NEW TRENDS IN DIABETIC NEPHROPATHY

SHORT ARTICLES

OF LECTURES AND ROUND TABLES

THESSALONIKI 9 – 10 OCTOBER 2009

Lecture

ADAMANTIOS KORAIS AND GREEK EDUCATION

President: **A. Diamantopoulos**

Speaker: **N.P. Terzis**

This paper is concerned with four distinctive elements: a) biographical information on Korais, b) basic concepts of Korais's philosophy, c) the content Korais gives to Greek Education, and d) evaluation of the accomplishments and the traits of Korais's work.

During the first part some biographical aspects on Korais's life will be presented, his studies at the renowned Evangelical School of Smyrna, his "free studies" in Amsterdam, his studies at the famous Medical Faculty of Montpellier, and his next placement at the City of Light, Paris, where he died. The knowledge of the course of his life will allow us to understand his personality, his actions and the impact of his work.

In the second part, some key-concepts of the philosophy Korais formed, will be analysed. Some of these concepts had a leading role in his life. These concepts are: a) *mesotis*, b) *metakenosis* και c) *biotic*. With the first, we refer to his basic philosophical attitude, which is clearly founded onto Aristotle's political philosophy, according to which the two ends of the range must be putted aside. The second general concept of Korais's thought is *metakenosis*. According to this the wisdom of our ancient ancestors not only was it comprehended, but it was also progressively transformed by the Western civilization. This achievement, in combination with the limitation of Modern Greeks to comprehend it because of their ignorance due to the Ottoman occupation, did not allow them to transcend themselves and to develop the Ancient Greek thought. This concept basically concerns the need for Modern Greeks to "re-borsrow" from the Westerns what the Westerns had previously borrowed by Modern Greeks' ancestors. At a practical level, *metakenosis* literally refers to the transfer of wine from the one barrel (the barrel of Westerns) to a second barrel (this of Modern Greeks). This is further supported by Korais by arguing that western authors' works must be translated in Greek. The third principle thought in Korais's personal philosophy is the *biotic.* The *biotic* refers to the modes of living in a community; *biotic*, nonetheless is not only related to society, but to politics as well (i.e. the way a political society is organized and functions) the basis of which is "ethics" that cannot be coloured understood in terms of formal moralism, but as a way of matching "practical philosophy".

The third part of this paper relates to the structural elements of Greek Education, as they appear in his work and his actions. These elements refer to the theory of education, the curriculum, the pedagogical style of educators and their competence, the relation between the ancient and the modern Greek world, the socio-political intentions of modern Greek education, and most importantly the language issue. The misapprehension of Korais's convictions on this issue by followers of *archaism* and the subsequent radical *demoticism* of that period, consists a proof of a polemic that aimed at self-justification, despite the honest and creative dialogue –a symptom not unknown in our society.

Finally, an effort will be made to evaluate the work and Korais's deeds, which will be included diachronically to the continuity of the phenomenon of Greek Education from the Ottoman period to the establishment of the Hellenic State.

Lecture

GENETICS OF DIABETES AND ITS COMPLICATIONS. WHERE DO WE GO NEXT?

President: **Z. Skouras**

Speaker: **C. Polychronakos**

Background: Type 1 and type 2 diabetes (T1D and T2D) are highly heritable: The l_S (the ratio of risk to a sibling over population prevalence) is 2-3 for T2D and ~15 for T1D. Independently of risk to diabetes, the risk of complications (for any given level of glycemic control) is also heritable. About half of the T1D heritability is due to strongly predisposing and strongly protective HLA alleles at the major histocompatibilty complex locus on chromosome 6p. The remaining is made up of a large number of weak effects.

Recent technological developments (high-density genotyping arrays) have enabled the examination of most of the common human genetic variation in large population samples of T1D, T2D and diabetic nephropathy (DNA). Approximately 40 loci are now known for T1D and a similar number for T2D. Two novel loci were also identified for DN, recently. Interestingly, a locus that determines glycated haemoglobin (HbA1c) levels was also discovered.

The problem with the genetic evidence obtained so far is that the high-density arrays target relatively common variants (minor allele frequency >0.01). These loci, collectively, explain a small part of the l_S. With larger and larger samples, more loci of smaller and smaller effects will undoubtedly be discovered, their potential total number being in the hundreds, or even thousands.

2. The future: Rare, highly penetrant variants and personalized medicine. A signal in a genetic study can be weak because the biological effect is small, or because the allele is rare, although it may confer strong biological effects. Of the large number of weaker genetic effects that are needed to explain diabetes heritability, it is likely that a considerable portion is weak only because of allele rarity. Among those rare alleles, some could have extremely high penetrance. Each of these variants will explain a large portion of the individual risk but only for a small fraction of the patient population, indicating that what appears to be a homogenous phenotype may have distinct molecular aetiologies, responding differently to a given therapeutic intervention. For type 1 diabetes, we have started with copy-number variations (CNVs) both because they are prime candidates for biological effects and relatively easy to detect. Analysis of fluorescence signals from the *Illunmina* Infinium array in 1,000 cases and 4,000 controls with the PennCNV algorithm, detected 11 CNV loci with significantly higher frequency in cases than in controls and large odds ratios (>>2). Collectively, these involve 6% of all cases. Confirmation with direct methods is in progress.

If confirmed, this will be a first step towards personalizing diagnosis at the molecular level, a step necessary for the meaningful evaluation of novel (as well as existing) therapeutics.

Round table

PATHOGENETIC MECHANISMS IN DIABETIC NEPHROPATHY

Presidents: **I. Magoula, H. Migdalis**

HYPERGLYCEMIA AND TGF – VEGF GROWTH FACTORS

Speaker: **F. Iliadis**

Increase expression of glucose transporter GLUT1 in glomerular cells leads to intracellular hyperglycemia. Intracellular hyperglycemia causes increased mitochondrial production of reactive oxygen species (ROS). ROS reduce the activity of the key glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH). The level of all the glycolytic intermediates those are upstream of GAPDH, increase. Increased levels of glyceraldehyde-3-phosphate form methylglyoxal, the major intracellular AGE precursor. Also form of diacylglycerol activates PKC. Moreover, levels of the glycolytic metabolite fructose-6 phosphate increase, which increases flux through the hexosamine pathway, where fructose-6 phosphate is converted by the enzyme GFAT to UDP–*N*acetylglucosamine (UDP-GlcNAc). Finally, inhibition of GAPDH increases intracellular levels of the first glycolytic metabolite, glucose. This increases flux through the polyol pathway, where the enzyme aldose reductase reduces glucose, consuming NADPH in this process.

Increased flux through the polyol pathway. When the glucose concentration in the cell becomes too high, aldose reductase converts glucose to sorbitol, which is oxidized to fructose, later. In this process, aldose reductase consumes the cofactor NADPH. NADPH is also the essential cofactor for regenerating a critical intracellular antioxidant, reduced glutathione. By reducing the amount of reduced glutathione, the polyol pathway increases susceptibility to intracellular oxidative stress.

PKC activation. When PKC is activated by intracellular hyperglycemia, it has a variety of effects on genes expression.

Increased hexosamine pathway (HBP) activity. UDP (uridine diphosphate) *N*-acetyl glucosamine gets put onto serine and threonine residues of transcription factors, and results in pathologic changes in genes expression.

AGEs formation. AGEs can act intracellularly or circulate and act on cell surface receptors such as the receptor for AGEs (RAGEs). The structural components of the connective tissue matrix and, in particular, basement membrane components such as type IV collagen are prime targets, but other long-lived proteins can also undergo advanced glycation, including plasminogen activator 1, and fibrinogen. ECM proteins are susceptible to AGE modification because of their slow turnover rate. The formation of intermolecular and intramolecular crosslinks with collagen as a result of the glycation process leads to structural alterations, leading to increased stiffness and resistance to proteolytic digestion. The composition of ECM is also modified by AGEs, with increased expression of ECM proteins, including fibronectin, types III, IV, and VI collagen and laminin, possibly mediated through upregulation of key profibrotic cytokines such as TGF-b and connective tissue growth factor. Through the interaction with RAGEs, AGEs trigger the activation of secondary messenger pathways such as protein kinase C. A key target of RAGE signaling is nuclear factor-B (NF-B), which is translocated to the nucleus where it increases transcription of a number of proteins, including intercellular adhesion molecule-1, E-selectin, endothelin-1, tissue factor, vascular endothelial growth factor (VEGF), and proinflammatory cytokines.

Overall, hyperglycaemia leads to intracellular activation of the PKC, HBP and polyol pathways, and also leads to accumulation of ROS and production of AGEs. These pathways lead to activation of the transcription factors USF1/2, AP-1, CREB, NF-kB, NFAT5, OREBP and Sp1. These

transcription factors have been demonstrated to increase the expression of genes encoding the transforming growth factor b (TGFb), as well as genes for a range of other proteins implicated in inflammation and extracellular matrix turnover, including thrombospondin 1, the chemokine CCL2, osteopontin, fibronectin, decorin, plasminogen activator inhibitor 1 and aldose reductase. That, ultimately culminate in extracellular matrix accumulation, inflammation and glomerulosclerosis in the diabetic kidney.

TGF-b as the mediator of matrix accumulation. Proof-of-concept studies have provided the crucial evidence that renal TGF-b is the causative agent of mesangial matrix expansion and renal insufficiency in diabetic nephropathy. The chief component of TGF-b signaling that is relevant to kidney disease is the Smad3 pathway.

VEGF as the mediator of diabetic proteinuria. Growing evidence suggests that podocyte-derived vascular endothelial growth factor (VEGF) and perhaps other angiogenic or permeability factors are directly involved in the proteinuria of diabetes rather than in extracellular matrix build-up. VEGF is significantly up-regulated in the diabetic state and participates in the development of the podocytopathy, especially the genesis of albuminuria. Stimulation of VEGF secretion by podocytes can increase macromolecular permeability of the glomerular capillary by affecting blood flow and glomerular endothelial cell function as well as possibly having a novel autocrine effect to alter podocyte synthesis of GBM constituents and foot processes.

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DIABETIC NEPHROPATHY: THE ROLE OF ENDOTHELIAL DYSFUNCTION

Speaker: **K. Paletas**

Vascular endothelium regulates vascular tone and maintains free flow of blood in vessels. Recently has been appreciated that endothelial dysfunction (VED) plays a key role in diabetic nephropathy. Specifically, it has been shown that diabetic subjects with renal disease have reduced generation and bioavailability of NO, which is the key vasodilator involved in keeping the endothelium healthy, and increased production of reactive oxygen species. Nitric Oxide (NO) is produced enzymatically by intracellular nitric oxide synthase (NOS), which converts L-arginine to citrulline and nitric oxide. The enzymatic production rate is dependent on several factors, including the bioavailability of arginine, cofactors such as tetrahydrobiopterine and NADPH, the presence of the endogenous NOS inhibitor, asymmetric dimethylarginine (ADMA) and also the amount of NOS protein. The eNOS uncoupling in VED leads to eNOS mediated production of ROS that further damage the endothelial cells by upregulating the proinflammatory mediators and adhesion molecules. In recent studies has been demonstrated a correlation between diabetes and VED, especially in diabetic endothelium there is uncoupling of the eNOS reaction due to excessive ADMA concentrations contributing to renal redox stress and remodeling. In diabetes, glucotoxicity induces increased production of advanced glycosylation end products (AGEs), increased glucose auto-oxidation reactions, activation of polyol-sorbitol pathway and the diacylglycerol-protein kinase C pathway and the direct scavenging of glucose on nitric oxide. Each is important in the production of excessive ROS contributing to the multiple microvascular complications and specifically to diabetic nephropathy.

VED has been revealed to be involved in diabetic nephropathy by inducing nodular glomerulosclerosis, mesangial expansion and decreasing the glomerular filtration rate. Diabetes-induced VED could be one of the culprits implicated in the pathogenesis of diabetic nephropathy since the mediators of endothelial dysfunction such as ang-II, ET-1, AVP, urotensin-II, ADMA, caveolin, CRP, leptin, resistin and Rho-kinase are getting upregulated and actively involved in nephropathy. Thus, it may be suggested that diabetes-induced VED upregulates various mediators that ultimately lead to diabetic nephropathy.

TRPC6 CHANNEL SIGNALING IN RESPONSE TO ANGIOTENSIN II TYPE 1 RECEPTORS IS ESSENTIAL FOR THE PRESERVATION OF THE PODOCYTE CYTOSKELETON

Speaker: **A. Greka**

Canonical Transient Receptor Potential 6 (TRPC6) channel mutations have been linked to proteinuric kidney disease. While gain of function mutations in TRPC6 have pointed to increased calcium influx as a putative mechanism of podocyte injury through disruption of the actin cytoskeleton, little is known about the specific pathways that regulate this process. Additionally, calcium influx into podocytes has been previously described in response to Angiotensin II signaling, but the channels responsible for the observed calcium influx remain elusive. Here we report that application of Angiotensin II (ATII) on Angiotensin Type 1 Receptor (AT1R) expressing podocytes generates a current with properties consistent with TRPC6 channels. Furthermore, AT1R activation results in a slow and transient TRPC-like augmentation in intracellular [Ca^{2+}] that is abrogated by Losartan. Importantly, the ATII-induced intracellular [Ca^{2+}] elevation is markedly reduced after gene silencing of TRPC6, but not after the antagonism of the NMDA Receptor, AMPA Receptor, or L-Type Ca^{2+} channels.

Interestingly, AT1R expressing podocytes loose their neuronal-like structure and instead display a polygonal shape characterized by markedly reduced primary processes, and a massive assembly of F-actin into stress fibers. Concomitantly with TRPC6 channel – mediated calcium influx, AT1R activation results in cortical actin formation. Intriguingly, TRPC6-deficient AT1R podocytes showed pervasive loss of actin stress fibers and focal contacts, severe collapse of the cytoskeleton, and significant reduction in cell size. Our studies have identified TRPC6 as the elusive principal Ca^{2+} conduit in the podocyte in response to Angiotensin II – mediated signaling. These results support the idea that TRPC6 may be an important mediator of podocyte injury in acquired forms of proteinuria, where the Renin – Angiotensin axis (RAS) is known to play an important role. Furthermore, TRPC6 appears to be essential in the preservation of the podocyte actin cytoskeleton and overall morphology. This observation suggests that while increased channel activity leads to podocyte injury and proteinuria, loss of channel activity may have similarly deleterious effects on podocytes and the filtration barrier.

Lecture

"MECHANISMS OF PROGRESSION AND MANAGEMENT"

President: **E. Mogensen**

Speaker: **G. Remuzzi**

Diabetic nephropathy (DN) is the leading cause of end-stage renal failure in Western countries and carries an increased risk for cardiovascular mortality. Studies have identified a number of factors that play a part in the development of DN. Among them, hypertension and proteinuria are the most important. In the early stages of DN, when albumin is present in the urine in very low quantities (microalbuminuria) and an increase is seen in blood pressure, there is no loss of filtrate and patients respond well to prophylactic measures. Along this line, the Bergamo Nephrologic Diabetes Complication Trial (BENEDICT) showed that microalbuminuria can be prevented by ACE inhibitor therapy. Once incipient or overt nephropathy has been detected, treatment is aimed at reducing the risk of disease progression and preventing cardiovascular complications. Treatment should include lifestyle modifications (such as weight loss, exercise, and reduction of protein, salt, and alcohol intake), smoking cessation, tight glucose control (target glycosylated hemoglobin, <7 percent), and control of blood pressure. Inhibition of the renin–angiotensin system with an ACE inhibitor or angiotensin II–receptor antagonist is warranted to decrease both blood pressure and albuminuria; the dose should be titrated upward to the moderate or high range, as tolerated, to achieve a systolic pressure below 130 mm Hg and a diastolic pressure below 80 mm Hg. Although data from clinical trials provide stronger support for the use of angiotensin II–receptor antagonists than for the use of other agents in patients with type 2 diabetes and microalbuminuria or macroalbuminuria, in the absence of a direct comparison of the two strategies, either of these classes of medication are a reasonable first choice.

Round table

STRUCTURAL AND FUNCTIONAL LESIONS IN DIABETIC NEPHROPATHY

Presidents: **G. Karkavelas, E. Dafnis**

HISTOPATHOLOGIC RENAL DAMAGE IN EARLY AND LATE DIABETIC NEPHROPATHY

Speaker: **G. Karayannopoulou**

Diabetic nephropathy affects all four components of kindney:the glomeruli,tubules,interstitium and the blood vessels.

Abnormalities of glomerular structure are the most consistent changes identified in biopsy material from patients with clinical diabetic nephropathy. In the earliest stages of injury there are no easily detectable glomerular alterations. Glomeruli may be enlarged but are otherwise morphologically normal. The earliest detectable histologic changes are a widening of the mesangium,due to increased accumulations of matrix, which is best demonstrated with a PAS stain. This process is generally global and diffuse. This process of diffuse mesangial sclerosis – or diffuse intercapillary glomerulosclerosis as termed by Kimmelstiel and Wilson – is the hallmark lesion of diabetic glomerulopathy. Increased cellularity in the widened mesangium is not a usual histologic finding.

The lesions of diffuse mesangial sclerosis are progressive. One of the advanced manifestations of mesangial sclerosis is the distinctive nodular accumulations of mesangial matrix , known as Kimmelstiel-Wilson nodules.

The nodules are irregularly distributed among glomeruli, and usually only 1 or 2 nodules are found in a particular glomerular tuft. They may vary considerably in size, and somewhat in shape, most often being round to oval. They are acellular to pausicellular and sometimes have a distinctive laminated quality that is highlighted by silver methenamine stains. While often considered to be the most characteristic lesion of diabetic nephropathy, it must be remembered that such lesions are not pathognomonic of a diabetic process. Nodular glomerulosclerosis indistinguishable from that in diaberic nephropathy may also be encountered in renal biopsies from patients with monoclonal immunoglobulin or light chain deposition disorders, some cases of membranoproliferative glomerulonephritis, rare cases of resolving post-infectious glomerulonephritis and amyloidosis.

Related to the process of nodular mesangial sclerosis is the presence of mesangiolysis and capillary microaneursym formation.

Three other lesions characterize the histologic appearance of diabetic glomerular injury. The first is diffuse thickening of basement membranes, which is best appreciated with PAS stains. The other two lesions are characterized by accumulations of hyaline – homogenous eosinophilic acellular material. When this material is located between the basement membranes of Bowman's capsules and the adjacent parietal epithelium, it has been termed "capsular drop". When hyaline is found within capillary lumina and particularly when it is adherent to the capillary wall, it has been termed the "fibrin cap" or exudative lesion.

Tubules. The commonest change in the tubules is an accumulation of lipid in the cells lining the proximal parts .Very occasionally the cells of pars recta are loaded with glycogen.(the Armani-Ebstein change).In advanced cases there is usually considerable atrophy of tubules.

Interstitium. The interstitium is often fibrotic and may contain many chronic inflammatory cells. These changes are most frequenty present when diabetes has been present for a long time.

Blood vessels. Changes in the blood vessels are very commonly present. There is marked hyaline thickening of the wall of the arterioles,this characterteristically affects the efferent arterioles as well as the afferent arterioles.The intima of the arteries is commonly thickened.

Immunofluorescence Microscopy. Diffuse linear staining of glomerular and tubular basement membranes with antibodies to IgG and albumin may be seen. Glomerular and arteriolar deposits of hyaline contain IgM and C3.

Electron Microscopy.The earliest observable alteration is uniform thickening of the glomerular basement membranes,which may have a three to four fold increase,measuring as much as 1200 to 1500 nm. Glomerular visceral epithelial cells show effacement of foot processes. The mesangial matrix is expanded with increased mesangial cells without immune comlexes. Areas of hyalinosis appear electron dense,often present in sclerotic areas,often contain lipid and should not be confused with immune complex-type deposits.

EARLY AND LATE RENAL CHANGES IN DIABETIC NEPHROPATHY

Speaker: **A. Raptis**

Glomelural hyperfiltration is a phenomenon found early in the clinical course of diabetes. Renal functional changes in diabetic nephropathy conventionally have been linked to progression of urinary albumin excretion. Early in 1980s' it was well known that alterations in renal function occur at the time of diagnosis of type 1 diabetes mellitus (T1DM). Glomelural filtration rate (GFR) and plasma renal flow were found to be higher than normal in young patients with T1DM compared to non-diabetic controls. Many investigators then examined if these early changes in renal function can define progression to overt diabetic nephropathy. Most, but not all investigators reported that elevated GFR is an independent predictor of future microalbuminuria and macroalbuminuria. The coupling of glomelural hyperfiltration and normoalbuminuria in short duration diabetes followed by the pairing of diminished renal function and proteinuria later quite naturally led to the hypothesis that declining renal function and increasing albumin excretion are progressing in parallel. Mongensen and colleagues have best articulated this model. Under this paradigm, renal function is normal or even elevated when the appearance of microalbumnuria signals the onset of diabetic nephropathy. Subsequently, urinary albumin excretion increases to overt nephropathy and causes renal function to decline as it progresses to proteinuria.

More contemporary research findings, using serum cystatin-C-based estimates of GFR (cC-GFR), have challenged this paradigm. In the 2nd Joslin Kidney Study on the Natural History of Microalbuminuria, over one-third of patients with T1DM and microalbuminuria at the time of enrollment already had evidence of mild (cC-GFR <90) or moderate (cC-GFR <60 ml/min) renal function impairment. Rather, the process of renal function loss appears to begin prior to the onset of proteinuria.

Renal haemodynamic and metabolic factors are involved in the pathophysiology of hyperfiltration in diabetic patients. Hyperfiltration has been found to be more prevalent in those with recent onset of diabetes and poor glycaemic control. Under normal physiologic conditions, there are autoregulatory mechanisms which protect the glomerular capillaries from changes in systemic arterial pressure. There are data demonstrating that hyperglycaemia alters the normal process of autoregulation within the glomerulus, resulting in reduction of afferent and, to a much lesser degree, efferent arteriolar tone. This causes transmission of systemic pressure to the glomerular capillary and higher glomelural trans-capillary hydraulic pressure and contributes to an increase in single –nephron and whole-kidney GFR. This is associated with more severe degree of structural glomerular damage. Additional mechanisms by which hyperglycaemia disrupts capillary vasoregulation include enhanced production of nitric oxide, which lead to both afferent and efferent glomerular arteriolar vasodilation, and increased TGF-β1 production, which enhance the production of reactive oxygen species. Moreover, hyperglycaemia increases the production of angiotensin II particularly by the local tissue renin-angiotensin-aldosterone system (RAAS), which contributes to diabetic hyperfiltration by mediating elective vasoconstriction of the efferent arteriole. The increased intraglomerular pressure produces shear-stress forces on the endothelium and stimulates mesangial matrix formation and mesangial cell proliferation by increasing formation of types I and IV collagen, laminin, fibronectin, TGF-β1, and angiotensin II receptor mRNA.

A recent meta-analysis of ten cohort studies, following 780 patients in a median follow-up of 11,2 years, indicated that type 1 diabetic patients with hyperfiltration are 2,7 times more likely to progress to incipient nephropathy than those with normofiltration and the patients who progress, had a higher baseline GFR.

Uncertainty remains as to whether glomerular haemodynamic changes similar to those found in patients with T1DM are also present in type 2 diabetes. In studies of Pima Indians, GFR was found to be 15% higher in recent-onset type 2 diabetic patients. However, these changes were no longer significant when corrected for body surface area and were not confirmed by another smaller study. Additionally, in longitudinal studies the GFR was found to be elevated at the onset of type 2 diabetes mellitus and remained so until the onset of overt nephropathy.

In conclusion, patients with T1DM who have glomerular hyperfiltration or elevated GFR subsequently have an increased risk of developing diabetic nephropathy. However, additional studies using patient level data are required to examine whether this increased risk would persist after adjustment for the confounding effects of glycaemic control, diabetes duration and blood pressure.

Lecture

DIABETIC RENAL DISEASE: CHANGING PARADIGMS

President: **M. Sion**

Speaker: **E. Mogensen**

Interestingly, in type 1 diabetes hyperfiltration is a marker for poor prognosis related to metabolic control. Thus hyperfiltration is a marker for bad development but microalbuminuria (below the proteinuric level) is also associated with a poor prognosis. It is well-known that patients with essential hypertension may sometimes have microalbuminuria and it is also known that it predicts a poor prognosis.

It was originally believed that microalbuminuria only predicted renal disease. However, surprisingly it predicts as well cardiovascular disease and early mortality. The story about blood pressure and progression of renal disease is interesting because it was earlier believed that a certain high blood pressure was mandatory for preservation of the renal function. This appeared to be a completely wrong concept.

Important data regarding microalbuminuria suggest that patients with microalbuminuria should receive anti-hypertensive treatment, even patients with so-called normal blood pressure. This was confirmed in several trials and was also included in the guidelines. Reducing blood pressure is important but it appeared to be especially beneficial to block the renin-angiotensin-system. It is evident that albuminuria is a continuous variable and is also a risk factor. Earlier it was suggested to use ACE-inhibitors or ARBs. Now, based on a good theoretical background, it is clear that it is possible to use a combination.

In the history of hypertension, it was earlier believed that diastolic blood pressure was most important but later on it was generally accepted that systolic is a better predictor as the goal for treatment, but new data suggest pulse pressure may be even better.

Not only is microalbuminuria an important risk marker but it is clear as well that regression of microalbuminuria is a good marker for a better prognosis in patients. Microalbuminuria is believed to be the strongest risk factor but new studies actually suggest that, along with other factors, a simple parameter such as self-rated health is crucial.

Regarding new developments, it is clear that new studies have led to several advancements in the management of patients. For instance the Steno II Study shows positive effect on mortality by multifactorial intervention. Similarly, the ADVANCE Study also showed positive effect on mortality by more intensified anti-hypertensive treatment with an ACE-inhibitor. Results from glucose arm in the ADVANCE Study also showed positive effect on renal disease. This is important in the light of the ACCORD Study showing increased mortality with too strict glycemic control with a goal of 6% in HbA1c.

Lecture

MICROALBUMINURIA TODAY: EPIDEMIOLOGY DATA AND CURRENT PATHOGENETIC VIEWS

President: **D. Karamitsos**

Speaker: **G. Viberti**

Prof. G. Viberti did not send the abstract.

Lecture

THE ROLE OF THE PODOCYTES IN THE PATHOGENESIS AND TREATMENT OF PROTEINURIA

President: **D. Vlachakos**

Speaker: **P. Mundel**

The highly dynamic foot processes contain an actin-based contractile apparatus comparable to that of smooth muscle cells or pericytes. Mutations affecting several podocyte proteins lead to the rearrangement of the actin cytoskeleton, disruption of the filtration barrier and subsequent renal disease. Proteins regulating the plasticity of the podocyte actin cytoskeleton are therefore of critical importance for sustained function of the glomerular filter. Synaptopodin is the founding member of a unique class of actin and alpha-actinin binding proteins. Synaptopodin is strongly expressed in dynamic cell compartments such as podocytes foot processes and telencephalic dendrites. Synaptopodin is a proline-rich linear protein and is highly susceptible to proteolytic degradation. Synaptopodin is a critical regulator of the podocyte actin cytoskeleton and bigenic heterozygosity for CD2AP and synaptopodin results in spontaneous proteinuria and FSGS-like glomerular damage. It is well established that the immunosuppressive action of the calcineurin inhibitor cyclosporine A (CsA) stems from the inhibition of NFAT signaling in T cells. CsA is also used for the treatment of proteinuric kidney diseases. As it stands, the antiproteinuric effect of CsA is attributed to its immunosuppressive action. Here we discuss the novel finding that the beneficial effect of CsA on proteinuria is not dependent on NFAT inhibition in T cells, but rather results from the stabilization of the actin cytoskeleton in kidney podocytes. CsA blocks the calcineurin-mediated dephosphorylation of synaptopodin, thereby preserving the phosphorylation-dependent synaptopodin-14-3-3 beta interaction. Preservation of this interaction, in turn, protects synaptopodin from cathepsin L-mediated degradation. Our results unveil a novel physiological role of cathepsin L in the cytoplasm, where it mediates the phosphorylation-dependent proteolysis of the actin-organizing protein synaptopodin. These data also identify the podocyte as a novel direct target of calcineurin and CsA, independent of the inhibition of NFAT signaling in T cells. Altogether, these results represent a significantly extended view of calcineurin signaling and shed new light on the treatment of proteinuric kidney diseases. Novel calcineurin substrates such as synaptopodin are promising starting points for drugs that avoid the serious side effects of NFAT inhibition caused by long-term CsA treatment.

Satellite symposium

THE ROLE OF RENIN INHIBITORS IN THE LOWERING OF PROTEINURIA FOR RENAL AND CARDIOVASCULAR PROTECTION OF PATIENTS WITH DIABETIC NEPHROPATHY

President: **Ch. Zamboulis, K. Siamopoulos**

Speakers: **G. Bakris, G. Remuzzi**

It is well known and accepted that reduction of blood pressure to levels well below 140/90 mmHg slows progression of diabetic nephropathy. While this benefit is seen in all patients it is particular evident in those with proteinuria at levels above 300 mg/day that also have a >30% reduction in this level[1]. There are four outcome trials in patients with advanced proteinuric nephropathy that all demonstrate a benefit of > 30% proteinuria reduction along with blood pressure leading to the slowest progression of nephropathy[2].

While most commonly used agents that lower blood pressure also reduce proteinuria, the antihypertensive agents found to produce the greatest benefit are the blockers of the renin angiotensin aldosterone system (RAAS). RAAS blockers are known to reduce proteinuria by multiple mechanisms apart from simple blood pressure reduction. They reduce intraglomerular pressure and affect cytokines that interact with angiotensin II at the level of the podocyte and hence stabilize membranes in the podocytes and attenuate fibrotic mechanism in the interstitium[3].

Recent studies have not supported the use of combined RAAS blockade with converting enzyme inhibitors and angiotensin receptor blockers to provide added benefit for cardiovascular risk[4]. However, the question on nephropathy progression is still an open question. Data from the COOPERATE trial have been called into question and are not valid. The NEPHRON-D trial currently ongoing in the United States will definitively answer the question about nephropathy progression and dual RAS blockade[5].

More effective RAAS blockade can be achieved by blocking the rate limiting enzyme of the RAAS system, renin. Aliskerin, the first renin inhibitor available, effective blocks renin activity and hence, reduces the production of angiotensin II and aldosterone more than traditional RAS blockers. This observation may explain why, when it is combined with an ARB, there is greater reduction in proteinuria among patients with diabetic nephropathy and no additional blood pressure reduction[6]. Whether this will translate into better cardiovascular or renal outcomes will be evident in the ALTITUDE trial[7].

It can be argued that renin inhibitors may be ideal initial agents as they do not have problems with angioedema like ACE inhibitors and more effectively reduce aldosterone compared to either ACE inhibitors or ARBs. Unfortunately, given the current environment and the evidence for other classes of RAS blockers it may never be tested in a head-to-head trial against one of these agents to answer this question.

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Lecture

PATHOGENETIC ROLE OF AUTONOMIC NEUROPATHY IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

President: **E. Pagkalos**

Speaker: **T. Didangelos**

Cardiovascular autonomic neuropathy has a prevalence of ~ 17–22% in patients with type 1 and type 2 Diabetes Mellitus, and its presence has been associated with substantially increased risk of cardiovascular and all-cause mortality in patients with type 2 diabetes[1]. Approximately 65% of people with diabetes die of cardiovascular disease, and cardiovascular disease is the leading cause of death among patients with type 2 diabetes who are diagnosed with cardiovascular autonomic neuropathy. The clinical manifestations of cardiovascular autonomic neuropathy (CAN) include orthostatic hypotension, abnormalities in heart rate control, and decreased heart rate variability (HRV) on 24-h monitoring or provocative testing. CAN may be present at diagnosis of diabetes[2].

Microalbuminuria is strongly associated with increased risk of cardiovascular complications, including atherosclerotic coronary artery disease, stroke, peripheral vascular disease, and cardiovascular mortality. Abnormalities in 24-h ambulatory blood pressure pattern, particularly loss of normal sleep decrement in systolic pressure ("nondipping"), are associated with diabetic microalbuminuria. It has been hypothesized that autonomic neuropathy impairs both renal function and the normal diurnal blood pressure pattern.

The pathological mechanism linking subclinical neuropathy and future microvascular complications (nephropathy and retinopathy) has not been fully clarified, yet. Early glycemic damage to the autonomic nerves may, in parallel, directly damage the retinal and glomerular blood vessels. However, as pupillary dysfunction (early sign of autonomic dysfunction) predicts microvascular complications even after adjusting for glycemic control, it has been postulated that early autonomic nerve dysfunction affects vascular tone and results in changes to both glomerular and retinal blood flow, thus predisposing to microalbuminuria and retinopathy. It has been hypothesized that damage to the autonomic nerves supplying the renal vasculature may lead to increased renal blood flow, glomerular hyperfiltration, and, thus, microalbuminuria. Similarly, increased retinal blood flow during acute hyperglycemia and an overall decreased retinal blood flow in diabetes mellitus may contribute to the development of retinopathy. Neurally mediated changes in retinal blood flow may further contribute to the development of retinopathy.

A causative role of autonomic neuropathy in the development of nephropathy in patients with diabetes Type 1 and 2 has been proposed based on clinical studies results and supported by the knowledge of a neural control of renal function. Some studies clearly showed an association between microalbuminuria and disturbances in autonomic function. Abnormalities of circadian blood pressure (BP) rhythm, detected with ambulatory blood pressure measurement (AMBP) in normotensive microalbuminuric patients were related to autonomic and renal dysfunction. Recent data indicate that autonomic dysfunction associated with blunted diurnal variations in arterial BP is already present in patients with high normal urinary albumin excretion (UAE) compared to those with low normal UAE. It has been suggested that the abnormalities in BP profile can be attributed to the presence of autonomic dysfunction itself. High normal level of albumin excretion, raised BP, and poor glycemic control have been suggested as the most important predictors of the development of microalbuminuria. Torbjornsdotteret al.[3] obtained 24-h blood pressure recordings and renal biopsy specimens in 41 normoalbuminuric patients with type 1 diabetes and found that nocturnal mean arterial blood pressure correlated directly with early evidence of nephron basement membrane thickening and mesangial ma-

trix hyperplasia.

HRV was associated with microalbuminuria, in another study, independent of blood pressure level, suggesting that cardiovascular autonomic impairment may be involved in the pathogenesis of diabetic renal disease through mechanisms independent of blood pressure[4]. The renal vasculature is extensively innervated by the sympathetic nervous system. These findings are consistent with the hypothesis that impairment of autonomic function leads to increased renal blood flow, glomerular hyperfiltration, and sodium excretion, all of which accelerate progression to diabetic microalbuminuria. Alternatively, the metabolic and vascular changes associated with diabetes may adversely affect both renal and cardiovascular autonomic function through other mechanisms.

We did find that CAN was associated with left ventricular diastolic dysfunction (LVDD) with preserved systolic function and microalbuminuria in type 1 and 2 diabetes mellitus[5]. Moreover, early administration of ACE inhibition or angiotensin receptor blockade improved CAN, LVDD and microalbuminuria after 1 year of treatment.

Furthermore, intensive multifactorial management aimed at control of BP, lipids, HBA1c, use of aspirin, vitamins E and C, and ACE inhibitors reduced CAN by 68% and microalbuminuria[6].

In conclusion, diabetic CAN is a serious complication found in one fourth of type 1 and one third of type 2 diabetic patients and it is associated with increased mortality. Cardiovascular autonomic neuropathy is associated with microalbuminuria in patients with Diabetes Mellitus and maybe plays a pathogenetic role in the development of diabetic nephropathy.

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Round table

THERAPEUTIC APPROACH OF DIABETIC NEPHROPATHY

Presidents: **E. Koulouridis, A. Alaveras**

GLYCEMIC CONTROL AT EARLY DIABETIC NEPHROPATHY

President: **E. Koulouridis, A. Alaveras**

Speaker: **S. Bakatselos**

Diabetic nephropathy is the most common cause of renal failure in developed countries, although the proportion of patients with diabetes who progress to end-stage renal disease is declining. The lifetime risk of nephropathy is roughly equivalent in type 1 and 2 diabetes.

A Finnish study reported a prevalence of end-stage renal disease of 2,2% at 20 years and 7,7% at 30 years. These prevalence rates are significantly lower than previous estimates of 30 to 40%, and probably reflect, at least in part, the impact of improved glycemic management.

The efficacy of strict glycemic control depends in part upon the stage of nephropathy at which it begins, and the degree of normalization of glucose metabolism. The evidence is best established in type 1 diabetes.

The earliest clinical manifestation of renal involvement in diabetes is an increase in albumin excretion (microalbuminouria), a stage at which renal histology may be relatively normal or may reveal glomerulosclerosis. The microalbuminouria may resolve spontaneously in a substantial proportion of patients, mainly in type 1 diabetics, or may progress to overt nephropathy, defined as a positive for protein urine dipstick (macroalbuminouria).

Glycemic control and nephropathy in type 1 diabetes

The association between glycemic control and nephropathy in type 1 diabetic patients was confirmed in the prospective **Diabetes Control and Complications Trial** (**DCCT**). Patients with no nephropathy at baseline were evaluated in the primary prevention study, while those with established nephropathy in the secondary intervention study.

The mean HbA_{1c} during the 6,5 years of the study was 7,2% with intensive therapy, as opposed to 9,1% with conventional therapy.

Primary prevention

At mean follow-up of 6,5 years, intensive therapy was associated with a significantly lower rate of new onset microalbuminouria (16,4% versus 23,9%, risk reduction 39%). There was also a significant reduction in new onset macroalbuminouria (3,2% vs 7,2%, risk reduction 51%).

Similar to the case with retinopathy, metabolic memory applies to the primary prevention of nephropathy (**EDIC** Follow-up study). Thus, previous intensive treatment with near-normal glycemia during the DCCT had an extended benefit in delaying the onset and progression of diabetic nephropathy.

The observations that combined renal and pancreas transplantation (the latter to restore normoglycemia) can prevent, and that intensive insulin therapy can delay recurrent diabetic nephropathy in renal allografts, is further evidence of the protective effect of meticulous glycemic control.

Secondary prevention

Intensive insulin therapy is also effective at a somewhat later stage, after microalbuminouria

has developed. There were only 73 patients in DCCT with microalbuminouria at baseline and too few of these patients progressed to macroalbuminouria, to demonstrate significant benefit from intensive insulin therapy.

A meta-analysis of 226 patients in 7 earlier trials of type 1 diabetes also found benefit. Among patients with either no albuminouria or microalbuminouria, the odds ratio for increasing albumin excretion was 0,34 in patients receiving intensive therapy, compared to those receiving conventional therapy.

Established macroalbuminouria

In contrast to the benefit of aggressive therapy in patients with microalbuminouria, it has been suggested that strict glycemic control with intensive insulin therapy may not slow the rate of progressive renal injury once overt dipstick-positive proteinouria has developed (equivalent to albuminouria>300 mg/day).

However, results from pancreatic transplant recipients in whom true euglycemia is restored, suggest that this may not be correct at prolonged follow-up. In the absence of restored normoglycemia (which can currently be achieved only with pancreas or islet transplantation), the apparent lack of substantial benefit in overt diabetic nephropathy from strict glycemic control alone suggests that other factors (such as intraglomerular hypertension and glomerular hypertrophy) may contribute to the progressive glomerular injury. At this late stage, there is often marked glomerulosclerosis.

Glycemic control and nephropathy in type 2 diabetes

Hyperglycemia is an important risk factor for the development of microvascular disease in patients with type 2 diabetes, as it is in patients with type 1. This has been shown in several observational studies. In addition, improving glycemic control improves microvascular outcomes.

In the **Kumamoto study** intensive therapy was beneficial. A lower incidence of the development or progression of nephropathy was achieved after 6 years (7,7% vs 32% in the primary prevention and 19,2% vs 44% in the secondary intervention).

In the **UKPDS**, the risk for any diabetes-related endpoint in newly diagnosed patients was 12% lower in the intensive therapy group (p=0,029). Most of the risk reduction in this group was due to a 25% risk reduction in microvascular disease. The results of the post-trial monitoring phase of the study show that a sustained period of good glycemic control has lasting benefit in reducing microvascular disease (metabolic memory). Also, there was no evidence of a threshold effect for HbA_{1c}; a 1% fall in HbA_{1c} was associated with a 35% reduction in microvascular endpoints.

In the **ADVANCE trial** 5571 type 2 diabetic patients receiving intensive therapy to lower HbA_{1c} (mean 6,5%) had a reduction in the incidence of nephropathy defined as development of macroalbuminouria, doubling of the serum creatinine to at least 2,26 mg/dl, the need for renal-replacement therapy, or death due to renal disease (4,1% vs 5,2%, hazard ratio 0,79), compared to 5569 patients receiving standard therapy (HbA_{1c} 7,3%).

In the **Veteran's Affairs Diabetes Trial (VADT)** type 2 diabetic patients receiving intensive therapy (HbA_{1c} 6,9%) did not have a reduction of major nephropathy outcomes, which were predefined as secondary endpoints, compared with standard therapy (HbA_{1c} 8,4%). The lack of benefit of intensive control in VADT may be due to the duration of diabetes (secondary prevention) in subjects participating (mean 11,5 years versus newly diagnosed in UKPDS), and the time required to show benefit (delayed benefit may require longer follow-up, mean 5,6 years versus 10 in UKPDS). In addition, aggressive treatment of hypertension and hyperlipidemia in all VADT participants may have contributed to the inability to show a microvascular benefit of intensive glucose control.

In conclusion, the results of the Kumamoto, UKPDS and ADVANCE trials are consistent with those of the DCCT**:** intensive therapy improves the outcomes of early diabetic nephropathy.

INHIBITION OF PRODUCTION OF ADVANCED GLYCATION END PRODUCTS AND PROTEIN KINASE C

Speaker: A. Zantidis

Diabetic nephropathy is a leading cause of end stage renal disease (ESRD) in western societies and in 2003 there were 7,9 million patients diagnosed with diabetic nephropathy in the USA, France, Germany, Italy, Spain, UK and Japan[1,2]. Clinical practice guidelines for the management of diabetic nephropathy mainly target hyperglycemia and hypertension[3]. Despite mltifactorial intervention existing measures are not effective for some patients and novel treatment strategies are needed. The elucidation of the molecular mechanism of diabetic nephropathy has prompted new drugs for the disease. The diabetic renal damage is now believed to be the result of a complex interaction between metabolic and hemodynamic abnormalities[1-5]. The production of advanced glycation end products (AGES) and the activation of protein kinase C (PKC) seem to play an important role in diabetic nephropathy.

Protein kinase C seems to be activated by most of the known contributors for development and progression of diabetic nephropathy including hyperglycemia, angiotensin II, oxidative stress, lipis and AGES. Subsequently it promotes the over expression of TGF β the major prosclerotic cytokine of the diabetic kidney disease and VEGF another cytokine relevant to increased glomerural permeability[2,4,6]. The PKC isoforms α and β appear to be involved in diabetic nephropathy[1,5]. The development of the PKC β I and II specific inhibitor ruboxistaurin (LY333531) attenuated overexpression of TGF β and delayed the progression of albuminuria in pilot trials when given 32 mg/day[1,2]. As yet there are no specific CPK α inhibitors suitable for clinical investigation[1]. Nevertheless, the AGES cross link breaker Alagebium (ALT-711) is found to reduce PKC α expression in animal models of type 1 diabetes. It remains to be seen if ALT-711 acts in part via PKC α inhibition apart from the reduction of AGES[1,3].

Advanced glycation end products are another major contributor in diabetic nephropathy. Exogenous AGES derive from tobacco and heated food. Endogenous AGES form by non-enzymatically reaction of reducing sugars with amino groups in proteins. The best characterized AGES are pentosidine and carboxymethyllysine (CML) which are only two of a large heterogenous group of molecules. Despite of their heterogeneity a common consequence of their formation is covalent cross linking to long live proteins such as collagen and interaction with their numerous membrane receptors (e.g. RAGE). Cross linking results in basic membrane thickening, altered adhesion/signaling and atherosclerosis.[4,7]. Binding to AGE receptors induce inflammation, apoptosis, oxidative stress, activation of PKC, and upregulation of VEGF, MCP-1 and TGF β[1,3,4,7]. AGE inhibition can be achieved by lowering intake of exogenous AGES, by tight glycemic control, by inhibition of AGES formation, by AGES cross link breakers and finally by receptor blockage[5]. Smoking cessation, dietary interventions and glucose lowering agents (mainly metformin) can reduced exogenous AGES and also the major initiator of their formation, hyperglycemia. Many AGES formation inhibitors have developed over the last decades. Aminoguanidine is the most studied inhibitor and it was found that in combination with ramipril can reduce albuminuria in type I diabetic patients in ACTION I. Other inhibitors such as ALT 946, ORB 9195, LR 90 have provided evidence of the of AGES blockage in diabetic nephropathy although their clinical utility, same as aminoguanidine, is remained to be seen. Pyridoxamine which inhibits the conversion of AGE intermediates to AGES has entered a phase II clinical trial in humans since it was found that reduces albuminuria and glomerural lesions. Benfotiame inhibites AGES by reducing accumulation of triosephosphatate intermediates. Alagebrium (ALT 711) is a cross link breaker which was found to reduce albumin excertion rate, blood pressure, renal collagen

accumulation and glumerosclerosis. ALT 711 may have therapeutic potential for clinical use. Neutralizing RAGE antibodies and soluble RAGE (sRAGE), which is a circulating Cterminal spice variant that blocks the full length RAGE receptor, also have shown renoprotective actions. Interestingly ACE inhibitors reduce AGES and increase sRAGE, thiazolidinediones reduce endothelial expression of RAGE and low molecular weight heparin (LMWH) can bind to RAGE and block it. Renal transplantation seems to increase AGES clearance in patients with ESRD and this may contributes to a better survival[1-7].

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ENDOTHELIN INHIBITION IN PATIENTS WITH DIABETIC NEPHROPATHY

Speaker: **S. Douma**

Diabetic nephropathy is observed in about one third of patients with diabetes mellitus. It is characterized by albuminuria, hypertension, glomerular filtration rate (GFR) reduction, and is associated with a dramatic increase of cardiovascular events. Several factors have been implicated in the pathogenesis of diabetic nephropathy; among them, the interaction of hemodynamic changes with metabolic factors seems to play a central role. Hemodynamic changes include increased systemic arterial and endoglomerular pressure and the activation of vasoactive factors, such as the renin-angiotensin system and endothelin. Several lines of evidence indicate that the initiating factor is endothelial dysfunction, a term that is synonymous with decreased bioavailability of nitric oxide (NO).

Increased expression of endothelin, mainly endothelin-1 (ET-1), has been observed in patients with diabetes mellitus. ET-1 over-expression affects primarily glomerular and tubular epithelial cells. Experimental data suggests that ET-1 over-expression results in hyperfiltration and interstitial damage, which in turn accelerates the progression of renal impairment through marked vasoconstriction, hypertrophy, hyperplasia and accumulation of extra-vascular tissue at mesangial cells. Inflammation seems to hold a significant role during this perplexed process, while ET-1 promotes inflammation via ET-A receptors.

Diabetic nephropathy represents a major therapeutic challenge and effective management during the initial stages of this devastating entity is of utmost importance, not only for the prevention or attenuation of disease progression, but for the reduction of cardiovascular events as well.

Inhibitors of endothelin receptors, both selective (ET-A) and non-selective (ET-A and ET-B) have been tested in experimental and small clinical studies, and have been shown to reduce albuminuria, inflammation and fibrosis. Selective ET-A inhibition seems to be superior to the non-selective inhibition, since ET-B receptors mediate enhanced NO activity and subsequent natriuresis. Despite theoretical advantages, the first large clinical study (ASCEND) with a selective ET-A inhibitor (Avosentan) in 286 patients with diabetic nephropathy was prematurely terminated due to fluid retention and edema. However, albuminuria was significantly reduced, providing a hopeful sign for future studies in this area.

In conclusion, it seems possible that combination therapy, aiming at different pathophysiological factors might attenuate the progression of diabetic nephropathy. Up to now, inhibition of the renin-angiotensin system (ACE-inhibitors or angiotensin receptor blockers), successful blood pressure and glucose control remain established therapeutic options. Endothelin inhibition has still much to prove. In fact, endothelin inhibition has yet proved beneficial only in pulmonary hypertension, although endothelin has been implicated in the pathogenesis of almost every cardiovascular disorder.

Lecture

OBESITY AND GLOMERULAR DISEASE

President: **A. Agrafiotis**

Speaker: **D.S. Goumenos**

Obesity represents a major health problem with increasing incidence in the Western world[1]. It is the phenotypic hallmark of metabolic syndrome that is characterized by insulin resistance, hyperinsulinemia and dyslipidemia. This syndrome contributes to the development of type 2 diabetes, hypertension, cardiovascular disease and chronic kidney disease[2].

The clinical evidence of renal involvement in obesity is manifested by the presence of proteinuria that usually precedes GFR decline by several years[4]. Experimental studies in obese animals show that angiotensin II and insulin are implicated in the development of glomerular hyperfiltration whereas leptin, a hormone produced in adipose tissue, contributes to the development of renal injury via induction of growth factors and cytokines[3]. These findings suggest that the adipose tissue itself may exert systemic effects through secretion of a variety of hormones and cytokines.

The type of kidney disease that has become recognized with increasing incidence over the last decade in kidney biopsies of obese patients with proteinuria is the so-called "obesity related glomerulopathy" (ORG) characterized by glomerulomegaly with or without focal segmental glomerulosclerosis (FSGS)[4,5].

We recently showed that in severely obese patients morphological changes are observed even with lack of proteinuria or microalbuminuria[6]. Eighteen patients with body mass index (BMI) >50 kg/m^2 who underwent a variant of biliopancreatic diversion with Roux-en-Y reconstruction and consented to undergo a renal biopsy during the surgical procedure were studied. Enlarged glomeruli, thickening of the glomerular basement membrane (GBM), scattered paramesangial deposits and reduction in the slit pore frequency were the main features identified on light and electron microscopy. The observed changes resemble to those described at the early stages of diabetic nephropathy.

The definition of glomerulomegaly is not uniform in the different studies. Chen et al[7] defined glomerulomegaly as glomerular volume of more than 3.27x10^6 μm^3 whereas Kambham et al found that the mean glomerular diameter in patients with ORG was 226±24.6 μm compared to 168±12 μm in controls[4]. In our cohort, 78% of patients had a glomerular volume>3.27x10^6 μm^3 with a mean glomerular volume of 4.28x10^6±2.17 x10^6 μm^3 and a mean diameter of 195.2±37 μm that falls in between those with ORG and the control group. The latter suggests that the development of ORG may be a gradual process starting with progressive glomerulomegaly before microalbuminuria and proteinuria ensue.

In a similar study of patients undergoing bariatric surgery by Serra et al, the mean glomerular area was 27,425±7,473 μm^2 compared to 19,086±4,727 μm^2 of normal weight controls[8]. A larger mean glomerular area (30,943±10,983 μm^2) was observed in our study but this could be explained by the higher mean BMI of our patients (59.3 kg/m^2 vs. 52 kg/m^2). A significant correlation between body weight and glomerular size was also observed. The correlation with body weight rather than BMI suggests that the weight *per se* is the most significant factor.

Intrinsic changes to the GBM have been demonstrated in our and previous studies. A significant positive correlation was observed between GBM thickness and HbA1C. It is well known that strict control of blood glucose by intensive insulin treatment delays the onset and slows the progression of diabetic nephropathy[9,10]. Furthermore, GBM thickness correlated with serum cholesterol and triglycerides. Serum lipids that are a manifestation of the metabolic syndrome may have a direct pathogenic effect on the GBM.

Perhaps the most consistently reported changes are those of podocytes. Studies have shown increased foot process width foot process fusion, decreased podocyte number and podocyte hypertrophy[7,8]. In our study, we demonstrated an earlier stage of GBM injury where there is a reduction in the slit diaphragm frequency as a result of foot process effacement. This is accompanied by an increase in the proportion of pores with electron dense slit diaphragm as opposed to a normal filamentous appearance. The absence of proteinuria at this stage may be explained by the fact that those changes are very early and precede the more prominent changes such as podocyte swelling and fusion.

In conclusion, histological changes resembling those of early diabetic nephropathy are observed in patients with morbidly obesity. Further research is required in order to identify the potential reversal of histological changes after surgical treatment of clinically severe obesity and substantial weight loss.

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Round table

RISK FACTORS FOR DIABETIC NEPHROPATHY

Presidents: **A. Efthimiadis, A. Xatzitolios**

ARTERIAL HYPERTENSION IN DIABETES MELLITUS AND DIABETIC NEPHROPATHY

Speakers: **C. Savopoulos, G. Ntaios, A. Hatzitolios**

The incidence of arterial hypertension is 1.5-3 times higher in diabetics compared to non-diabetics. Moreover, the co-existence of hypertension and diabetes mellitus (DM) increases cardiovascular risk and mortality (up to 86%) in diabetics. The manifestation and pathophysiology of hypertension are different between DM-I and DM-II patients. In particular, hypertension complicates approximately 30% of DM-I patients usually many years after the diagnosis of DM and it signifies the presence of diabetic nephropathy (DN). On the contrary, 20-60% of DM-II patients already suffer from hypertension at the time of diagnosis. Hypertension occurs in 71% of non-albuminuric patients, in 90% of patients with microalbuminuria and in 93% in macroalbuminuria. The prevalence of hypertension in DM-II is 1.5 times higher compared to non diabetics of similar age and body mass index (BMI).

Insulin resistance is the main pathophysiologic mechanism of hypertension in diabetics via several pathways like hyperglycemia, endothelial dysfunction, dyslipidemia, visceral obesity, microalbuminuria, inflammation and hyperplasia of smooth muscle and mesangial cells.

Several studies in diabetic patients, like UKPDS, HŒT, Steno-2, PROCAM, ALLHAT, Syst-Eur and SHEP showed that hypertension is a major cardiovascular risk factor and that its optimal management results in significant reduction of cardiovascular mortality. The first step in the management of hypertension is to exclude rare causes of secondary hypertension, to recognize the degree of involvement of target organ damage, and identify other modifiable risk factors like dyslipidemia and obesity.

Algorithm of antihypertensive management in diabetics

• Treat when blood pressure (BP) exceeds 130/80mmHg in two instances with at least one week time difference. When BP: 130-139/80-89 without microalbuminuria, lifestyle changes and reevaluation after three months. In the presence of microalbuminuria, start immediately medical treatment together with lifestyle changes.

• Low dose of diuretics in patients without microalbuminuria.

• If systolic BP >20 mmHg and/or diastolic BP >10 mmHg, treat with combination of drugs.

• In patients with microalbuminuria or albuminuria, treat with drugs that act on the Renin-Angiotensin axis.

• In patients with coronary heart disease, treat with b-blockers.

• If target BP is not achieved, add a second drug (diuretic, if it was not the first choice) or increase dose.

• If target BP is not achieved with two drugs, add a third one.

Lifestyle changes

Diabetics should comply more strictly due to their metabolic profile and increased cardiovascular risk. In particular, they should reduce salt and alcohol consumption, body weight, smoking cessation, increase physical activity and calcium, magnesium and potassium intake.

Medical treatment

Antihypertensive drugs should not influence metabolism, not alter the perception of hypoglycae-

mia, not induce postural hypotension, not worsen peripheral vascular disease, not deteriorate (and if possible, protect) renal function, not worsen (and if possible, improve) cardiovascular prognosis and not compromise sexual potency.

Since there is no ideal drug to fulfil all these criteria, one should focus on whether the existing drug categories differ concerning their organ-protective action in diabetic patients. Based on large trials like PROGRESS, BENEDICT, IRMA2, RENAAL, IDNT and meta-analyses, the American Diabetes Association (ADA) guidelines for the management of hypertension in diabetics recommend the ACE or AT-1 inhibitors as the first choice, diuretics as the first drug to be added and then beta blockers (in patients with coronary heart disease) or calcium blockers. Similarly, JNC-VII recommends ACE inhibitors or AT-1 as the first choice and then addition of another if necessary. In more details, ADA recommends ACE inhibitors in DM-I patients with microalbuminuria (Collaborative Study Group, reduction of end stage renal disease and mortality). Similarly, ACE inhibitors are the drug of choice in patients with microalbuminuria with or without hypertension. In DM-II, ACE or AT-1 inhibitors are the first choice in patients with hypertension and microalbuminuria, whereas AT-1 inhibitors in patients with hypertension, macroalbuminuria and renal insufficiency.

Recently, the AVOID study showed that Aliskiren (the first renin antagonist) had a positive effect on the degree of proteinuria in diabetic patients. The effect of Aliskiren in cardiovascular and renal compications in diabetics is currently being studied in the Altitude study, which is incorporated in the ASPIRE HIGHER clinical trials program.

Combination of antihypertensives

The reduction of systolic and diastolic BP is 12-15 and 6-8mmHg respectively when one drug is administered. Therefore, taken into account that the BP target is lower in diabetics, combinations of two or more drugs of different categories are frequently necessary, as it was shown in several trials. In particular, the HOT study showed that three or more drugs were necessary to achieve the diastolic BP target (<80 mmHg). Increased age, BMI and microalbuminuria are associated with difficulty in satisfactory BP control.

The combination of drugs has several advantages since it reduces BP more significantly, increases the proportion of 24hrs control, reduces the dose of each component and their adverse effects. Moreover, the steady combinations lead to improved compliance.

Recently, the COACH study showed that the combination of olmesartan/amlodipine is useful for the management of hypertension and at the same time, it reduces the levels of several inflammatory markers. The ACCOMPLISH study showed the amlodipine/benazepril combination is superior to diuretic/benazepril in hypertensive diabetics, leading to larger reduction of BP and cardiovascular complications.

In conclusion, the combination of two or more drugs is necessary to achieve the target BP in patients with increased cardiovascular risk like diabetics. In these patients and when BP is >20/10 mmHg than the target level, treatment should start with two drugs in a steady combination to increase compliance. The combination of calcium blockers and ACE or AT-1 inhibitors is has many advantages since it increases the synergistic antihypertensive effect and target organ protection, reduces the cardiovascular risk and the adverse effects.

Suggested reading

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DYSLIPIDEMIA AND ATHEROSCLEROSIS IN DIABETES MELLITUS AND DIABETIC NEPHROPATHY

Speaker: **V.G. Athyros**

Dyslipidemia of diabetes mellitus (DM) is characterised by a) Increased flow of free fatty acids (FFA) to the liver, b) Increased level triglycerides (TGs), c) Increased concentration of small dense molecules of low density of lipoprotein (sdLDL), d) Decreased concentration of HDL cholesterol. (Fig. 1)

The increased levels of FFA, which are observed in this lipid aberration, are probably related to the weakness of incorporation or the decreased withholding of FFA in the adipose tissue. The increased flow of FFA from the peripheral tissues to the liver within the insulin resistance syndrome leads to hepatic composition of TGs, which in turn causes high concentration and "excretion" to the blood of very low density of lipoprotein (VLDL) particles that contain mainly TGs. Moreover, the increased availability of FFA in the liver, which is observed in diabetic dyslipidemia, causes toxicity, which inhibits the glucose related insulin excretion and deteriorates insulin resistance, leading to a vicious cycle.

It is highly possible that the elevation in TG levels in DM has multiple causes and is not simply caused by the increased flow of FFA to the liver. The VLDL molecules contain mainly TGs. These are hydrolysed in the plasma by lipoprotein lipase (LL) and VLDL is gradually transformed to IDL and finally LDL particles, containing more cholesterol esters than TGs. In cases with high TG levels, like diabetic dyslipidemia, the TG content of LDL molecules is higher than that observed in normal lipoprotein metabolism. However, LDL TGs are finally further metabolised by LL or hepatic lipase (HL) and form sdLDL particles. sdLDL particles are related to increased cardiovascular disease

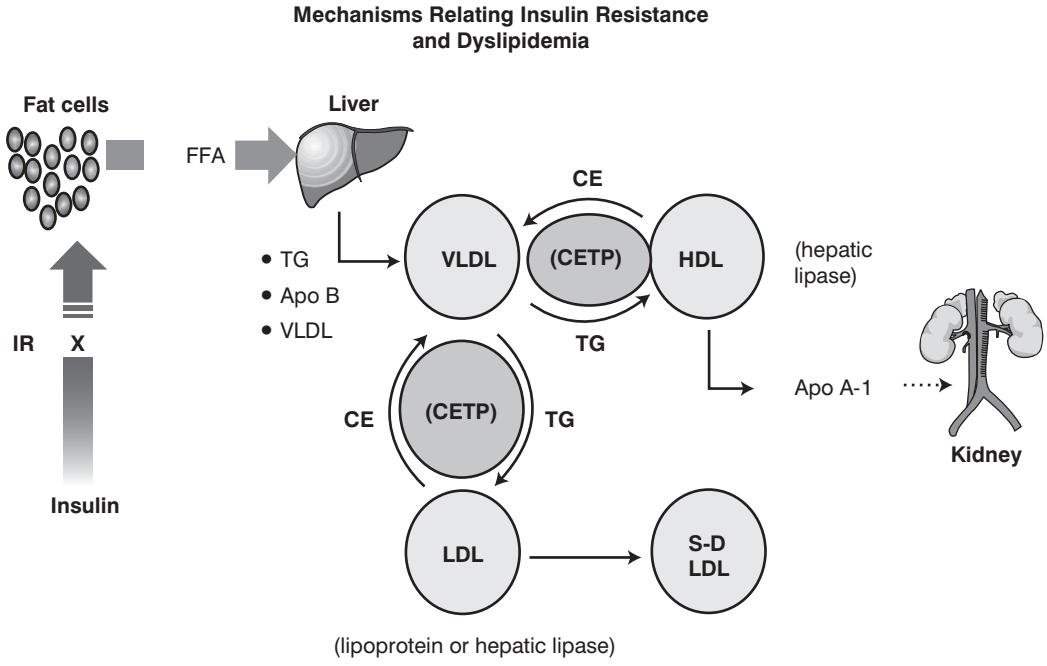

Fig. 1

(CVD) risk and a number of studies have shown that they have pro-atherogenic properties such as: a) Decreased removal rate of LDL particles from the circulation via the LDL receptor, b) Increased engagement within the arterial wall, g) Increased tendency to oxidation.

Low HDL cholesterol concentration in patients with DM is often considered consequence of elevated TG levels. The enzyme cholesterol ester transport protein (CETP) is implicated in an exchange of cholesterol ester with TGs between VLDL and HDL particles. Thus, VLDL become richer in cholesterol ester, while they should not be so, and HDL particles become richer in TGs and depleted of cholesterol esters, while they should not be so. This process is performed at a very high level in subjects with hypertriglyceridaemia and negatively affects HDL cholesterol concentration and reverse cholesterol transport. Another possible explanation for low levels of HDL cholesterol is that the altered flow of lipids in the liver, related to insulin resistance, may decrease the hepatic production apolipoprotein A, main constitutive protein of HDL particles, and a rate limiting factor for HDL cholesterol.

Subjects with DM, even if they have normal levels of fasting TGs, exhibit an abnormal response to a fatty meal (postprandial lipaemia). In insulin resistance conditions, the antilipolitic action of insulin (activates LL) is inadequate. Also, insulin resistance decreases the expression of LDL receptors and increases the composition of cholesterol and "excretion" of VLDL from the liver to the blood stream. This induces a competition between chylomicron remnants and VLDL remnants for hepatic receptors. This leads to a reduced (postprandial) chylomicron clearance and thus postprandial hypertriglyceridaemia, which is an independent CVD risk factor.

All the above increase CVD risk and subjects with DM exhibit a 2 to 4-fold increase in CVD risk, in comparison to those without DM. This is the reason that DM is characterized as coronary heart disease equivalent (high risk situation).

There is a further deterioration of lipoprotein metabolism in patients with DM and impaired renal function (diabetic nephropathy). These patients with reduced GFR have even lower HDL cholesterol, more elevated TGs, more VLDL particle remnants, higher Lp (a), and higher levels oxidised LDL particles.

Treatment dyslipidemia with statins decreases CVD mortality in the general population almost by one third. Statins have similar or even greater effectiveness in normalizing lipid profile in subjects with diabetic dyslipidemia with or without renal function impairment. Statins are well tolerated by subjects with diabetic dyslipidemia at average doses (>20 mg/day atorvastatin or simvastatin). In regard to reduction of CVD morbidity and mortality the results of statin treatment are very impressive and superior to that in primary and secondary CVD prevention patients without diabetes. The studies CARDS, GREACE and TNT, that used 10, 24 and 80 mg/day of atorvastatin, respectively, showed exceptionally high clinical benefit compared to placebo, usual care or small dose atorvastatin, respectively. Besides, two of these studies (GREACE and TNT) showed in subjects with mildly impaired renal function a substantial improvement in GFR, instead of the expected deterioration over time. On the contrary, studies as the "4 D" with atorvastatin and the AURORA with rosuvastatin that included diabetic patients with end-stage renal function (GFR <15 mL/minute) could not induce significant decrease in CVD or total morbidity and mortality.

Consequently, if we wish our diabetic patients to gain from the use of statins these should be prescribed early, time at which renal function is within normal range or has just started to decline. Thus, there will be multiple clinical benefits.

INSULIN RESISTANCE AND DIABETIC NEPHROPATHY

Speaker: **P.A. Sarafidis**

From the early decades of the 20th century several clinicians have described the coexistence in some individuals of certain disorders, i.e. type 2 diabetes mellitus, elevated blood pressure, obesity and dyslipidemia, using several terms to name this "syndrome".[1] However, the modern era of what we now call the "metabolic" or the "insulin resistance" syndrome rather started in 1988 when G.M. Reaven described "syndrome X", proposing that insulin resistance (IR) is the common etiological factor for a group of disturbances, including impaired glucose tolerance, hyperinsulinemia, elevated triglycerides, low HDL-cholesterol and hypertension.[2] In the years that followed an enormous amount of studies investigated the possible epidemiologic and pathophysiologic associations of IR and its pathogenetic role for several diseases;[1] among these studies, several examined the role of IR in the development of renal injury, especially in patients with diabetes.

The first epidemiologic reports on the associations between IR and kidney damage, published in the mid '90s, focused on microalbuminuria, which was then considered as the first sign of diabetic nephropathy.[3] Most of these early case-control studies showed that IR was significantly higher in microalbuminuric than in normoalbuminuric subjects with diabetes or other components of the metabolic syndrome. Similar trends were observed in cross-sectional studies, in the majority of which IR or elevated fasting insulin levels were independently associated with the presence of macroalbuminuria.[3] These observations were followed by studies on the epidemiologic associations of IR and hyperinsulinemia with chronic kidney disease (CKD). Case-control studies, suggested that IR was higher in patients with CKD, than patients with components of the metabolic syndrome without CKD or controls.[4,5] The relation of IR with CKD was clearly shown by a large cross-sectional study in a subgroup of the NHANHES III population, including 6453 adults.[6] In this population, after adjustment for multiple confounders, the odds ratios of prevalent CKD (defined as estimated glomerular filtration rate <60 ml/min/1.73 m^2) were significantly and progressively increasing from the lowest to the highest quartiles of fasting insulin and HOMA-IR levels, whereas absolutely no change in the prevalence of CKD was noted with increase of fasting glucose within the pre-diabetic levels. Importantly, the association between IR and CKD was also documented in a prospective study, including 10,096 subjects with normal renal function at baseline from the Atherosclerosis Risk in Communities (ARIC) study.[7] After a follow-up of 9 years, the odds ratios of CKD were progressively and significantly increasing with the level of IR measured with the HOMA-IR index, even after adjustment for several confounders (age, gender, race, education, BMI, ethanol and tobacco use, coronary heart disease, and physical activity).

These epidemiologic associations of IR and CKD are strongly supported by numerous background studies providing several different pathways through which IR and compensatory hyperinsulinemia can evoke renal injury.[8] Insulin is an important trophic factor, with growth effects known for more than 30 years; current knowledge suggests that insulin exerts important proliferative effects also on renal glomerular and mesangial cells.[8] These effects are both indirect and direct, as insulin strongly stimulates the secretion of insulin-like growth factor 1 (IGF-1), but it can also act through the IGF-1 receptor.[9] Previous in vitro studies provided evidence for this concept with regards to renal cells, showing that physiological concentrations of IGF-1 and pharmacological concentrations of insulin induce growth and inhibit apoptosis of mesangial cells and increase extracellular matrix protein synthesis.[10] Insulin was also shown to stimulate the production of several other growth factors from renal mesangial and tubular cells, including transforming growth factor-β (TGF-β),[8] which is currently believed to play a key role in the fibrogenic processes of diabetic nephropathy. In addition, insulin interferes in several

sites with the renin-angiotensin system (RAS), potentially increasing its deleterious renal effects; among other actions, insulin was shown to stimulate the production of angiotensinogen and angiotensin I from various cell types, and increase the expression of angiotensin II AT1 receptors in the kidney.[8] Insulin physiologically stimulates the production of both endothelin-1 (ET-1) and nitric oxide (NO) from endothelial cells; however, in insulin-resistant states the effect of insulin on endothelial NO secretion is severely impaired, whereas the stimulating effect on ET-1 production is sustained.[11] This imbalance between the production of ET-1 and NO in subjects with IR could also contribute towards renal damage, since activation of ET-1 system in the renal level, as well as alterations in NO bioavailability are both suggested to play roles in the progression of diabetic nephropathy.[12]

Apart from the above, current knowledge suggests that IR and hyperinsulinemia may be connected with oxidative stress in the form of a vicious circle, i.e. oxidative stress impairs insulin action through interference with normal intracellular insulin signalling and chronic hyperinsulinemia enhances oxidative stress, through alterations in enzymes responsible for the production and degradation of reactive oxidant species. Oxidant stress was also proposed to promote renal injury through various mechanisms, i.e. decrease in NO bioavailability and increase in advanced glycation end products and lipid peroxidation products.[13] Finally, another pathway that could connect IR with renal damage is the elevation of plasminogen activator inhibitor type 1 (PAI-1) activity, which is considered as a secondary component of the metabolic syndrome.[1] Increased PAI-1 levels exert fibrogenic actions at the kidney level as they inhibit the plasmin-mediated matrix metalloproteinase activation, and up-regulate TGF-β expression.[14]

In the presence of the aforementioned data, during the last 15 years several animal and human studies have investigated whether the pharmacological reduction of IR with the use of hypoglycemic compounds of the thiazolidinedione drug class could decrease the rate of renal injury progression. In animal models of IR or diabetes all members of this class (i.e. troglitazone, rosiglitazone, and pioglitazone), were shown to reduce urine albumin excretion and to produce important functional and morphologic changes, such as reduction of glomerular hyperfiltration, prevention of intrarenal arteriolosclerosis, and prevention of glomerulosclerosis and tubulointerstitial fibrosis.[15] These beneficial effects were confirmed by several clinical studies in patients with diabetes and normo-, micro- or even macroalbuminuria, in which thiazolidinediones were shown to significantly reduce the levels of urine albumin or protein excretion.[15] The above important findings call for clinical trials in patients with diabetes including hard renal outcomes as primary endpoints to fully elucidate the clinical value of thiazolidinediones as an additional therapeutic option for diabetic nephropathy.

Overall, several observational studies have shown clear associations of IR/hyperinsulinemia with microalbuminuria and CKD. Background studies strongly support these associations, providing several mechanisms through which IR/hyperinsulinemia can harm the normal kidney. Further, accumulating animal and human data suggest that pharmacological reduction of IR could slow down the progression of diabetic nephropathy. These findings suggest that IR is an important and potentially modifiable risk factor for the development and progression of nephropathy in patients with diabetes. Future studies should further delineate the mechanisms through which IR induces renal injury, and the type of the interventions that best ameliorate this important risk factor.

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CARDIOVASCULAR RISKS IN DIABETIC NEPHROPATHY

Speaker: **S. Liakos**

Introduction

Diabetic nephropathy, a major complication of diabetes, is characterized by progressive renal injury and increased cardiovascular mortality. Diabetes mellitus (diabetes) affects approximately 100 million people worldwide[1]. Cardiovascular disease is a major chronic complication of both type 1 and type 2 diabetes and people with diabetes have an approximately 2–4-fold increased risk of cardiovascular events, as compared with control populations[2,3]. In addition to cardiovascular disease, people with diabetes are also at high risk of developing microvascular complications, the most clinically important being end-stage kidney disease or diabetic nephropathy. Importantly, the combination of diabetes and nephropathy increases cardiovascular disease risk by 20–40-fold[4,5]. Diabetic nephropathy can be classified on its severity. Diabetics initially have no kidney disease at all. The earliest detectable level of kidney disease is microalbuminuria, where there is a minute amount of protein that is excreted in the urine (not detectable by urine dipstick). The next stage is proteinuria, which is defined as easily measurable levels of protein in the urine, but without disturbance of measures that generally mark renal failure (creatinine and urea). Lastly, the disease process may develop into renal failure, otherwise known as uremia[6,7].

Albuminuria as a marker for cardiovascular disease

In the normal population, cardiovascular risk increases in a continuous fashion along with progression from normal to overt proteinuria levels[7-10]. Shown in a prospective 5-year survey of more than 20 000 subjects in the United Kingdom, microalbuminuria and proteinuria were independently associated with risk of cardiovascular disease and death[10]. This relationship is also true for people with diabetes, with post-hoc analyses of three recent large clinical trials showing that albuminuria not only determines renal outcomes, but also cardiovascular outcomes[11-13]. In one of these three studies, the reduction of albuminuria with therapeutic interventions resulted in protection against cardiovascular disease as well as the development of progressive renal impairment[12].

Albuminuria leading to vascular inflammation

There are many physiological abnormalities that are attendant in end-stage kidney disease that has led to the identification of mechanisms that may link cardiovascular disease and renal failure[14]. High on the list of possible mechanisms are factors such as: hypertension, dyslipidemia[15], activation of the renin-angiotensin system[16]; medial calcification of the vascular tree, malnutrition and inflammation[17,18].

However, while these factors are present in end-stage nephropathy, not all of them are universally present in the early stages of albuminuria. Inflammation is associated with the microalbuminuric state, with albuminuria now recognized to reflect generalized vascular damage[8]. Importantly, inflammation underlies all stages of atherosclerotic lesion formation, including early atherogenesis where inflammatory cells adhere and infiltrate the subendothelium[19]. Critical proteins expressed by endothelial cells that bind the inflammatory cells are the cell adhesion molecules.

Cell adhesion molecules, inflammation, and atherosclerosis

Two important cell adhesion molecules expressed by endothelial cells that play a major role in the pathogenesis of atherosclerosis are vascular cell adhesion molecule-1 (VCAM-1) and intercellu-

lar cell adhesion molecule-1 (ICAM-1). Expression of both ICAM-1 and VCAM-1 have been demonstrated in atherosclerotic plaques[20-23] with focal expression evident at lesion prone areas and at the borders of atherosclerotic lesions[24,25]. There have been many in vitro studies showing high glucose milieus increase both ICAM-1 and VCAM-1 endothelial cell expression at both the protein[26] and messenger ribonucleic acid (mRNA) levels, via a cell signaling mechanism related to activation of protein kinase C.

Soluble cell adhesion molecules and diabetic cardiovascular disease

Soluble ICAM-1 and/or sVCAM-1 show good correlation with cardiovascular disease in diabetic subjects. In a recent study, type 2 diabetics showed high sICAM-1 levels, independent of known cardiovascular risks, and predicted all cause as well as cardiovascular mortality over 10 years[27]. Increased levels of sVCAM-1 were also associated with increased risk of mortality in type 2 diabetes, as seen in the Hoorn study[28]. In the Hoorn study, 631 type 2 diabetic and control subjects with higher sVCAM-1 levels at the beginning of the study period had increased risk of cardiovascular death during 8 years of follow-up even after adjustment for age, sex, glucose tolerance, hypertension, cardiovascular disease, high density lipoproteins, low density lipoproteins, homocysteine, microalbuminuria, von Willebrand factor, C-reactive protein, and glomerular filtration rate. The effect was magnified in type 2 diabetics, thus indicating that sVCAM-1 is independently associated with the risk of cardiovascular mortality.

Traditional cardiovascular disease risk factors Hypertension

Abnormal lipid profile
Central obesity
Smoking
Left ventricular hypertrophy/dysfunction
Coronary ischemia

Nontraditional cardiovascular disease risk factors

Elevated c-Reactive protein
Elevated von Willebrand factor
Elevated plasminogen activator inhibitor-1
Elevated thromomodulin
Elevated homocysteine
Elevated interleukin-6
Absent nocturnal drop in blood pressure
Insulin resistance
Elevated white blood cell count
Prolonged Q-T interval
Lipoprotein (a).

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Lecture

RENAL, PANCREAS AND ISLET TRANSPLANTATION FOR THE DIABETIC PATIENT WITH END-STAGE RENAL DISEASE

President: **G. Vergoulas**

Speaker: **C. Fourtounas**

Diabetes Mellitus is a worldwide epidemic and the leading cause of kidney failure, blindness, stroke, heart attack and amputations. Several years ago, diabetes mellitus was considered as a relative or absolute contraindication for renal transplantation, due to increased rates of morbidity and mortality mainly due to cardiovascular and infectious complications. However, recent data show a substantial survival benefit of renal transplantation for patients with diabetes and advanced Chronic Kidney Disease (CKD).

Early referral of diabetic patients with CKD for evaluation by a transplant centre for pre-emptive transplantation has been considered as the optimal procedure, with special emphasis on the detection of coronary and peripheral vascular disease, even in the absence of clinical symptoms.

For patients with type 1 diabetes, Living Related (LR) renal transplantation and Simultaneous Pancreas/Kidney (SPK) transplantation offer superior and approximately equivalent long-term patient and renal allograft survival and reduce mortality by almost two thirds compared with remaining on dialysis therapy (hemodialysis or peritoneal dialysis).

For patients with type 2 diabetes not only LR, but even deceased donor (DD) renal transplantation are superior to dialysis. SPK transplantation can be offered only in a specific group of type 2 diabetics, with special characteristics similar to type 1 diabetics.

As glycemic control may become difficult after renal transplantation due to immunosuppression (steroids), successful pancreas transplantation protects the patient from both hyper- and hypo-glycemia and normalizes glycosylated hemoglobin values without the need for exogenous insulin administration. In addition the patient may avoid the long-term catastrophic complications of diabetes on the allograft (allograft diabetic nephropathy). Although SPK has been considered as the optimal procedure, there have been reports from experienced transplant centers with excellent results with Pancreas after Kidney (PAK) Transplantation. However, there is always the need for two separate operations with the PAK procedure and with all the possible consequences.

The initial enthusiasm with islet transplantation as a therapy for type 1 diabetes mellitus has been decreasing, as there is a need for islets administration from multiple donors and the long term insulin free interval results are rather disappointing. In addition, there is a substantial risk for sensitization after islet transplantation, despite the use of aggressive immunosuppresion. Islet transplantation should be still considered only as an experimental procedure for type 1 diabetes and has no place in diabetic patients with advanced CKD.

The main focus of the transplant community is to improve the outcomes (both patient and allograft survival) for renal transplant recipients with diabetes. Regarding immmunosuppression, there is a trend for steroid free protocols in both renal and SPK transplantation with equivalent, if not better results, compared with the classic triple protocols with steroids. Glycemic control remains also important after renal transplantation, with the same targets as in the general diabetic population for fasting and postprandial glucose levels and glycosylated hemoglobin values. Hypertension and proteinuria after transplantation should be treated aggressively with angiotensin converting enzyme inhibitors or angiotensin receptor blockers, but serum potassium, serum creatinine and hemoglobin values should be closely monitored. Finally, there is a need for special emphasis on aggressive lipid control with statins, avoidance of excessive weight gain after transplantation, smoking cessation, regular exercise

and daily aspirin administration, which have been shown to reduce the overall cardiovascular risk.

Although the therapeutic options for the diabetic patient with advanced CKD have been improving during the recent years, there are many unanswered questions that should be addressed in the near future. The most appropriate therapy for these patients (LR, DD, SPK, or PAK transplantation) should be individualized, taking under consideration the patient's needs and overall medical condition and the transplant center's experience with all these procedures.

Round table

LATE ONSET COMPLICATIONS OF DIABETIC NEPHROPATHY

Presidents: **A. Efstratiadis, J. Stefanidis**

SECONDARY HYPERPARATHYROIDISM IN DIABETIC NEPHROPATHY

Speaker: **M. Nikodimopoulou**

Diabetic nephropathy, a "microvascular" chronic complication of diabetes that affects between 20 and 40% of diabetes patients, is today the commonest cause of chronic kidney disease (CKD) and end stage renal disease (ESRD) in the Western World[1]. A major complication of diabetic nephropathy that is accompanied with a high cardiovascular morbidity and mortality is secondary hyperparathyroidism (SHPT). SHPT is a very early finding in CKD that progresses as glomerular filtration rate (GFR) decreases[2].

Studies have shown that early loss of 1-hydroxylation of 25-hydroxyvitamin D in the kidney is a key event in causing SHPT that precedes phosphate retention, hypocalcemia and a compensatory i-PTH secretion. PTH achieves calcium and phosphate homeostasis by the following mechanisms: controlling the release of calcium into the blood from bone through osteoclastic stimulation of bone resorption causing renal osteodystrophy; by stimulating the kidneys to reabsorb calcium and convert 25-hydroxyvitamin D produced in the liver to the active form, which in turn stimulates gastrointestinal absorption of calcium; and by reducing renal tubular reabsorption of phosphorus[3-5].

The compensatory rise in PTH levels during the progression of CKD is primarily due to the decrease in vitamin D levels and not to substantial changes in serum calcium or phosphate[6]. This biochemical SHPT is accompanied by parathyroid hyperplasia. Without therapy, the parathyroid glands can develop areas of micronodular change that can progress to macronodular adenomas. Parathyroid nodularity is irreversible and is associated with decreased levels of vitamin D receptors (VDRs) and calcium-sensing receptors with the development of autonomous function. Parathyroid nodular hyperplasia is often refractory to medical therapy in patients with severe SHPT and may require parathyroidectomy[7-8].

As a result of parathyroid-induced changes, excess bone resorption leads to loss of bone mineral, increased release of calcium and phosphorus from bone, increased risk for vascular and visceral calcification, and increased risk for cardiovascular complications and mortality in patient with CKD receiving dialysis[9]. Thus, interventions to treat and/or or prevent SHPT should be initiated early in patients with CKD because SHPT contributes notably to morbidity and may contribute to the high mortality of patients with progressive renal failure[10].

In the setting of CKD, effective treatment of SHPT includes the administration of active vitamin D to restore 1,25-dihydroxyvitamin D levels, to improve VDR activation and to normalize the PTH-vitamin D endocrine feedback loop that is critical for maintenance of mineral homeostasis. Active vitamin D therapies suppress PTH levels by 2 mechanisms: preventing parathyroid gland enlargement and decreasing PTH synthesis[11]. Activation of VDRs also decreases PTH secretion indirectly by increasing serum calcium to activate the calcium-sensing receptor and by directly stimulating the expression of the calcium-sensing receptor within the parathyroid glands. A major goal for the treatment of elevated PTH is to normalize serum PTH without causing hypercalcemia or hyperphospatemia[12].

Structural and functional categories of vitamin D therapies relate to their ability to directly activate VDRs. Active vitamin D therapies include calcitriol and paricalcitol. Inactive vitamin D therapies require activation by the kidney or liver before they can bin with high affinity to VDRs. An important representative is alfacalcidol[13].

Calcimimetics are a distinct therapeutic category for SHPT. Cinacalcet is a calcimimetic agent that binds the calcium-sensing receptor of the parathyroid gland, resulting in diminished secretion of PTH[14].

Hyperphosphtemia progresses as kidney function gradual decline and is associated with increased cardiovascular risk of mortality in predialysis CKD. All phosphate binders effectively reduce serum phosphorus levels when taken with a meal, although there are differences in tolerability and dosing convenience. Pharmakologic agents used to treat hyperphosphatemia may be classified as calcium-based (e.g. calcium carbonate) and non-calcium-based (e.g. sevelamer hydrochloride, lanthanum carbonate)[15].

In conclusion, SHPT is an early and major complication of CKD. Early detection of elevated PTH, usually in the absence of elevated serum phosphorus and reduced calcium, and appropriate therapeutic intervention for active vitamin D deficiency is essential. The initial treatment involves use of active vitamin D in combination with phosphate binders.

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PATHOGENESIS OF ANEMIA IN DIABETIC NEPHROPATHY

Speaker: **M. Papaioannou**

Diabetic nephropathy is a long term, microvascular complication of diabetes mellitus (DM) that is characterized by progressive angiopathy of capillaries within the renal glomeruli. With increased life expectancy of patients with DM, diabetic nephropathy has become the most common cause of end stage renal disease and renal anemia in developed countries. Anemia also occurs in patients with non-diabetic , chronic kidney disease (CKD), but it develops later in the course of the disease and is usually less severe than in patients with diabetic nephropathy who have similar degrees of renal impairment. The etiology and pathogenesis of anemia in diabetic nephropathy is multi-factorial. *Chronic inflammation*, *diabetic neuropathy, nephrotic syndrome*, *elevated levels of advanced glycation end products (AGEp)*, *medications* and *low testosterone levels* are the main pathogenetic factors related to DM and/or CKD, which result to *abnormal iron homeostasis, Epo deficienc and hypo-responsiveness to Epo,* all contributing to the development of anemia.

Recent analyses of the National Health and Nutrition Examination Survey IV suggest that up to 50% of patients with CKD stages 2-5, have *absolute or relative (functional) iron deficiency.* Functional iron deficiency is more common and is strongly associated with up-regulation of inflammatory cytokines and impaired tissue responsiveness to erythropoietin which can inhibit iron transport from tissue stores to erythroblasts. Increased levels of inflammatory cytokines such as interleukin -6 enhance production and secretion of hepcidin, a hepatic protein that inhibits intestinal iron absorption and impairs iron transport from the reticulo-endothelial system to bone marrow. In addition, Epo, which normally enhances iron transport from macrophages to the blood stream, is impaired, thereby exacerbating relative iron deficiency.

Both, *Epo deficiency and hypo-responsiveness to Epo* contribute to anemia in diabetic patients with CKD. The cause of *erythropoietin deficiency* in these patients is thought to be reduced renal mass with consequent depletion of the hormone. However, in diabetic patients a normochromic, normocytic anemia due to reduced Epo release can occur before evidence of renal impairment is present. Factors that have been suggested to play a role for the earlier onset of anemia in patients with diabetes include : *severe symptomatic neuropathy*, causing efferent sympathetic denervation of the kidney with a defect of "anemia-sensing" mechanisms and loss of appropriate Epo production, *damage to the renal interstitium* and *inhibition of Epo release*. *Hypo-responsiveness to Epo* is defined clinically as a requirement for high doses of erythropoietin in order to raise blood Hb level, in the absence of iron deficiency. It is believed to represent impaired anti-apoptotic action of Epo on pro-erythroblasts. Possible causes of erythropoietin hypo-responsiveness, in the absence of iron deficiency, include systemic inflammation and microvascular damage in the bone marrow. Modulation of erythropoietin receptors as a result of glycation may also render erythropoietin ineffective

Nephrotic syndrome is not uncommon in patients with diabetic nephropathy and can occur even in early stages of CKD. The presence of nephrotic syndrome contributes to the development of anemia in diabetic nephropathy by inflammatory – mediated mechanisms as discussed above and absolute iron deficiency. Iron excretion increases in early stages of kidney disease, in patients with diabetes and albuminuria and is exacerbated by development of nephrotic – range proteinuria. In nephrotic syndrome, many non-albumin proteins are excreted in the urine, including transferrin and erythropoietin, leading to both iron – and erythropoietin – deficiency –caused anemia in patients with diabetes. Evidence for increased transferrin catabolism in nephrotic syndrome may contribute to iron deficiency-caused anemia.

Advanced glycation end products (AGEp) are a diverse group of end –product molecules that are formed as a result of non-enzymatic, covalent binding of glucose residues to the free amino groups of proteins, lipids and nucleic acids. Increased accumulation of these products can promote non-enzymatic glycation of red blood cell membrane glycoproteins and hemoglobin, which leads to impaired deformability of red cells in diabetes mellitus.

Angiotensin converting enzyme inhibitors and Angiotensin receptor antagonists may cause a reversible decrease in Hb concentration in patients with diabetes and CKD. A direct blockade of the pro-erythropoietic effects of Angiotensin II on red cell precursors, degradation of physiological inhibitors of hematopoiesis, and suppression of IGF-1 are the main mechanisms. *Metformin* , one of the most commonly used oral anti-diabetic agents, has been associated with malabsorption that leads to vitamin B_{12} deficiency.

Promptly diagnosing and treating anemia in patients with diabetes may result in an improved quality of life and decreased morbidity and mortality. Until definitive evidence of optimal hemoglobin levels is available, treatment should aim to achieve levels of 10.5g/dl-12.5g/dl, as recommended by the National Institute for Health and Clinical Excelence.

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Lecture

END STAGE OF DIABETIC NEPHROPATHY: THERAPEUTICAL MANAGEMENT WITH CHRONIC MAINTENANCE HEMODIALYSIS

President: **N. Zirogiannis**

Speaker: **A. Sioulis**

Diabetic nephropathy is the most common leading cause of end-stage renal disease (ESRD) worldwide and therefore diabetes remains the main cause that induces ESRD for ever one third of all patients beginning renal replacement therapy in USA and Europe.

The risk to develop ESRD is higher in type-I than in type-II diabetics patients, but 50-60% approximately of diabetic subjects receiving renal replacement therapy have type-II diabetes, because of the higher prevalence of type-II diabetes.

Diabetic nephropathy is most likely to occur in patients who have worse glycemic control, hypertension, glomerular hyperfiltration or a genetic predisposition. The lifetime risk of nephropathy is roughly equivalent in type 1 and type 2 diabetes.

The earliest clinical manifestation of renal involvement in diabetes is an increase in albumin excretion (microalbuminuria), a stage at which renal histology may be relatively normal or may reveal glomerulosclerosis. However, regression of microalbuminuria to normoalbuminuria occurs spontaneously in a substantial proportion of patients with type 1 and type 2 diabetes.

Diabetic nephropathy is a clinical syndrome charactirized by persistent albuminuria >300 mg/24h, on at least two occasions separated by 3-6 months. Patients invariably develop associated hypertension, a progressive increase in proteinuria and relentless decline in glomerular filtration rate (GFR).

Patients with diabetes do appear to be more vulnerable to uremic symptoms, fluid retention and hyperkalemia at an earlier stage than non diabetic subjects.

For this reason diabetic subjects should initiate dialysis at an earlier stage than patients with other causes of ESRD, when serum creatinine approximately 5.5 mg/dl or GFR 10-20 ml/min, according to the recommendation in the National Kidney Foundation Dialysis Outcome Quality Initiative (DOQI) guidelines.

In 2002, diabetes mellitus (DM) accounted for 44% of all new cases of treated ESRD in the US, while the number of people who began treatment for ESRD-DM increased 162%, from 16.648 in 1990 to 43.638 in 2002.

Early in the course of kidney disease, medications are used to help preserve kidney function and delay the need for dialysis or transplantation. These early treatments are directed at the underlying kidney disease, secondary factors (such as hypertension) that promote kidney disease progression, and the complications of chronic kidney disease.

The aims of these therapies of diabetic nephropathy are particularly the importance of glycemic control, the decrease of proteinuria and the antihypertensive therapy with emphasis on the use of angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. In addition to data from controlled trials, further proof of benefit from these therapies is the observation that the incidence of end-stage renal disease among patients with type 1 diabetes may be decreasing.

As the kidneys lose their ability to function, fluid and waste products begin to build up in the blood. Dialysis should begin before kidney disease has advanced to the point, where life-threatening complications occur. This usually takes many months or years after kidney disease is first discovered, although sometimes severe kidney failure is discovered for the first time in people who were not previously known to have kidney disease.

It is the best to begin dialysis treatment when diabetic patients have advanced kidney disease, but

while they still feel well.

The choice of the suitable method (hemodialysis or peritoneal dialysis) is based on the particular specification for each diabetic patient and the selection between the two types of dialysis will became generally based upon other factors, including their preferences, home supports, and underlying medical problems. Older diabetics subjects (age >55 years old) survive longer on hemodialysis than the peritoneal dialysis.

In general, technique survival and hospitalization rates are better for patients receiving hemodialysis than peritoneal dialysis, while in addition also provides more efficient solute and water removal and can be performed at home, maintaining patient independence.

The most common complication of hemodialysis is low blood pressure and can be accompanied by light-headedness, shortness of breath, abdominal cramps, nausea or vomiting.

Further analysis of data showed that the increased death rate in elderly diabetic patients may have resulted from the presence of cardiovascular or/and peripheral vascular disease.

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Lecture

END-STAGE DIABETIC NEPHROPATHY: TREATMENT MANAGEMENT WITH PERITONEAL DIALYSIS

President: **P. Pasadakis**

Speaker: **E. Balaskas**

Introduction

Since the late 1990s diabetic nephrophathy (DN) has been steadily increasing and diabetes mellitus (DM) remains the leading cause of end-stage renal disease (ESRD) in many countries today. During the last several years, the survival of diabetic patients with ESRD has improved, but the survival rate in this subgroup remains the lowest primarily because of a higher prevalence of cardiovascular comorbidity and complications[1]. Indeed, multiple complications such as hypertension, ischemic heart disease, left ventricular hypertrophy, arrhythmia, orthostatic hypotension, arteriosclerosis obliterans, diabetic retinopathy, hyperglycemia, and dyslipidemia exist even before the pre-dialysis stage. In addition, clinical factors following initiation of dialysis, such as malnutrition, anemia, inadequate dialysis, and hyperphosphatemia, are strongly associated with mortality risk for dialysis patients[1-3]. It was observed an independent graded association between reduced estimated glomerular filtration rate (GFR) and the risk of death and cardiovascular events[4], and also, cardiovascular disease (CVD) accounts for about 50% of deaths in type 2 DM[5]. Therefore, the combination of chronic kidney disease (CKD) and DM synergistically leads to the development of a hight risk for CVD[3].

While transplantation remains the treatment choice of all ESRD patients, both dialysis modalities, hemodialysis (HD) and peritoneal dialysis (PD) are suitable for most diabetic patients. In fact, PD may provide several advantages for diabetic patients, but in contrast to the utilization of HD, utilization of PD is still very low in many countries (<10%), more than 30 years since its introduction as an alternative modality to HD[6].

In this mini-review, we discuss, in-brief, the selection of dialysis modality, the advantages and disadvantages of PD, the treatment targets for diabetic patients on PD, patient and technique survival, comparisons with HD, and interventions that may improve the survival of diabetic PD patients.

Selection of dialysis modality

As compared with HD, PD offers equal or better patient survival across all subgroups of patients needing dialysis, during the first 2 years[7,8]. This observation may be attributable to a lower prevalence of infections and congestive heart failure (CHF) and better preserved residual renal function (RRF) in PD patients[1,9,10]. Mortality studies comparing PD and HD show that DM, age and comorbidity all significantly modify the effect of treatment modality on patient survival[7]. In all populations, CHF is a powerful independent predictor of mortality[10]. The cumulative probability of CHF was 55,9% in the incident HD population at 2 years as compared with 40.8% in the incident PD population[1].

In a review of 9 mortality studies comparing PD and HD, Vonesh et al.[7] summarized the key results:

1. Among younger patients (USA, Canada, Denmark) with and without DM, PD is associated with survival equivalent to or better than that for HD.

2. The relative risk (RR) of death for PD as compared with HD varies with time on therapy: PD has an equal or lower mortality rate during the first 1-2 years, and then, results vary by subgroup.

3. In the USA, HD is associated with better survival among diabetic patients over age 45, while in Canada and Denmark, no such difference is observed.

4. The RR for death varies according to the type of analysis (as-treated vs intent-to-treat).

5. In the USA, there appears to be a temporal trend of improved outcomes associated with PD that exceeds the trend associated with HD.

6. DM, age, and comorbidity all significantly modify the effect of treatment modality on patient survival.

All these above observations make apparent that PD should be the initial modality of dialysis for all ESRD patients. Older patients with DM and patients without DM may switch modality to HD or undergo Kidney transplantation in 1-2 years and younger patients with DM may stay on PD longer[10].

Advantages and disadvantages of PD

Table 1 lists potential benefits of PD in diabetic PD patients[6]. One of the most important of these, with a significant impact on patient outcome, is superior preservation of RRF, which is reported to decline 24%-80% faster in patients on HD than in those on PD[10]. The combination of DM and HD may further augment the risk and, therefore, a "PD first" approach appears to be a rational way to maximize the maintenance of RRF[6]. However, PD is also associated with certain negative factors inherent to the use of this modality in diabetic patients (Table 1).

Table 1. Advantages and disadvantages of PD in the treatment of ESRD patients with DM

Advantages	Disadvantages
• Home-based continuous therapy	*Continuous glucose absorption:*
• Better blood pressure control	• Hyperglycemia
• Fewer epidoses of hypotension	• Obesity
• Fewer episodes of arrhythmia	• Hyperlipidemia
• Better preservation of RRF	• Increased peritoneal permeability (accumulation of glucose degradation products)
• No need for vascular access	*Complications specific to PD*:*
• Lack of pain from needle puncture	• Encapsulating peritoneal sclerosis
• No need for heparinization	• Peritonitis
• Fewer episodes of progressive retinopathy	• Exit-site infection
• Fewer episodes of blood-borne disease	• Other (loss of ultrafiltration, hernias, peripheral vascular disease etc)
• More liberal diet in the early stages	
• Advantages in lifestyle	

*No evidence for greater incidence in diabetic patients

Therapy that is more individualized and linked to the concept of integrated ESRD care allows for the two dialysis modalities and kidney transplantation all to be considered at each phase of active ESRD treatment[6].

Treatment targets

Strategies for managing diabetic patients on PD include proper control of glycemia, ultrafiltration, blood pressure, and metabolic status. In addition, prevention of cardiovascular complications, nutrition optimization, and preservation of RRF are also important[12]. No study specifically addresses glycemic control targets for diabetic PD patients. The most recent recommendations from the American Diabetes Association suggest these targets for diabetic patients[13].

- Fasting plasma glucose: <130 mg/dL
- Postprandial plasma glucose: <180 mg/dL
- HbA1c: <7%
- Blood pressure: < 130/80 mmHg
- Low-density lipoprotein cholesterol (LDL): <100 mg/dL
- Triglycerides: <150 mg/dL

Notably, 60%-80% of glucose PD solution instilled is absorbed (daily intake of 100-300 g glucose), and the use of non-glucose-based dialysis solutions (e.g. icodextrin or amino acids) may be a helpful addition, but the result are still conflicting[12,14]. Increased albumin leakage through the capillary wall is a central pathophysiologic feature of diabetic microangiopathy and leads to increased urinary and peritoneal protein loss in diabetic PD patients and finally to protein-energy malnutrition, peritoneal small – solute transport increase, and ultrafiltration decrease. The use of angiotensin converting – enzyme inhibitors (ACEIs) or angiotensin II receptor-blockers (ARBs) may have potential benefits on peritoneal protein permeability, and peritoneal transport characteristics. For malnourished diabetic PD patients, use of 1.1% amino-acid dialysis solution is beneficial as nutritional support. RRF and blood pressure control both contribute to survival in PD patients, especially in diabetics, and the use of either ACEIs or ARBs has been shown to better preserve RRF[9].

Patient and technique survival

A review of the literature comparing the survival of diabetic ESRD patients on PD and HD showed the great disparity in the results[15]. In some studies, the overall survival rates of diabetic patients on PD and HD were similar, but in other reports, a more favorable outcome was associated with one of the two modalities[1,2,6-8,16-19]. We don't report here numbers, but these mixed outcomes are potentially explained by heterogeneity in patients backgrounds, such as sex, age, onset and duration of DM, year of dialysis start, duration of dialysis, severity of cormobid conditions, and by statistical techniques used to analyze the data.

Several studies have compared survival rates between diabetic and nondiabetic patients[7]. In general, the presence of DM is always associated with worse results, which may be even worse in patients with ESRD[2,6-8,16-23]. The most recent studies show better patient survival rate, both in older or younger diabetic PD patients, especially in those under 55 years of age, with a 5 years survival from 47% to 68%[16,23]. Analysis of the USRDS data showed that the PD: HD death rate ratio varied by age and sex[18]. For male diabetics there was little or no difference in the mortality risk, for diabetics patients under 50 years of age, PD was associated with a significantly lower risk of death, while older female diabetics patients on PD had significantly higher risk of death than did those on PD[18]. In a recent, long-term study, the best survival occurred in nondiabetic patients on PD, diabetics patients on PD had survival rate equal to that of nondiabetic patients on HD and diabetic patients on HD had the worst survival rate[6]. Some reports have shown that PD in diabetics is associated with survival equal to that in HD for the first 2-3 years[2,8,19,22].

Based on the foregoing data, it may be concluded that PD offers equal or better survival in diabetic patients than HD does, especially during the early years on dialysis.

Interventions to improve clinical outcomes

Aside from the choice of PD as an RRF-preserving modality, other factors that may improve the prognosis in diabetic PD patients should be taken into considerations:

• *Control of blood sugar:* use of insulin (intraperitoneal or subcutaneous) or a combination with oral hypoglycemic agents such as peroxisome proliferator-activated receptor gamma (PPARG) agonists, and non glucose –based solutions[3,12].

• *Control blood pressure* (ACEIs or ARBs)[6]

• *Effective fluid removal:* icodextrin (increased ultrafiltration volume and reduction in blood pressure), mainly in high transport diabetic patients[6,24].

• *Prevention of peritoneal fibrosis during long-term PD:* intraperitoneal antioxidant (e.g. N-acetylcysteine) and ARBs may preserve better the functional and structural integrity of the peritoneal membrane with improved ultrafiltration capacity and decreased peritoneal thickening[6,11,25].

• *Lipid-lowering drugs, such as statins:* another promising group of drugs for reducing CVD.

Finally, Table 2 summarizes the potential benefits of icodextin, a new, available the last years,

PD solution. These benefits are clinically important, and they justify common use of the new solutions in diabetic patients[3,6,12,22].

Table 2. Potential benefits of icodextin in the treatment of ESRD patients with DM[6]

1. Increase in ultrafiltration volume (in the presence and absence of peritonitis)
2. Better blood pressure control
3. Increase in solute clearance
4. Better glycemic control
5. Less requirement for insulin
6. Better control of hyperlipidemia
7. Better preservation of RRF
8. It does not affect peritoneal permeability and act in a protective way on the peritoneum

Conclusions

Evidence suggests that PD should be the initial modality of dialysis in all ESRD patients. Older diabetic patients and patients without DM may switch modality to HD or undergo kidney transplantation to 1-3 years after PD start and younger patients with DM may stay on PD longer. To summarize, strict blood sugar control, tight control of blood pressure with ACEIs or ARBs and the use of statins or antioxidants may improve the survival of diabetic PD patients. In addition, PPARG agonists and the new PD solutions may also be effective in reducing CVD risks. PD preserves RRF better than HD does and the use of ACEIs or ARBs may further help. Less frequent use of high glucose PD solutions and use of non-glucose PD solutions, ACEI, ARB and antioxidants may help preserve peritoneal membrane function. As outlined, PD offers cardiovascular, retinal, metabolic, renal, and peritoneal benefits. In addition, the advantages of PD are accentuated with the use of icodextrin.

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Lecture

TRPC6 CHANNEL SIGNALING IN RESPONSE TO ANGIOTENSIN II TYPE 1 RECEPTORS IS ESSENTIAL FOR THE PRESERVATION OF THE PODOCYTE CYTOSKELETON

President: **P. Mundel**

Speaker: **A. Greka**

Canonical Transient Receptor Potential 6 (TRPC6) channel mutations have been linked to proteinuric kidney disease. While gain of function mutations in TRPC6 have pointed to increased calcium influx as a putative mechanism of podocyte injury through disruption of the actin cytoskeleton, little is known about the specific pathways that regulate this process. Additionally, calcium influx into podocytes has been previously described in response to Angiotensin II signaling, but the channels responsible for the observed calcium influx remain elusive. Here we report that application of Angiotensin II (ATII) on Angiotensin Type 1 Receptor (AT1R) expressing podocytes generates a current with properties consistent with TRPC6 channels. Furthermore, AT1R activation results in a slow and transient TRPC-like augmentation in intracellular [Ca^{2+}] that is abrogated by Losartan. Importantly, the ATII-induced intracellular [Ca^{2+}] elevation is markedly reduced after gene silencing of TRPC6, but not after the antagonism of the NMDA Receptor, AMPA Receptor, or L-Type Ca^{2+} channels.

Interestingly, AT1R expressing podocytes loose their neuronal-like structure and instead display a polygonal shape characterized by markedly reduced primary processes, and a massive assembly of F-actin into stress fibers. Concomitantly with TRPC6 channel – mediated calcium influx, AT1R activation results in cortical actin formation. Intriguingly, TRPC6-deficient AT1R podocytes showed pervasive loss of actin stress fibers and focal contacts, severe collapse of the cytoskeleton, and significant reduction in cell size. Our studies have identified TRPC6 as the elusive principal Ca^{2+} conduit in the podocyte in response to Angiotensin II – mediated signaling. These results support the idea that TRPC6 may be an important mediator of podocyte injury in acquired forms of proteinuria, where the Renin – Angiotensin axis (RAS) is known to play an important role. Furthermore, TRPC6 appears to be essential in the preservation of the podocyte actin cytoskeleton and overall morphology. This observation suggests that while increased channel activity leads to podocyte injury and proteinuria, loss of channel activity may have similarly deleterious effects on podocytes and the filtration barrier.

Satellite symposium

CARDIO-RENAL SYNDROME IN TYPE 2 DIABETES AND DIABETIC NEPHROPATHY: THE ROLE OF VITAMIN D RECEPTORS

President: G. Remuzzi

Speakers: G.L Bakris

Diabetes is the most common cause of nephropathy and end stage kidney disease in the world. With the incidence of type 2 diabetes, hypertension and obesity growing in all industrialized countries, this problem is only going to get worse. This combination of these metabolic abnormalities is the leading cause of morbidity and mortality in industrialized countries[1]. Additionally, when starting hemodialysis, the prevalence of coronary artery disease is approximately 40% and the prevalence of left ventricular hypertrophy (LVH) is approximately 75%. LVH, in general, is an ominous prognostic sign and an independent risk factor for arrhythmias, sudden death, heart failure, and myocardial ischemia.

Approximately one billion people worldwide have 25-hydroxyvitamin D deficiency or insufficiency, and more than half of middle-aged vitamin D–deficient patients develop cardiovascular disease (CVD). In hypertensive patients, low serum vitamin D levels increase the risk of CVD by 60%[2]. In women with type 2 diabetes, the prevalence of vitamin D deficiency is a third higher than in control subjects, and low vitamin D levels nearly double the risk of developing CVD compared with diabetic patients with normal vitamin D levels[3]. Therefore, understanding the mechanism of accelerated atherosclerosis induced by vitamin D deficiency may be crucial for treating CVD in diabetics. To that end, understanding the contribution of vitamin D or lack thereof contributing to glucose-independent factors modulating macrophage cholesterol deposition is also critical to our understanding of the development of cardiovascular disease (CVD) in diabetics.

Apart from elevated blood pressure, hypervolemia, anemia, activation of local endocrine systems, such as renin-angiotensin-aldosterone system (RAAS) and the endothelin system, low vitamin D and/or high parathyroid hormone (PTH) is a permissive factor for the development of cardiac hypertrophy and interstitial fibrosis. Vitamin D therapy regresses myocardial hypertrophy in hemodialysis patients with secondary hyperparathyroidism[3,4].

Although separating the effect of vitamin D and PTH on the cardiovascular system is difficult, there is more evidence to suggest vitamin D plays an independent role in CVD. Vitamin D receptor (VDR) exists in the heart[5,6]. Vitamin D is able to regulate several genes related to cardiomyocyte hypertrophy, such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). A clinical study revealed the association between low calcitriol, high ANP level and congestive heart failure[7].

Vitamin D is well known as immunomodulatory agent. Vitamin D insufficiency is associated with increased inflammation markers like C-reactive protein (CRP) and matrix metalloproteinase (MMP)[8]. In addition, Park et al demonstrated that calcitriol therapy resulted in LVH regression in hemodialysis patients and decreased ANP as well as plasma renin activity, which is consistent with a report that demonstrated vitamin D is a renin inhibitor[9.10].

Given these benefits on CV risk and potential for reductions in kidney disease progression an ongoing trial is assessing the effects of these therapy on nephropathy progression assessed by changes in albuminuria. The VITAL study is a randomized double-blind trial that tests the hypothesis, does paricalcitol persistently reduces albuminuria in diabetic subjects already receiving angiotensin-converting enzyme inhibitor (ACEI) and/or angiotensin receptor blocker (ARB). Patients included in the study have a diagnosis of type 2 diabetes, urinary albumin/ creatinine ratio (UACR) between 100-3,000 mg/g, estimated glomerular filtration rate (eGFR) between 15-90 ml/min/1.73 m^2, serum

calcium <9.8 mg/dl, and parathyroid hormone (PTH) between 35-500 pg/ml. The study will finish in November 2009 and the baseline characteristics of the 281 subjects are: 69% men, mean age 64.9 ± 10.4 years, eGFR 40.7 ± 16.7 ml/min, median UACR (interquartile range) 612.3 mg/g (281-1,181 mg/g) and PTH 98.4 ± 63.8 pg/ml[11].

In addition, a recent study adds to our knowledge about vitamin D as the "unexplained inflammatory factor" when LDL cholesterol is lowered and yet CV risk is high. Oh and colleagues demonstrate that active vitamin D suppresses foam cell formation by reducing acetylated or oxidized low-density lipoprotein cholesterol uptake in diabetics[12]. Through downregulation of macrophage stress-related c-Jun N-terminal kinase signaling and suppression of endoplasmic reticulum stress, active vitamin D reduces peroxisome proliferated–activated receptor-γ expression, suppresses CD36 and scavenger receptor A-1 expression, and prevents macrophage cholesterol deposition. Deletion of vitamin D receptor confirmed acceleration of foam cell formation. Thus, vitamin D is truly more like a hormone than a vitamin and may provide the link to help in our understanding of kidney disease progression and CVD risk reduction

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