Invited Article
Diabetes Angiopathy in Children

F **Chiarelli**, D **Di Marzio**

Abstract

Objective: To review currently available evidence on the molecular mechanisms, risk factors and outcomes of mico- and macrovascular complications in children with type 1 diabetes mellitus. ***Data sources and study selection:*** Medline, Pubmed, and Cochrane Library searches of internationally published English language journals, from 1985 to February 2010 using the terms "diabetes", "children", "complications", "angiopathy" and "management". ***Data extraction:*** All articles involving diabetes vascular complications in children were included in the review. ***Data synthesis:*** During the natural history of diabetes several molecular, receptorial and cellular factors provide a continuous mechanism of vascular damage. In children with diabetes this state is present at an early age so that accelerated atherosclerosis is associated with an increased risk of micro- and macrovascular complications as compared to the non-diabetic paediatric population. The major long term complications of diabetes can be divided into micro- (nephropathy, retinopathy and neuropathy) and macrovascular complications. Childhood remains a period during which intensive education and treatment may prevent or delay the onset and progression of these complications. ***Conclusions:*** Although the prognosis has improved considerably in recent years due to the advances in the therapeutic interventions, systematic regular screening continues to have a pivotal role in the management of complications in diabetic children. Primary prevention to all risk factors for vascular complications is essential and intervention is indicated if necessary even in childhood.

Diabetes mellitus (DM) is a chronic disease characterised by hyperglycaemia which is the earliest trigger in the development of vascular damage in these patients. During the process of the development of atherosclerosis process, several molecular, receptorial and cellular factors provide a continuous mechanism of vascular complications. In diabetic children this state seems to be enhanced and facilitated so that accelerated atherosclerosis is associated with an increased risk of cardio-vascular events compared to the non-diabetic population. The major long-term complications of both type 1 (T1DM) and type 2 diabetes (T2DM) can be divided into microvascular (represented by nephropathy, retinopathy and neuropathy) and macrovascular (cerebrovascular, coronary artery and peripheral vascular disease).

The outcomes include visual impairment and blindness due to diabetic retinopathy, renal failure and hypertension due to diabetic nephropathy, pain, paraesthesiae, muscle weakness and autonomic dysfunction due to diabetic neuropathy and cardiac disease and stroke due to macrovascular disease.[1]

Clinically evident diabetes-related vascular complications are rare in childhood and adolescence; however, early functional and structural abnormalities may be present even within a few years after the onset of the disease. Intensive education and treatment during childhood and adolescence may prevent or delay the onset and

---

Department of Paediatrics, University of Chieti, Via dei vestini, 5, I-66100 Chieti, Italy

F **Chiarelli** *MD, PhD*
D Di **Marzio** *MD*

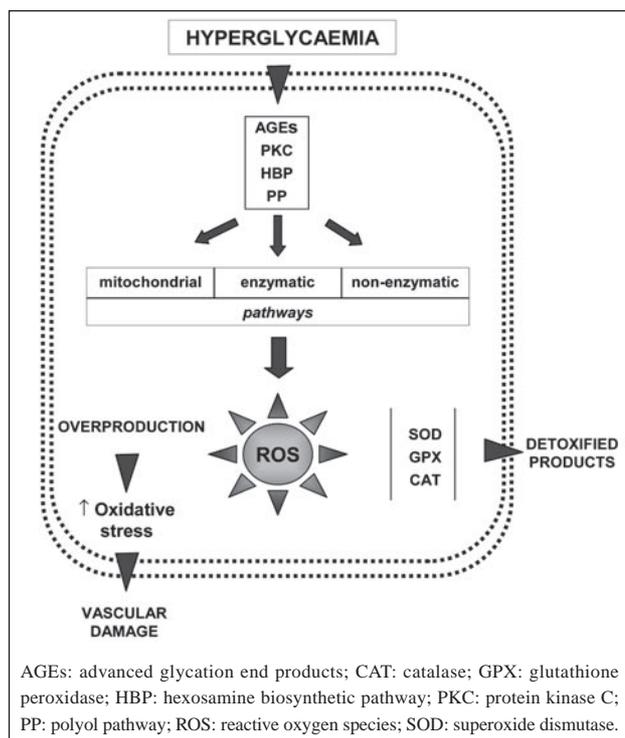**Correspondence to:** Prof. F **Chiarelli**

progression of complications.[2]

T2DM is an increasing problem in childhood, however type 1 remains by far the most common type of diabetes in this age group in the Western world. In this review we have focused on T1DM, because this will have the greatest implication for patients diagnosed in childhood. Inflammation has an important role in the development of vascular damage.[3,4] Leuocyte adhesion molecules, chemokines, interleukins and growth factors are the mediators of this action.[5] The first event in the pathogenesis of angiopathy is the endothelial damage. Risk factors such as hyperglycaemia, oxidised low-density lipoprotein (LDL) cholesterol, hypertension and aging increase the production of free radicals. The alteration of the oxidant-antioxidant status results in loss of nitric oxide, vasoconstriction and a chronic activation of inflammatory response.[6]

Molecular Mechanisms Involved in Diabetic Angiopathy

The cellular chronic toxicity of hyperglycaemia is related to the activation of four molecular pathways: (1) polyol pathway (PP); (2) production of advanced glycation end products (AGEs); (3) activation of protein kinase C (PKC) and (4) hexosamine biosynthetic pathway (HBP). The final common effect of these molecular pathways seems to be represented by increased oxidative stress.[7] The alteration of the cellular oxidant-antioxidant status is able to induce a diffuse endothelial dysfunction and contribute to the progressive development of micro- and macrovascular complications (Figure 1).[8]

Oxidative stress derives from an excessive production and/or insufficient removal of highly reactive molecules, such as reactive oxygen species (ROS).[9] The generation of ROS is increased in subjects with diabetes, particularly in those with poor glycaemic control, whereas antioxidant defenses are generally reduced.[10] Under normal circumstances, ROS are generated mainly in the mitochondria where at least 0.2% of oxygen is converted to radicals[11] and there is evidence that mitochondrial ROS production plays a key role in the pathogenesis of diabetic vascular complications.[7] Besides the mitochondrial pathway, enzymatic and non-enzymatic ones are involved in the generation of ROS in diabetes, and they appear to be interrelated to the four main pathways activated by hyperglycaemia.[12] NADPH oxidase, xanthine oxidase, cyclooxygenase, cytochrome P_{450}-dependent oxygenases and uncoupled endothelial nitric oxide synthase (eNOS)

AGEs: advanced glycation end products; CAT: catalase; GPX: glutathione peroxidase; HBP: hexosamine biosynthetic pathway; PKC: protein kinase C; PP: polyol pathway; ROS: reactive oxygen species; SOD: superoxide dismutase.

Figure 1 Schematic representation of oxidative stress pathways. Hyperglycaemia activates PKC, PP, HBP and is associated with an increased production of AGEs. All these factors can induce oxidative stress through the mitochondrial, enzymatic and non-enzymatic pathways. The overproduction of ROS may induce cellular and vascular damage.

can all contribute to an overproduction of ROS.[13-15] At the molecular level, ROS induce the activation of stress signaling pathways and apoptosis (NF-kB, MAPK, JAK-STAT), gene expression, transcriptional factors and redox-sensitive kinases, as well as molecular damage of proteins, DNA and lipids and accelerated formation of AGEs.[16] ROS-induced peroxidation of lipids alters the structure and fluidity of biological membranes, whereas both protein and lipid oxidation can cause secondary loss of catalytic function of enzymes and promotes their degradation.[14] On the other hand, several cellular and mitochondrial enzymes are involved in the detoxification of oxygen reactive molecules. Superoxide dismutase (CuZnSOD present in cytosol and MnSOD in mitochondria) together with glutathione peroxidase (GPX) in the cytosol, and catalase (CAT) in peroxysomes, constitute the main enzymatic antioxidant defence system.[17] Diabetes can reduce the levels and activity of these enzymes, thus suppressing defense responses and, in this way, it can further contribute to oxidative stress and vascular damage.[18]

In the diabetic kidney, ROS enhance basal vascular tone, inflammatory cell infiltration, impaired endothelium-dependent relaxation and contribute to glomerular hypoxia.[19] In the early stages of diabetic glomerulopathy, oxygen radicals contribute to prostaglandin E2 (PGE_2) synthesis through the activation of the NF-kB expression in human mesangial cells. Many of these effects are normalised by uncoupling of oxidative phosphorylation and by overexpression of MnSOD.[20] ROS contribute also to the pathogenesis of neuropathy as clearly demonstrated by several experimental studies. ROS-dependent nerve dysfunction seems to be related to neurovascular defects associated with hypoxia.[21] Increased activity of NADPH oxidase has been detected in the retina of diabetic rats, thus suggesting a role of increased oxidative stress also in retinopathy.[22] In patients with diabetes, endothelial cells are the main source of ROS, which overwhelm the cellular antioxidant capacity and impair cellular function.[23] Impaired endothelium dependent vasodilatation has been demonstrated in vascular beds in patients with type 1 and type 2 diabetes.[24] In addition, vascular smooth muscle cell function is impaired with a reduction of response to circulating vasodilators in subjects with diabetes mellitus.[25] Oxidative stress also induces proliferation of vascular smooth muscle cells and promotes their migration into atherosclerotic lesions. The oxidative stress-related dysfunction of both endothelial and smooth muscle cells have a pivotal role in the progression of diabetic macroangiopathy.

Risk Factors for the Development of Complications

The development and progression of angiopathy in diabetic patients should be blocked at an earlier age if possible. Optimal blood pressure and glycaemic control, as well as normal lipid profile should be aimed for. Longer duration of diabetes, older age and puberty are important risk factors for the development of vascular complications in diabetic children.[26] Furthermore, attention to all risk factors is important and intervention is indicated in the high risk patients beginning from a young age (Table 1). Hypertension is a well-established risk factor for vascular complications. Epidemiologic evidence linking hypertension to the development of vascular complications is not limited to adults, and studies in children have provided convincing relations between hypertension and the subsequent development of vascular lesions. Observational

Table 1 Factors increasing the risk of vascular complications in diabetic subjects

Hypertension
Microalbuminuria
Dyslipidaemia
Impaired glucose control
Family history of premature angiopathy
Obesity
Smoking
Lack of exercise

studies in children indicate that the effects of high blood pressure begin well before adulthood.[27] The prevalence of hypertension is approximately doubled in diabetic patients compared with non- diabetic controls.[28,29] In T1DM, hypertension can be essential hypertension or related to diabetic kidney disease. Both are major risk factors for micro- and macro-vascular complications, and it may be difficult to differentiate them in risk calculations in clinical trials. The risk for angiopathy increases significantly when hypertension and diabetes coexist.[30] Hypertension has a greater impact on vascular complications and absolute risk of death is significantly higher for patients with diabetes as compared to normal subjects.[31] Moreover, hypertension accelerates the risk for nephropathy,[32] retinopathy[33] and neuropathy.[34]

While patients with T2DM who are frequently obese have a characteristic lipid profile (increased triglycerides, normal or high levels of LDL, reduced HDL),[35] those with T1DM usually have a normal lipid profile and yet the development of atherosclerosis is still accelerated.[36] Well controlled T1DM is not usually associated with blood lipid disturbance. In the DCCT/EDIC study dyslipoproteinaemia is associated with the development of microalbuminuria and retinopathy.[37,38] A family history of premature cardio-vascular disease (CVD) (usually before age 55 years) confers an additional and independent risk of future vascular complications[39] such as retinopathy[40] and nephropathy.[41] Epidemiologic studies have established obesity as a risk factor for premature vascular complications and the American Heart Association (AHA) classifies obesity as a major risk factor for angiopathy. This is not surprising, given the frequent association of obesity with hypertension, dyslipidaemia, insulin resistance, and frank diabetes. Higher body mass index (BMI) increases the incidence and severity of retinopathy,[42] neuropathy,[43] microalbuminuria[44] and macroangiopathy.[45]

Smoking is one of the main independent risk factors for micro- and macrovascular diseases. Evidence linking tobacco exposure to the development of atherosclerosis and CVD has accumulated from pathologic studies in animals and epidemiologic observations in humans.[46,47] Normally, more than 75% of blood glucose is cleared into skeletal muscle by insulin,[48] and insulin resistance in muscle is the primary defect leading to diabetes.[49]

Physical inactivity and decreased exercise capacity have been linked to higher mortality in patients with diabetes.[50] Puberty has long been recognised as a main risk period for the development of microangiopathic complications, although the underlying mechanism still remains unknown.[51] Normal pubertal development is associated with dramatic changes in insulin sensitivity and fasting insulin concentrations rise to a peak in both sexes at Tanner stages 3 and 4 before declining to prepubertal values during early adult life. The increased insulin resistance during puberty is mainly related to high growth hormone levels. Impaired fibrinolytic function in diabetes correlates with the severity of vascular disease and is a risk factor for myocardial infarction in both diabetic and non-diabetic subjects.[52-54]

Vascular Complications

Microvascular complications, represented by retinopathy, nephropathy and neuropathy account for the major causes of development of blindness, end-stage renal disease and peripheral nerve dysfunction in T1DM children. Equally, they are disproportionately affected by macrovascular complications so that the mortality from ischaemic heart disease is increased 10-40-fold when compared to the general population.

Nephropathy

Diabetic nephropathy (DN) is usually defined as persistent albuminuria greater than 300 mg/24 hours (persistent total proteinuria >500 mg/24 hours) and is usually associated with hypertension, and an inexorable decline in glomerular filtration rate (GFR).[55] The first clinical sign of incipient nephropathy is microalbuminuria. This is defined as:

1. albumin excretion rate (AER) between 20 and 200 μg/min or AER 30-300 mg/24 hours in 24-h urine collections;
2. albumin concentration (AC) 30-300 mg/L (on early morning urine sample);

3. albumin/creatinine ratio (ACR) 2.5-25 mg/mmol or 30-300 mg/gm (spot urine) in males and 3.5-25 mg/mmol in females (because of lower creatinine excretion).

DN still remains as the major cause of morbidity and mortality amongst young adults with type 1 diabetes.[56] Kidney functional and structural abnormalities may be present a few years after the onset of the diabetes. Early detection of diabetic nephropathy and timely treatment of early signs of this complication have a pivotal role in the prevention of end-stage renal failure in children and adolescents with diabetes.[57] Therefore, regular screening for DN, are of foremost importance in paediatric diabetes care.

In all children with prepubertal onset of diabetes the screening for microalbuminuria should be undertaken 5 years after the onset or at age 11 years, or at puberty (whichever is earlier), and annually thereafter. In the case of pubertal onset of diabetes, complication screening should be initiated 2 years after onset, and annually thereafter. Screening should be either on timed overnight urine collections or on a spot urinary albumin/creatinine ratio. Other causes of microalbuminuria must be excluded (Table 2).

The duration and level of hyperglycaemia are important determinants of nephropathy. The Diabetes Control and Complication Trial (DCTT)[2] has confirmed beyond any doubt that the risk for the development and progression of diabetic complications is intimately related to glycaemic control as judged by HbA1c. These observations have led to the recommendation that children and adolescents should aim for near-normal glycaemia and this should be achieved as early as possible. Thus, good metabolic control represents the best primary preventive measure to delay the progression of all microvascular complications including nephropathy.

Table 2   Other causes of microalbuminuria to be excluded during the screening for diabetic nephropathy

Glomerulonephritis
Orthostatic proteinuria
Intercurrent infections
Urinary tract infections
Menstrual bleeding
Vaginal discharge
Strenuous exercise

Genetic susceptibility may be another factor influencing the development of diabetic nephropathy. Much attention has recently been paid to research on the molecular genetics of microangiopathy in patients with diabetes. An example of this research is a study on the effect of the apolipoprotein E genotype associated with high lipid fractions which can affect the progression of diabetic nephropathy.[58] Other genes such as heparin sulphate and aldose reductase have also been proposed as candidate genes. Further genetic studies will be crucial for the detection of patients at risk for later development of diabetic nephropathy from the onset of the disease. The ability to identify genetic markers of susceptibility to kidney disease would permit clinicians to focus care on these patients, aiming to achieving best possible glycaemic control and to initiate early intervention with protective drugs.

Secondary Intervention for Nephropathy

Effective antihypertensive therapy in patients with nephropathy prolongs the time to progression to end-stage renal disease.[59] A recent prospective study has shown improved prognosis of renal function from 5 to 7 years from onset of nephropathy to a median of 21.7 years,[60] predominantly due to aggressive antihypertensive treatment, with smaller contributions from improved glycaemic control. Angiotensin converting enzyme inhibitors (ACEI) are recommended for use in children and adolescents with hypertension.[61] They have been effective and safe in children in short-term studies.[62] Progression to overt nephropathy may be delayed but their place in protecting long-term renal function in children has not been established. Some authors suggest that, even without hypertension, ACEI should be considered when persistent microalbuminuria has been confirmed.[1] The clinical

beneficial effect of angiotensin II receptor blockers (AIIRA) in hypertension is similar to that observed with ACEI. Up to now AIIRA have not been extensively used in children with diabetes, but more evidence of the efficiency and safety must be available before AIIRAs can become an obvious choice for the treatment of diabetic nephropathy and hypertension in children and adolescents in the near future. Thus, interventions to prevent or delay the progression of diabetic nephropathy should be achieved as soon as possible in children after diagnosis of diabetes (Table 3).

Retinopathy

Diabetic retinopathy (DR) is the major cause of new cases of blindness in adults. It is well accepted that hyperglycaemia is the major pathogenetic factor for retinopathy.[63] The prevalence of diabetic eye disease is strongly related to the duration of diabetes, blood pressure and glycaemic control, although a multifactorial pathogenesis is likely. In contrast to diabetes-related renal changes, the opportunity for direct ophthalmological observation allows the classification of retinal changes by structural and not by functional aspects. Retinal blood vessels do not have autonomic nervous system innervation and attempt to maintain constant blood flow through a mechanism called autoregulation.

DR is conventionally divided into several phases: the preclinical phase represented by non-proliferative DR (NPDR; including mild, moderate and severe or preproliferative DR) and proliferative DR (PDR; subdivided into low, high and advanced risk).[64,65] Although in preclinical retinopathy structural abnormalities are hardly detectable, functional alterations such as increased retinal blood flow and venous dilatation can be observed.[66] The two major characteristics of early retinopathy are increased
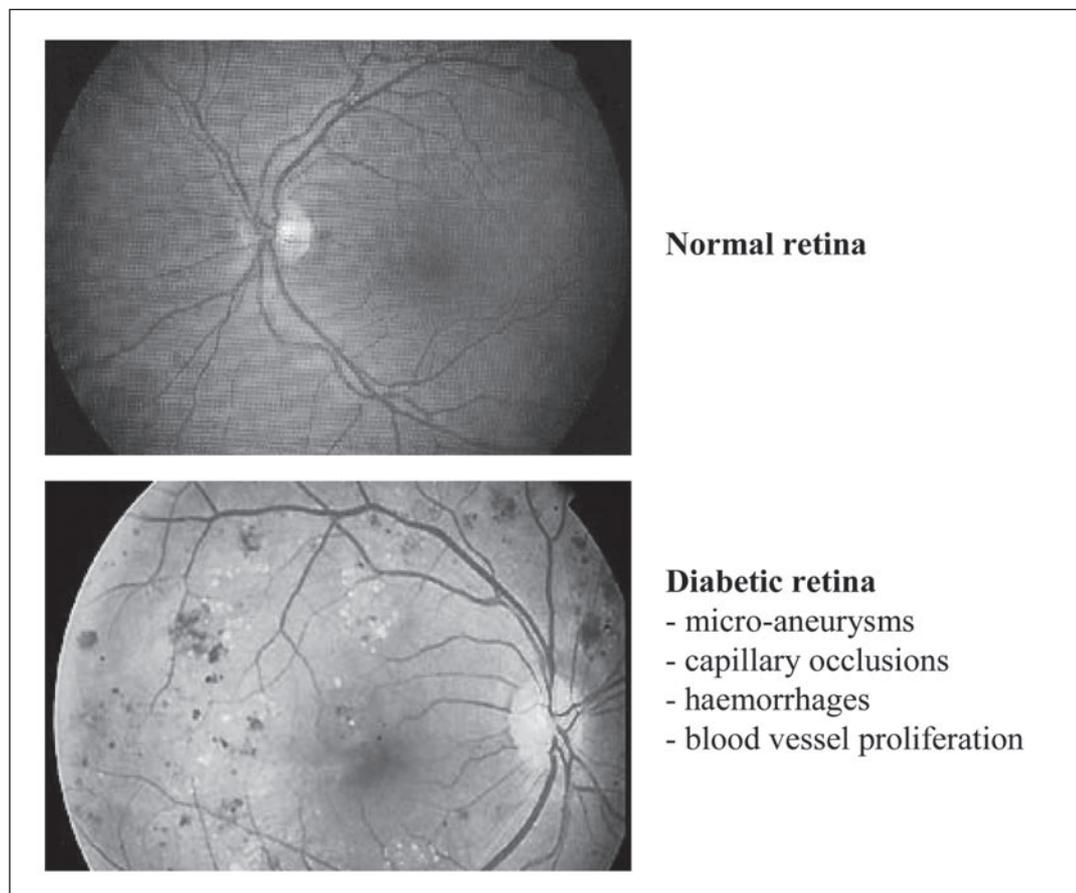
Table 3 How to prevent or delay progression of diabetic nephropathy

Conventional interventions	Pharmacological interventions
Aim for a near-normal glycaemia, and this should be achieved as soon as possible	Angiotensin converting enzyme inhibitors (ACEI)
Blood pressure should be mantained at less than the 95th centile for age	Angiotensin II receptor blockers (AIIRA)
Improve blood lipids levels	
Discourage excessive nutritional protein intake: not more than 1.0-1.2 g/Kg body weight/day	
Encourage physical exercise	
Strongly discourage smoking	

capillary permeability and progressive vascular closure. On a cellular level there is a loss of pericytes and thickening of the basement membrane followed by a proliferation and degeneration of endothelial cells; this would lead to focal thrombosis and vascular occlusion. The first morphological lesions are microaneurysms which evolve in areas of capillary hypoperfusion. This stage cannot be identified by ophthalmoscopy but can be demonstrated with sensitive examinations such as fluorescein angiography. As retinopathy progresses, the impairment of the blood-retinal barrier leads to the deposition of hard exudates (extracellular accumulation of lipids) and soft exudates ("cotton-wool spots", nerve fibre layer infarctions caused by obstruction of terminal retinal arterioles) and haemorrhages (Figure 2). These ocular changes can be seen by fluorescein angiography in almost all patients who have had diabetes for 20 years.[67]

Retinopathy may progress from this stage to vision-threatening proliferative retinopathy when partial ischaemia stimulates neovascularisation. These structurally and functionally deficient new vessels tend to rupture and lead to intra-retinal and vitreous haemorrhages that eventually lead to loss of sight and tractional retinal detachment resulting in blindness. Vision-threatening proliferative retinopathy may develop in up to 70% of youth-onset patients after 30 years of diabetes. These alterations are often associated with macular oedema that involves the breakdown of the blood retinal barrier and with the leakage of plasma from capillary into the macula. As fluid elements have been reabsorbed, lipid and lipoprotein components are deposited and lead to the formation of hard exudates that seriously impair central vision.[68] Although the prognosis has improved considerably in recent years due to the advances in laser therapy and vitroretinal surgery, early detection and treatment appear essential to yield the best results. Screening for DR should be started 5 years after the onset of diabetes or at age 11 and annually thereafter in the case of prepubertal onset; in the case of pubertal onset of diabetes, screening should be done 2 years after the onset and annually thereafter.[1]

Normal retina

Diabetic retina
- micro-aneurysms
- capillary occlusions
- haemorrhages
- blood vessel proliferation

Figure 2   Normal and diabetic retina in direct ophthalmoscopic observation.

Neuropathy

Diabetic neuropathy (Dn) still represents a major clinical and diagnostic challenge in the large spectrum of diabetes microvascular complications.[1] While diabetic nephropathy and retinopathy have a generally accepted screening programme to identify the onset of disease, there is a lack of similar basic understanding of Dn in terms of accurate diagnosis, prevention and treatment. The neuropathies associated with diabetes fall into two broad categories: focal and generalised neuropathies. Focal neuropathies include mononeuropathies such as carpal tunnel syndrome, palsy of the peroneal nerve, palsy of the third cranial nerve and proximal diabetic amyotrophy. Diabetic sensorimotor polyneuropathy is the most common generalised neuropathy and the simplified term "*diabetic neuropathy*" is commonly used. Dn is a polyneuropathy because of the diffuse damage to all peripheral nerve fibres, motor, sensory and autonomic. Such damage occurs insidiously and progressively and is characterised at first by sensory loss in a stocking and glove distribution and later by loss of motor function.

Autonomic nerve tests include heart rate response to deep breathing, rising to standing from a lying position, Valsalva manoeuvre, heart rate variation at rest, QT interval, postural changes in blood pressure and pupillary responses to light and dark adaptation. Peripheral nerve tests include quantitating vibration and thermal discrimination thresholds and nerve conduction. These are mostly used in research settings. Age- and gender- specific normal ranges need to be applied where relevant when interpreting results.

The duration and level of hyperglycaemia are important determinants of microvascular complications of diabetes, including neuropathy. The DCCT reported a 60% reduction in neuropathy in the intensively treated groups after five years and therefore, poor glycaemic control represents the most important risk factor for Dn, even in children and adolescents.[2] Recently, Tesfaye et al hypothesized that other risk factors besides hyperglycaemia are probably involved in the evolution of neuropathy and, in particular, provided convincing evidence that even slight improvements in lipid variables, blood pressure and body mass index are associated with a significantly lower risk of onset of Dn; this effect is similar to that of better glycaemic control on neuropathy.[69,70] These data suggest that vascular risk factors may accelerate the adverse effects of hyperglycaemia on the peripheral nerves in patients with diabetes, probably because they contribute to endothelial dysfunction. If this hypothesis can be confirmed in the near future, screening methods of Dn will consider not only neurological signs and symptoms as indicator of early nerve damage, but also lipid profile and body mass index as early vascular risk factors for Dn.

Macrovascular Complications

Children with T1DM are disproportionately affected by cardiovascular disease (CVD), compared with those without diabetes. For diabetic patients less than 35 years of age, the mortality for ischemic heart disease is increased 30-fold when compared to the general population.[71] Microvascular complications account for the major causes of development of blindness, end-stage renal disease and peripheral nerve dysfunction. Macrovascular disease is mainly associated with hypertension, dyslipidaemia, obesity, a hypercoagulable state and inflammation, all leading to atherosclerosis. The pathogenetic mechanisms of macrovascular complications include the production of advanced glycation endproducts (AGEs), NO decrease, increased oxidative stress, lipid peroxidation and platelet dysfunction. Macrovascular disease is the leading cause of death in patients with diabetes, causing 75% of deaths. It is clear that the progression of atherosclerosis is more aggressive and results in earlier development of macrovascular disease in patients with T1DM. Atherosclerosis starts early in childhood and adolescence as shown by increased intima-media thickness of the carotid arteries and aorta and silent coronary atherosclerosis measured by intravascular ultrasound in young adults with childhood onset diabetes.[71] One should aim for optimal blood pressure and glycaemic control, and maintenance of a normal lipid profile in diabetic children in order to prevent or delay the onset of macrovascular complications.

Conclusions

T1DM is a chronic disease with several related complications. Microvascular and macrovascular damage is accelerated in patients with DM and represents the leading cause of morbidity and death. From early childhood, several molecular, receptorial and cellular factors provide a continuous mechanism of vascular damage.[6] Angiopathy is associated with several mechanisms that are activated in response to noxious stimuli leading to a complex chronic inflammatory state. In DM, this inflammatory state seems to be enhanced so that accelerated vascular damage in diabetic patients is associated with a 3 to 4-fold increased risk of cardiovascular events as compared to the non-diabetic population.[72] Early prevention and therapeutic

measures to minimise the risk of vascular complications are very important for patients with T1DM. At present, the best therapeutic strategy is to maintain good glycaemic control and early screening and to provide prompt treatment when micro- and macrovascular complications are detected.[73] The continuous improvement in knowledge about the molecular and progression mechanisms of diabetic angiopathy is essential for the development of newer and better pharmacological therapies, targeted not only to just maintaining normoglycaemia but also to control the essential factors that contribute to the inflammatory state of diabetes.

References

1. Donaghue KC, Chiarelli F, Trotta D, Allgrove J, Dahl-Jorgensen K. ISPAD Clinical Practice Consensus Guidelines 2009 Compendium. Microvascular and macrovascular complications associated with diabetes in children and adolescents. Pediatr Diab 2009;10(Suppl 12):195-203.

2. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. J Pediatr 1994;125:177-88.

3. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-74.

4. Kher N, Marsh JD. Pathobiology of atherosclerosis-a brief review. Semin Thromb Hemost 2004;30:665-72.

5. Burke-Gaffney A, Brooks AV, Bogle RG. Regulation of chemokine expression in atherosclerosis. Vascul Pharmacol 2002; 38:283-92.

6. Santilli F, Cipollone F, Mezzetti A, Chiarelli F. The role of nitric oxide in the development of diabetic angiopathy. Horm Metab Res 2004;36:319-35.

7. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.

8. Mokini Z, Chiarelli F. The molecular basis of diabetic microangiopathy. Pediatr Endocrinol Rev 2006;4:138-52.

9. Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: Molecular and cellular mechanisms. Hypertension 2003;42:1075-81.

10. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19:257-67.

11. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. Circ Res 2000;86:E85-90.

12. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615-25.

13. King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol 2004;122: 333-8.

14. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: Basic mechanisms and in vivo monitoring of ROS. Circulation 2003;108:1912-6.

15. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies. Circulation 2003;108:2034-40.

16. Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage. Nature 2000;404:787-90.

17. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes 2003;52:1-8.

18. Ree SG. Cell signaling: H_2O_2, a necessary evil for cell signaling. Science 2006;312:1882-3.

19. Singh DK, Winocour P, Farrington K. Mechanisms of disease: the hypoxic tubular hypothesis of diabetic nephropathy. Nat Clin Pract Nephrol 2008;4:216-26.

20. Kiritoshi S, Nishikawa T, Sonoda K, et al. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. Diabetes 2003;52:2570-7.

21. Cameron NE, Cotter MA, Archibald V, Dines KC, Maxfield EK. Anti-oxidant and pro-oxidant effects on nerve conduction velocity, endoneurial blood flow and oxygen tension in non-diabetic and streptozotocin-diabetic rats. Diabetologia 1994;37: 449-59.

22. Ellis EA, Guberski DL, Somogyi-Mann M, Grant MB. Increased H2O2, vascular endothelial growth factor and receptors in the retina of the BBZ/Wor diabetic rat. Free Radic Biol Med 2000; 28:91-101.

23. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol 2000;130:963-74.

24. Matsumoto T, Kobayashi T, Kamata K. Relationships among ET-1, PPARgamma, oxidative stress and endothelial dysfunction in diabetic animals. J Smooth Muscle Res 2008; 44:41-55.

25. Son SM. Role of vascular reactive oxygen species in development of vascular abnormalities in diabetes. Diabetes Res Clin Pract 2007;77:S65-70.

26. Krolewski AS, Warram JH, Chrislieb AR, Busick EJ, Kahn CR. The changing natural history of nephropathy in type I diabetes. Am J Med 1985;78:785-94.

27. Berenson GS, Wattigney WA, Tracy RE, et al. Atherosclerosis of the aorta and coronary arteries and cardiovascular risk factors in persons aged 6 to 30 years and studied at necropsy (The Bogalusa Heart Study). Am J Cardiol 1992;70:851-8.

28. National High Blood Pressure Education Program Working Group report on hypertension in diabetes. Hypertension 1994; 23:145-58.

29. Sowers JR, Epstein M. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. An update. Hypertension 1995;26:869-79.

30. Rossing P, Hougaard P, Borch-Johnsen K, Parving HH. Predictors of mortality in insulin dependent diabetes: 10-year observational follow up study. Br Med J 1996;313:779-84.

31. Stamler J, Vaccaro O, Neaton JD, Wenworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Intervention Trial. Diabetes Care 1993;16: 434-44.

32. Elliott WJ, Maddy R, Toto R, Bakris G. Hypertension in patients with diabetes. Overcoming barriers to effective control. Postgrad Med 2000;107:29-32, 35-6, 38.

33. Merimee TJ. Diabetic retinopathy. N Engl J Med 1999;322:978-82.

34. Maser RE, Steenkiste AR, Dorman JS, et al. Epidemiological correlates of diabetic neuropathy. Report form Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes 1989; 38:1456-61.

35. Taskinen MR. Hyperlipidemia in diabetes. Clin Endocrinol Metab 1990;4:743-75.

36. Tomkin GH, Owens D. Insulin and lipoprotein metabolism with special reference to the diabetic state. Diabete Metab Rev 1994; 10:225-52.

37. Jenkins AJ, Lyons TJ, Zheng D, et al; DCCT/EDIC Research Group. Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. Kidney Int 2003;64:817-28.

38. Lyons TJ, Jenkins AJ, Zheng D, et al. Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Invest Ophthalmol Vis Sci 2004;45:910-8.

39. Grundy SM, Balady GJ, Criqui MH, et al. Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA Task Force on Risk Reduction. American Heart Association. Circulation 1998;97: 1876-87.

40. Soergel M, Kirschstein M, Busch C, et al. Oscillometric twenty-four-hour ambulatory blood pressure values in healthy children and adolescents: a multicenter trial including 1141 subjects. J Pediatr 1997;130:178-84.

41. Seaquist ER, Goetz FC, Rich S, Barbosa J. Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 1989;320:1161-5.

42. Dorchy H, Claes C, Verougstraete C. Risk factors of developing proliferative retinopathy in type 1 diabetic patients: role of BMI. Diabetes Care 2002;25:798-9.

43. De Block CE, De Leeuw IH, Van Gaal LF. Impact of overnight on chronic microvascular complications in type 1 diabetic patients. Diabetes Care 2005;28:1649-55.

44. Stone ML, Craig ME, Chan AK, Lee JW, Verge CF, Donaghue KC. Natural history and risk factors for microalbuminuria in adolescents with type 1 diabetes: a longitudinal study. Diabetes Care 2006;29:2072-7.

45. Koivisto VA, Stevens LK, Mattock M, et al. Cardiovascular disease and its risk factors in IDDM in Europe. EURODIAB IDDM Complications Study Group. Diabetes Care 1996;19:689-97.

46. Masetti S, Botto N, Manfredi S, et al. Interactive effect of the glutathione S-transferase genes and cigarette smoking on occurrence and severity of coronary artery risk. J Mol Med 2003; 81:488-94.

47. Ford ES, Malarcher AM, Herman WH, Aubert RE. Diabetes mellitus and cigarette smoking. Findings from the 1989 National Health Interview Survey. Diabetes Care 1994;17:688-92.

48. Helmrich SP, Ragland DR, Leung RW, Paffenbarger RS Jr. Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. N Engl J Med 1991;325:147-52.

49. Helmrich SP, Ragland DR, Paffenbarger RS Jr. Prevention of non-insulin-dependent diabetes mellitus with physical activity. Med Sci Sports Exerc 1994;26:824-30.

50. Moy CS, Songer TJ, LaPorte RE, et al. Insulin-dependent diabetes mellitus, physical activity, and death. Am J Epidemiol 1993;137: 74-81.

51. Donaghue KC, Fairchild JM, Craig ME, et al. Do all prepubertal years of diabetes duration contribute equally to diabetes complications? Diabetes Care 2003;26:1224-9.

52. Huber K, Christ G, Wojta J, Gulba D. Plasminogen activator inhibitor type-1 in cardiovascular disease. Status report 2001. Thromb Res 2001;103 (Suppl 1):S7-S19.

53. Haffner SM, D'Agostino Jr R, Mykkanen L, et al. Insulin sensitivity in subjects with type 2 diabetes. Relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. Diabetes Care 1999;22:562-8.

54. Meigs JB, Mittleman MA, Nathan DM, et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: The Framingham Offspring Study. JAMA 2000;283:221-8.

55. Mogensen CE, Keane WF, Bennett PH, et al. Prevention of diabetic renal disease with special reference to microalbuminuria. Lancet 1995;346:1080-4.

56. Rossing P. The changing epidemiology of diabetic microangiopathy in type 1 diabetes. Diabetologia 2005;48:1439-44.

57. Ruggenenti P, Remuzzi G. Kidney Failure Stabilizes after a Two-Decade Increase: Impact on Global (Renal and Cardiovascular) Health. Clin J Am Soc Nephrol 2007;146-50.

58. Joss N, Jardine A, Gaffney D, Boulton-Jones JM. Influence of apolipoprotein E genotype on progression of diabetic nephropathy. Nephron Exp Nephrol 2005;101:127-33.

59. Parving HH. Impact of blood pressure and antihypertensive treatment on incipient and overt nephropathy, retinopathy, and endothelial permeability in diabetes mellitus. Diabetes Care 1991; 14:260-9.

60. Astrup AS, Tarnow L, Rossing P, Pietraszek L, Riis Hansen P, Parving HH. Improved prognosis in type 1 diabetic patients with nephropathy: a prospective follow-up study. Kidney Int 2005; 68:1250-7.

61. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents: The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004; 114:555-76.

62. Wells T, Frame V, Soffer B, et al; Enalapril Pediatric Hypertension Collaborative Study Group. A double-blind, placebo-controlled, dose-response study of the effectiveness and safety of enalapril for children with hypertension. J Clin Pharmacol 2002;42:870-80.

63. Maguire A, Cusumano JM, Craig ME, Donaghue KC. The Case for Biennial Retinopathy Screening in Children and Adolescents: Response to Stefánsson. Diabetes Care 2006;29:178-9.

64. Grading diabetic retinopathy from stereoscopic color fundus photographs - an extension of the modified Airlie-House Classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophtalmology 1991;98:786-806.

65. Fundus photographic risk factors for progression of diabetic retinopathy. ETDRS report number 12. Early Treatment Diabetic Retinopathy Study Research Group. Ophtalmologhy 1991;98: 823-33.

66. Schmetterer L, Wolzt M. Ocular blood flow and associated functional deviations in diabetic retinopathy. Diabetologia 1999; 42:387-405.

67. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin Epidemiologic Study of Diabetic Retinopathy II. Prevalence and risk of diabetic retinopathy when age at diagnosis

is less than 30 years. Arch Ophthalmol 1984;102:520-6.

68. Klein R, Klein BE, Moss SE, Cruicckshanks KJ. The Wisconsin Epidemiologic Study of Diabetic Retinopathy XV. The long term incidence of macular edema. Ophthalmology 1995; 102:7-16.

69. Tesfaye S, Selvarajah D. The Eurodiab study: what has this taught us about diabetic peripheral neuropathy? Curr Diab Rep 2009; 9:432-4.

70. Laing SP, Swerdlow AJ, Slater SD, et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia 2003;46:760-5.

71. Jarvisalo MJ, Raitakari M, Toikka JO, et al. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. Circulation 2004;109:1750-5.

72. Orchard TJ, Stevens LK, Forrest KY, Fuller JH. Cardiovascular disease in insulin dependent diabetes mellitus: similar rates but different risk factors in the US compared with Europe. Int J Epidemiol 1998;27:976-83.

73. Nathan DM, Cleary PA, Backlund JY, et al; Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643-53.