caused the higher velocity by the nervous system compared to the control group (Schratzberger et al.,

2001). Contrary, VEGF induces angiogenesis and retina neovascularisation by what diabetic retinopathy is caused. Ranizumab and bevacizumab are monoclonal antibodies against VEGF. Several studies claimed their successful using in patients with diabetic retinopathy and macular edema (Avery et al., 2006). The prospective trial evaluated the efficacy and safety of **intravitreal bevacizumab** in eyes with macular edema secondary to central retinal vein occlusion. Eyes were treated with 3 intravitreal injections of 1.25 mg at monthly intervals. After 18 months of follow-up, visual acuity was increased and central retinal thickness was decreased while no drug-related systemic or ocular side effects were observed (Zhang et al., 2011). The influence on diabetic neuropathy have not been described.

Neuropathic pain can be relieved by magnetotherapy. Weintraub et al. (Weintraub et al., 2003) proved significantly improvement of the pain after 3 – 4 months of wearing the magnetic lining in the shoes. Static magnetic field can penetrate to the 20 mm height and influence the nociceptors in the epidermis and dermis. 45 children at the age 5 – 17 years with diabetic neuropathy had significantly better velocity of nervous fibers after treatment by dynamic magnetic fields (Nikolaeva et al., 2008).

## 5. Conclusion

Diabetes mellitus is chronic disease with rising incidence where supraphysiologic concentration of hyperglycemia alters the biostructures in nuclear, cellular, tissue and organ level. Diabetes duration and compensation are not exclusive factors playing role in the etiopathogenesis of chronic diabetic complications. Recent studies have shown the impact of gene polymorphisms and have suggested the influence of other factors (epigenetic, immunologic). The fact that prevalence of microangiopathic complications increase with diabetes duration is undeniable but it is true in general not in individual view. Improvement of diabetes compensation is still the only known therapeutic possibility recommended to delay or prevent diabetic complications. However, some patients despite adequate compensation have some forms of complications and on the other hand, some subjects despite poor compensation and long diabetes duration do not suffer from any complications. Here, the importance of individual approach to each subject is shown as each one has the unique combination of gene polymorphisms predisposing or protecting him to diabetic complications origin. By this new knowledge, the advance from general to individual approach is possible. Huge hope with proceeding research is presented by new diagnostic and therapeutic options like “therapy to measure” according to the individual demand.

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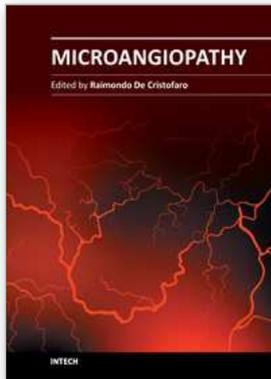
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## **Microangiopathy**

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Microangiopathies are pathological processes causing degenerative disorders of small vessels. The circulatory problems caused by microangiopathies may be responsible for failure of individual or multiple organs. These pathological processes are indeed one of the most common disorders characterized by high morbidity and mortality in the affected patients. Many studies have revealed very complicated processes both at cellular and molecular level. However, much work remains to define the diversity of different pathogenetic mechanisms leading to microangiopathic disorders to provide appropriate prevention and treatment strategies. The aim of this volume is providing illustrative examples of relevant mechanisms responsible for different forms of microangiopathies and how this body of evidences can be harnessed to define new strategies of therapeutic intervention.

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