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Diabetes mellitus is a common disease worldwide. Diabetic patients with un-controlled hyperglycemia undergo diabetic complications including heart disease, kidney disease, retinopathy and neuropathy which are irreversible. High blood glucose concentration is the pivot cause of generalized pathophysiological changes. Autoxidation of glucose produces many free radicals which can directly damage the cells. Chronic diabetic patients with the renal complication always develop nephropathy and reach the end stage of renal disease. Accumulation of extracellular matrix materials such as collagen, fibronectin etc. which are induced by transforming growth factor- β1 (TGF-β1), causes the renal sclerosis. TGF-β1 is a key factor in the development of diabetic nephropathy. It stimulates the mesangial cells and endothelial cells of glomerlar capillaries to produce collagen and other extracellular matrix materials. High concentrated glucose milieu induces an overproduction of TGF-β1 from mesangial cells in the glomeruli. The increase in the matrix materials results in renal vascular and tissue damage and loss of the renal functions. In addition, TGF-β1 could stimulate the mesangial cells to produce glucose transporter 1 (GLUT 1). The overproduction of GLUT 1 enhances the transportation of glucose into the masangial cells resulting in the recurrence of those processes. Advanced glycated end-products (AGEs) are synthesized from the reaction between glucose and protein or lipid resulting in cell and tissue damages. Not only that AGEs are increased, but reactive oxygen species (ROS) is also generated during the synthesis of AGEs. In addition, diacylglycerol (DAG) is synthesized during the glucose meatabolism. The increase in DAG, AGEs and ROS will activate protein kinase C (PKC) which is involved in the synthesis of many kinds of growth factors including TGF-β1 leading to nephropathy and other micovascular complications in

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diabetes mellitus. Sorbitol is also produced in polyol pathway in glucose metabolism. In diabetes, the accumulation of sorbitol in the eyes and kidneys causes changes in osmotic gradient that damage cells and tissues. The mechanisms of diabetic nephropathy development occur at the early stage of diabetes mellitus. Therefore, prevention and control of hyperglycemia are the prior essential treatment at the early diagnosis to inhibit key causal factors of diabetic nephropathy development. Early detection of excessive glucose in the body may be useful for those who tend to develop diabetes mellitus. The determination of glycosylated hemoglobin (Hb_{A1c}) level is a tool for early detection of excessive blood glucose.

Keywords: *Diabetic nephropathy, Involving factors.*

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Objectives:
1. To study causal factors of diabetic nephropathy development.
2. To understand the mechanisms of the development of diabetic nephropathy.

มาเรียม อยู่สุขสวัสดิ์. การเสื่อมสภาพของไตเนื่องจากโรคเบาหวาน: ปัจจัยที่เกี่ยวข้อง. จุฬาลงกรณ์เวชสาร 2550 พ.ย. - ธ.ค; 51(11): 507 - 25

โรคเบาหวานเป็นโรคที่พบได้บ่อยและพบได้ทุกภูมิภาคของโลก ผู้ป่วยโรคเบาหวานเรื้อรังมักจะมีอาการแทรกซ้อนซึ่งเกี่ยวข้องกับระบบของหลอดเลือดต่าง ๆ โดยเฉพาะอย่างยิ่งหลอดเลือดฝอยของไต ทำให้ไตไม่สามารถทำงานได้อย่างปกติ มีการเปลี่ยนแปลงทั้งพยาธิสภาพและการทำงานของไต สาเหตุจากการมีระดับของกลูโคสในเลือดสูง ขณะที่ร่างกายมีระดับกลูโคสในเลือดสูงขึ้นนั้น จะทำให้มีการเปลี่ยนแปลงทางชีวเคมีของร่างกายอย่างต่อเนื่อง ได้แก่ การเกิด autoxidation ของกลูโคส ทำให้เกิดอนุมูลอิสระต่าง ๆ จำนวนมาก มีการสูญเสียสมดุลของการกำจัดอนุมูลอิสระ ทำให้ร่างกายเกิดภาวะ oxidative stress อนุมูลอิสระที่เกิดขึ้นเป็นปัจจัยสำคัญที่กระตุ้นให้เกิดพยาธิสภาพได้โดยตรงและทางอ้อม อนุมูลอิสระจะ oxidize สารที่เป็นองค์ประกอบของเซลล์ ทำให้เนื้อเยื่อต่าง ๆ ถูกทำลายหรือเปลี่ยนแปลงโครงสร้างไป สภาพแวดล้อม ที่มีกลูโคสความเข้มข้นสูง ๆ จะกระตุ้นให้เกิดปฏิกิริยาชีวเคมีหลายอย่างและมีการสร้างสารบางชนิดที่ส่งผลต่อไตและอวัยวะอื่น ๆ มีการกระตุ้นให้ mesangial cell ในโกลเมอรูลัสของไตสร้าง transforming growth factor- β1 (TGF-β1) เพิ่มขึ้นซึ่งจะกระตุ้นให้ mesangial cell และ endothelial cell ของหลอดเลือดฝอยมีการสร้าง และสะสมสารพวก collagen เกิดการสูญเสียหลอดเลือดฝอยโกลเมอรูลัส (glomerulosclerosis) และทำให้ไตแข็งเกิดพยาธิสภาพของไตโดยรวมและไม่สามารถทำงานได้อย่างปกติ (nephropathy) การเพิ่มขึ้นของ TGF-β1 ยังกระตุ้นให้ mesangial cell มีการสร้าง glucose transporter 1 (GLUT 1) เพิ่มขึ้นด้วย ซึ่งทำให้มีการขนส่งกลูโคสเข้า mesangial cell เพิ่มขึ้น ส่งผลให้เกิดปฏิกิริยาและการเปลี่ยนแปลงต่าง ๆ เพิ่มขึ้นอย่างต่อเนื่อง มีหลักฐานยืนยันว่า TGF-β1 และ GLUT 1 อาจส่งเสริมการสร้างเพิ่มปริมาณซึ่งกันและกัน นอกจากนั้นการมีระดับของกลูโคสในเลือดสูงจะทำให้มีการสร้างสารพวก advanced glycated end-products (AGEs) ซึ่งเกิดจากการที่กลูโคสทำปฏิกิริยากับโปรตีนหรือไขมัน ทำให้มีการเปลี่ยนแปลงองค์ประกอบและโครงสร้างของเนื้อเยื่อ ซึ่งทำให้ไตเกิดพยาธิสรีรภาพได้อีกทางหนึ่ง อีกทั้ง AGEs ที่เกิดขึ้นนั้นจะจับกับโปรตีนทำให้โครงสร้างของเนื้อเยื่อไตผิดปกติไปได้อีกขั้นหนึ่งด้วย ไม่เพียงเท่านั้นในระหว่างปฏิกิริยาของการสร้าง AGEs นั้นเกิด reactive oxygen species อื่น ๆ ด้วย ทำให้เสริมการเกิดพยาธิสภาพของไตมากขึ้น นอกจากนั้นในกระบวนการสลายกลูโคสยังเกิดสารพวก diacylglycerol (DAG) เพิ่มมากขึ้น ทั้ง DAG, AGEs และอนุมูลอิสระสามารถกระตุ้น protein kinase C ซึ่งเป็นเอ็นไซม์ที่กระตุ้นปฏิกิริยา phosphorylation ของกระบวนการทางชีวโมเลกุลต่าง ๆ ในการสร้าง growth factors ต่าง ๆ รวมทั้ง TGF-β1 ซึ่งเป็นสารสำคัญที่ทำให้เกิด diabetic nephropathy และ microvascular complications การมีระดับกลูโคสในเลือดที่สูงขึ้นนั้นยังกระตุ้น polyol pathway ทำให้มีการสร้างและสะสมสารพวก sorbitol ในลูกตาและไตเพิ่มขึ้นและเกิดการทำลายของเซลล์เนื้อเยื่อเหล่านั้น

การเปลี่ยนแปลงต่าง ๆ ที่กล่าวมานี้เกิดขึ้นในระยะเริ่มแรกของการตรวจพบโรคเบาหวาน การมีระดับกลูโคสในเลือดสูงเป็นปัจจัยสำคัญของการเกิดพยาธิสรีรภาพของไต ดังนั้นหากผู้ป่วยไม่ควบคุมระดับกลูโคสในเลือดที่สูงนั้นและทิ้งไว้เรื้อรัง จะเป็นสาเหตุให้เกิดการเปลี่ยนแปลงของไตและอวัยวะอื่น ๆ ตามมา ซึ่งไม่สามารถจะรักษาให้กลับคืนสภาพเดิมได้ การตรวจหาระดับ glycosylated hemoglobin (Hb_{A1c}) เป็นอีกวิธีหนึ่งที่สามารถบอกได้ตั้งแต่เริ่มแรกถึงการมีกลูโคสเกินความจำเป็นแล้วและอาจพัฒนาไปสู่โรคเบาหวานได้หากไม่ควบคุมตั้งแต่ตรวจพบ การรักษาภาวะแทรกซ้อนต่าง ๆ ของโรคเบาหวาน เช่น การใช้ PKC inhibitor ล้วนเป็นการแก้ปัญหาที่ปลายเหตุ ถึงแม้ว่าจะเป็นสิ่งจำเป็นที่ต้องกระทำก็ตาม การตระหนักถึงมูลเหตุและมีการควบคุมอย่างเคร่งครัดเพื่อให้ระดับน้ำตาลในเลือดปกตินั้นเป็นสิ่งแรกที่ต้องคำนึงถึงและจัดการทันทีเมื่อตรวจพบ เพื่อป้องกันการเกิดการเปลี่ยนแปลงต่าง ๆ อย่างต่อเนื่องตามมาจนทำให้เกิดโรคไตอันเป็นโรคแทรกซ้อนของผู้ป่วยเบาหวานที่ไม่สามารถแก้ไขให้กลับสู่สภาพเดิมได้อีกต่อไป

คำสำคัญ : *การเสื่อมสภาพของไต, โรคเบาหวาน, ปัจจัยที่เกี่ยวข้อง*

Diabetes mellitus is a common disease worldwide. The prevalence of diabetes in the adult population is estimated at 5.1 % in 2003, and tends to increase to 6.3 % by 2025. Recently, the Southeast Asian region has 13.3 % high prevalence of diabetes and impaired glucose tolerance; and it is expected to be the highest in the world by 2025. [1] In Thailand, the estimated national prevalence of diabetes in Thai adults has been reported as 9.6 % (2.4 million people). [2] The most common diabetic complication is nephropathy. The prevalence of diabetic nephropathy in Thailand was about 43.8 % of all diabetic complications. [3]

Diabetic nephropathy is a renal complication that attacks a number of diabetic patients with uncontrolled hyperglycemia.Since it causes irreversible kidney failure leading to end-stage renal disease, it is a serious problem of diabetic patients. The approach to the causal factors and the mechanism of diabetic nephropathy development is helpful for those who are involved in the prevention and treatment of diabetic complications. High blood glucose concentration is the crucial cause of renal pathophysiological changes in diabetic patients. It induces oxidative stress and biochemistry disturbances that generate many causal factors of diabetic nephropathy including ROS, advanced glycated end-product (AGEs), diacyl glycerol (DAG), PKC, TGF-β1, polyol pathway (impair the ratio of NADH to NAD and accumulation of sorbitol in the renal glomeruli). Glucose transporter 1 (GLUT 1) is a factor which attributes to the development of diabetic nephropathy. This article review brings up some key causal factors of diabetic nephropathy development. However, other factors including angitensin II, molecular factors-induced growth factor synthesis, molecular binding are not stated in the comments. In the beginning, this article reviews the roles of hyperglycemia, hypertension and proteinuria related to molecular mediators of the nephropathy development. The latter is focused on some biochemical factors and mechanisms involving in the development of diabetic nephropathy.

1. Hyperglycemia

Diabetes melitus with poor blood glucose control contributes to the development of albuminuria. It has been elucidated that hyperglycemia is involved in morphological and functional abnormalities in diabetic kidney disease. Also, in extracellular ambient, glucose reacts non-enzymatically with primary amines of proteins, forming glycated compounds. When glucose is transported into cells by glucose transporters, it is partly metabolized to sorbitol via polyol pathway and then to hexosamines. All these biochemical pathways have been implicated in hyperglycaemia-induced kidney damage. Furthermore, excess glucose can directly exert toxic effects by activating intracellular signaling pathways and inducing a number of cytokines that injure the kidney.

1.1 Glucotoxicity

High glucose milieu has been confirmed that it directly alters the extracellular matrix deposition in the kidney. Some studies on the mesangial cells as well as tubular epithelial cells demonstrated that high glucose concentrations induce cellular hypertrophy and increase extracellular matrix components such as collagen, laminin and fibronectin. [4] A further mechanism, whereby high glucose concentrations lead

to matrix deposition, is the reduction of the activity of metalloproteinases, the enzymes responsible for the extracellular matrix degradation. [5, 6] In the mesangial cells, high glucose levels induce transcription and secretion of TGF-β1. [7, 8]

Kidney cells do not have an absolute requirement of insulin for glucose uptake. Therefore, the intracellular glucose level more directly reflects its plasma concentration. The importance of excess glucose entry into the mesangial cells is an increase in extracellular matrix production and over-expressed cellular glucose transporter GLUT-1. [9] The increase in GLUT-1 expression leads to the increase of basal glucose uptake, with consequential increase in aldose reductase expression and PKC activation. This results in the increase in extracellular matrix production. [10] When antisense GLUT-1 is used to protect the mesangial cells from glucose-induced GLUT-1 over-expression, high glucose concentrations fail to induce extracellular matrix proteins. [11] This finding indicates that the factors regulating glucose transporter expression or activity could influence glucose uptake and glucotoxicity. Glucose itself as well as TGF-β1 could up-regulate GLUT-1 expression in mesangial cell culture. [9, 12]

Glucose is metabolized to fructose-6-phosphate in glycolysis. Fructose-6-phosphate is converted to glucosamine-6-phosphate by a rate-limiting enzyme of glucosamine-fructose-6-phosphate amidotransferase in the hexosamine pathway. This leads to the formation of N-acetylglucosamine which is a component of membrane glycoproteins. Thus, the activation of the hexosamine pathway is implicated in the development of chronic diabetic complication associated with PKC activation and TGF-β1 over-expression. [13]

1.2 Non-enzymatic glycation

Chronic hyperglycaemia leads to non-enzymatic protein glycation. The glycation results from exposure of lysine amino-terminal groups of proteins to high glucose concentrations. The increase in the covalent binding of glucose into proteins results in the formation of Schiff base which subsequently forms stable ketoamines, the Amadori products. When these glycated proteins undergo other reactions, such as dehydration, cyclization, oxidation and rearrangement, they form advanced glycation end-products (AGEs). The reaction is not reversible, and AGEs gradually accumulate in the tissues. [14, 15]

The accumulation of AGEs in the kidney is parallel to the development of albuminuria, mesangial expansion and thickening of glomerular basement membrane in diabetes mellitus. The molecular cross-linking is also found in blood vessels that causes diabetic vascular complications. [16] AGEs have various AGE-specific receptors. AGE-binding proteins or receptors for advanced glycosylation end-products (RAGE such as AGE-R1, AGE-R2, AGE-R3), lysozyme and macrophage scavenger receptors transduce the action of AGEs. AGE-specific receptors are present in many cell types, including the mesangial cells, glomerular epithelial cells and tubular epithelial cells. [17] Interaction of AGE-modified proteins with AGE receptors results in the degradation of AGE proteins, and simultaneously induces the synthesis and release of cytokines, such as TGF-β1, platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF). Consequently, they result in the enhancement of collagen, laminin and fibronectin production. [18]

1.3 The polyol pathway

Hyperglycemic condition increases the production of ROS which is implicated in sorbitol accumulation. Glucose is reduced to sorbitol by the enzyme aldose reductase in the polyol pathway. Excessive flux of glucose in the polyol pathway results in the increase in the ratio of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD). This results in metabolic imbalances, mimicking the effects of the same redox change induced by hypoxia. [19] In chronic diabetes, sorbitol accumulates in various tissues including the renal glomeruli and tubules. The accumulation of sorbitol disturbs the cellular osmoregulation by depletion of myoinositol [20] and by changing in the cellular redox potential [21], resulting in permanent tissue damage in chronic diabetes mellitus. The polyol pathway is, therefore, involved in the pathogenesis of diabetic nephropathy [22] and associated with GLUT 1, [10] PKC activation and TGF-β1 production. [23] Vascular endothelial cells are also affected by sorbitol accumulation causing vascular complication in diabetes mellitus. [24]

2. Hypertension

Hypertension plays a critical role in the progression of diabetic nephropathy. The development of proteinuria mostly takes place parallel to the gradual rise in the systemic blood pressure. The increase in blood pressure is closely related to the speed of the decline in glomerular filtration rate. [25] Therefore, diabetic patients with normal albumin excretion who have higher arterial blood pressure eventually progress to microalbuminuria. [26,27] The elevated arterial blood pressure causes glomerular lesions. Antihypertensive therapy can prevent the occurrences of the proteinuria and the renal changes in diabetes mellitus. [28, 29]

3. Proteinuria

Proteinuria is a key feature of renal disease and a strong predictor of the progression toward end-stage renal failure. [30] Proteinuria not only reflects renal impairment and a key pathogenic element of disease progression but also the advancement of generalized vascular damage. An excessive protein overload can induce tubulo-interstitial damage and contributes to the disease progression. [31] Excessive tubular reabsorption of proteins and the consequent accumulation of proteins in tubular epithelial cells induce the release of vasoactive and inflammatory mediators, such as, TGF-β1 endothelin 1, osteopontin and macrophage chemotactic protein-1. These factors in turn lead to infiltration of mononuclear cells, causing injury to the tubulo-interstitium, and ultimately the renal damage. The changes in renal hemodynamics, either primary or reactionary to nephron loss, induce further proteinuria that contributes progressive renal impairment. [32, 33]

Microvascular complications in diabetes mellitus

Nitric oxide (NO) is a potent vasodilator molecule which is produced by the endothelial cells. Nitric oxide synthase (eNOS) catalyzes the reaction of L-arginine that changes to NO. NO inhibits the migration [34] and proliferation of vascular smooth muscle cells. [35] Furthermore, platelet aggregation [36] and adhesive molecule expression of leukocyte [37, 38] are inhibited by NO. The injured vascular smooth muscle cells, endothelial cells, and the activated vascular wall mast cells, fibroblasts,

macrophages, and leukocytes, as well as the oxidation of norepinephrine (NE) from the renal sympathetic nerves produce abundance of ROS. ROS then interacts with NO to form the potent cytotoxic ($OONO^-$). Peroxynitrite radicals interact with proteins in the kidney leading to the glomerular and tubular dysfunction (Figure 1).

The causal factors of coronary artery disease and progressive renal insufficiency adversely affect endothelial cell function and vascular smooth muscle cell function. They induce the formation of reactive oxygen species such as superoxide anion and hydrogen peroxide. These ROS result in the decreases in the vasodilators and the growth inhibitors such as

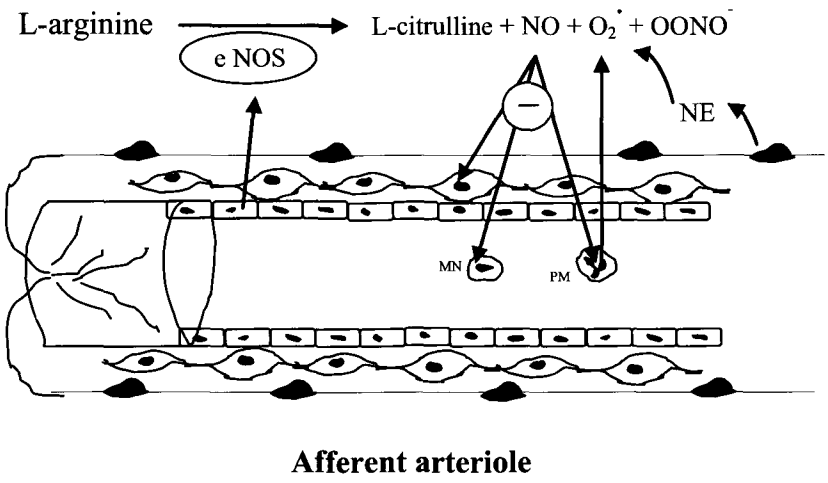

Figure 1. ROS reduces the biological effects of NO and induces the renal vascular complications.
Available from: http://www.hypertensiononline.org [2005,March 20]

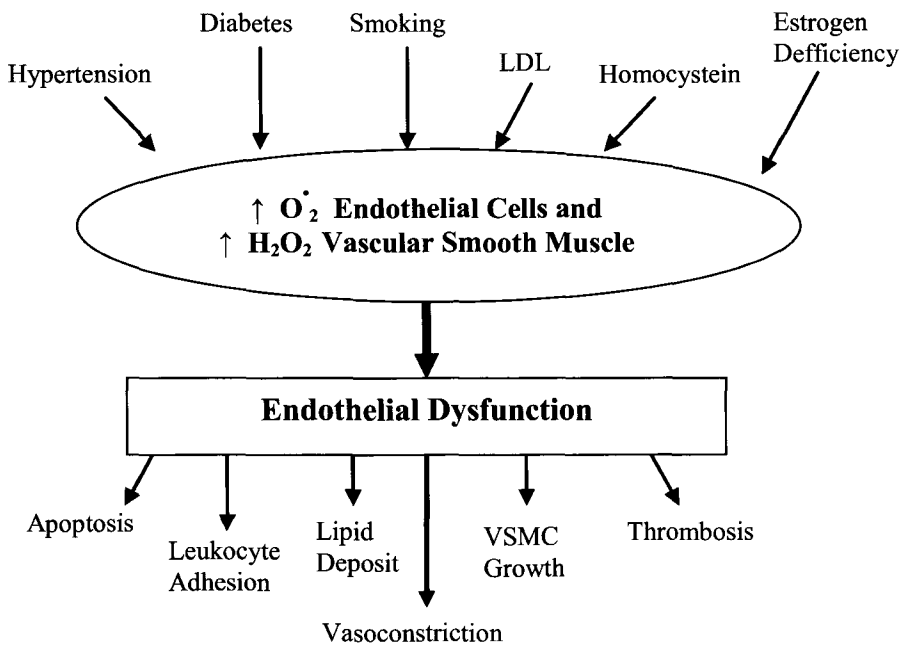

Figure 2. Risk factors of the endothelial dysfunction lead to the coronary artery disease and renal pathophysiology.
Available from: http://www.hypertensiononline.org [2005,March 20]

prostacyclin and nitric oxide but the increases in the formation of the endothelium-derived vasoconstrictors and growth promoters such as angiotensin II, endothelin-1, and plasminogen activator inhibitor (PAI-1). These changes lead to vascular complications, especially the consequential pathophysiology of the kidneys (Figure 2).

The progressive injury of the coronary and the kidney are involved in the increase in apoptosis or programmed cell death which contributes to the remodeling of vascular wall and the activation of cell adhesion molecules. The adherence of both mononuclear and polymorphonuclear leukocytes to the vascular wall results in the infiltration and the deposition of oxidized lipids in the vessel wall. This consequently causes vasoconstriction, hypertrophy and hyperplasia of the vascular smooth muscle cells and thrombus formation.

Microvascular damage is a common underlying change of diabetic complications including diabetic peripheral neuropathy, retinopathy and nephropathy. Chronic diabetics with microvascular complications and poor treatments often undergo lower extremity amputation, blindness, and end-stage renal disease. All of these are irreversible. The studies in diabetic animals have demonstrated that an over-activation of PKC results in the microvascular damage. [39] Protein kinase C_β (PKC_β) is an enzyme that acts as a signal transducer that involves in many biochemical processes. [39-42] There are at least 12 isoforms of PKC that are located in various tissues throughout the body. Two of the isoforms, specifically $\beta 1$ and $\beta 2$, are located within the nerves, eyes, and kidneys and have been hypothesized to play major roles in both the development and progression of microvascular complications in

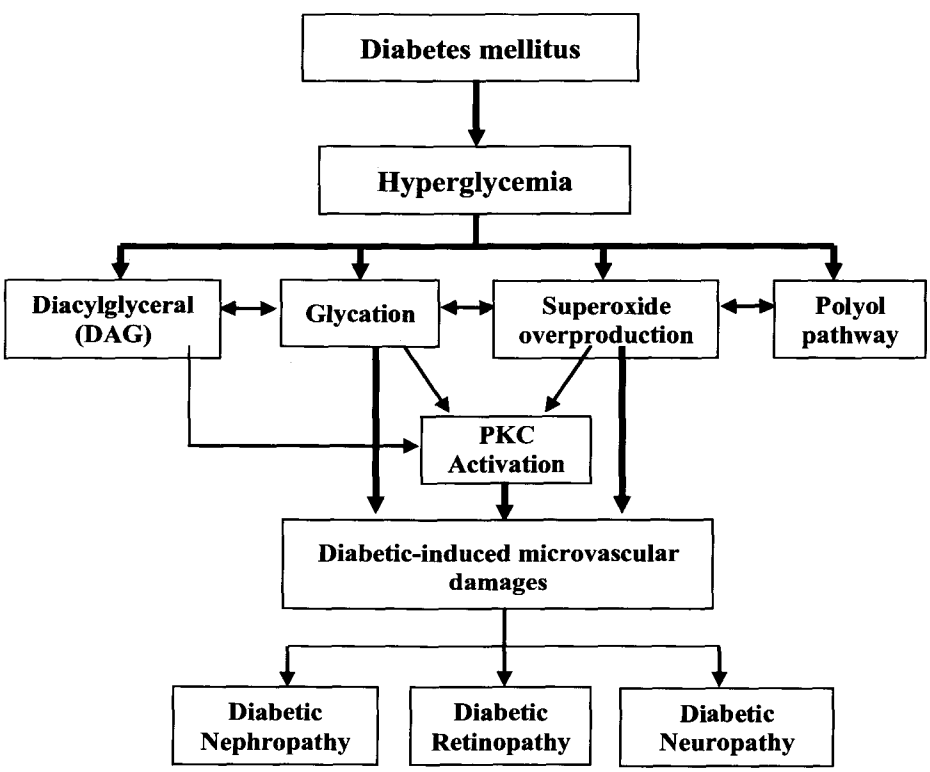

Figure 3. The relationship of the factors involving in the microvascular damage in diabetes mellitus. [49]

diabetic patients. [39] Hyperglycemia activates at least four metabolic pathways that contribute to diabetes-induced microvascular damage. In the normal physiological glucose concentrations, the activation of PKC occurs via cell signal transductions. It is associated with a complex sequence of biochemical interactions through G-protein coupled receptor-mediated pathway. [43-45] DAG is an intermediate substance of glucose metabolism which is an important mediator to signal the activation of PKC. [46] The activation of PKC accounts for the renal pathophysiological changes including an initial increase in the glomerular filtration rate, mesangial matrix expansion, glomerular capillary crowding, and glomerular occlusion. [47, 48] Other pathways, including glycation, polyol pathway and superoxide overproduction also play a role in diabetic microvascular complications. The relationship of these factors involving in microvascular damage that leads to the diabetic complications is shown in the following diagram.

Relationship of hyperglycemia, oxidative stress, TGF-β1, PKC and GLUT 1 in the development of diabetic nephropathy

Diabetic nephropathy is one of microvascular complications in diabetes mellitus which finally leads to end-stage renal failure. Its pathological features are thickening of the glomerular and tubular basement membrane. [50] Glomerulosclerosis is a conspicuous morphological change in diabetes mellitus. [51] Studies in streptozotocin-induced diabetic rats found the glycemic control is an important way in the prevention of the development of glomerulosclerosis. Poorly-controlled diabetic rats with constantly high plasma glucose level showed significant increase in basement membrane-like materials and mesangial cell mass in comparison with well-controlled diabetic rats. [52, 53]

Hyperglycemia generates diabetic nephropathy-inducing oxidative stress

Under physiological conditions, glucose normally undergoes oxidation, the process through which protein-reactive ketoaldehyde, hydrogenperoxide and highly reactive oxidants are produced. [16] In diabetes mellitus, the generation of reactive oxygen species is abundant. Hyperglycemia does not only generate more reactive oxygen species but also impairs antioxidant mechanisms leading to nephropathy in diabetes mellitus. [54-61] Several *in vitro* studies have confirmed that highly concentrated glucose directly induces the increase in oxidative stress in glomerular mesangial cells. [62, 63] A study in kidney-cortex tubules of diabetic rabbits indicated an increase in intracellular hydroxyl free radical generation and change in intracellular glutathione status. The intracellular glutathione redox state was diminished (GSH/GSSG) in spite of the elevation of glutathione reductase activity. [64] The activities of xanthine oxidase and catalase in the diabetic rat kidney were decreased. Insulin treatment could restore antioxidant enzyme activities. [65] In addition, other antioxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase also decreased in diabetes mellitus. Advanced oxidative stress in uncontrolled diabetes manifested marked alterations in tissue antioxidant status. [54, 57] In addition, hyperlipidemia, which results from the chronic hyperglycemia, can induce the nephropathy via the generation of reactive oxygen species. [61, 66-68]

The attachment of these reactive oxygen species to proteins contributes to protein fragmentation and cross-linking in diabetic tissues. [14, 69, 70] *In vitro* and *in vivo* studies indicated that lipid peroxidation also enhanced both diabetic animals and humans causing renal damages. [56, 61, 71] Furthermore, an elevation of free radicals can induce apoptosis, which also contributes to the development of diabetic nephropathy. [8, 72, 73] In addition, oxidative stress increases the production of TGF-β1 via the PKC activation. [5, 74]

Hyperglycemia stimulates TGF-β1 overproduction via PKC activation

Transforming growth factor-beta 1 (TGF-β1) is found an important mediator in the development of diabetic renal disease. [7] It stimulates the production and accumulation of glomerular matrix materials. [76, 77] The urinary levels of TGF-β1 increase in diabetic patients. [78] The neutralization of TGF-1 by anti-TGF-β1 antibody has been shown to attenuate renal hypertrophy and thus enhance extracellular matrix gene expression in STZ-induced diabetic mice. [9] In addition, a long-term administration of neutralizing anti-TGF-β1 antibody could prevent glomerulosclerosis and renal insufficiency in diabetic *db/db* mice, a genetic model of non-insulin dependent diabetes mellitus. [77] The stimulation of TGF-β1 expression with high glucose concentration milieu has been demonstrated in the culture of glomerular mesangial cells and proximal tubular cells. [7] The high glucose concentration ambient and mechanical stretch could stimulate the production TGF-β1 via a PKC-dependent mechanism. [79, 80] An *in vitro* study demonstrated that highly concentrated glucose ambient could induce TGF-β1 over-expression and increase extracellular matrix protein in the renal glomeruli. [15, 81] The highly concentrated glucose induces TGF- β1 gene expression via two adjacent activating protein-1 (AP-1) binding sites. [82] It enhances the binding activity of the nuclear proteins to the AP-1 binding sites which results in the increase of activity of TGF-β1 promoter. Protein kinase C (PKC) and p38 mitogen-activated protein kinase (p38 MAPK), which regulate the TGF-β1 promoter activity, are also activated by the highly concentrated glucose. [83] The events result in the over-expression of TGF-β1-mRNA and protein.

TGF-β1 induces overproduction of extracellular matrix materials via PKC activation

Under concentrated glucose conditions, the increased in *de novo* synthesis of DAG from glucose has been demonstrated in the glomerular mesangial cells cultured and in the glomeruli of diabetic rats. The increased DAG activates PKC resulting in the activation of the various intracellular signal transduction systems. [79] It has been shown that TGF-β1 over-expression stimulates PKC translocation and activation which accounts for the increase in the extracellular matrix materials. [80, 84] The inhibition of PKC effectively blocks highly concentrated glucose-induced fibronectin production. [73] A long-term administration of a PKC_{β} inhibitor inhibits the glomerular PKC activation, reduces urinary albumin excretion rates and prevents the mesangial expansion in diabetic db/db mice. [80] Inhibition of PKC_{β} could decrease the basal and TGF-β1-stimulated collagen I production in human mesangial cells. [85]

Highly concentrated glucose milieu induces GLUT 1 over-expression.

GLUT 1, which is a major glucose transporter of the mesangial cells, plays an important role as a glucose transporter in the pathogenesis of glomerulosclerosis. It is up-regulated in diabetic kidneys. [86-88] An over-expression of GLUT1 could increase aldose reductase, a protein kinase C_{α} and the native GLUT1 transcription in both normal glucose and highly concentrated glucose condition in the mesangial cells. [89] A study in a mesangial cell culture demonstrated that high concentrated glucose milieu (20 mM) facilitated the GLUT 1 expression in the rat mesangial cells and the uptake of glucose analog ^{3}H 2-deoxyglucose (^{3}H 2-DOG) when compared with mesangial cells in the physiological glucose concentration milieu (8 mM). The transport of glucose into the cells has been shown to be a rate-limiting for extracellular matrix production in the mesangial cells. [79] A study in STZ-induced diabetic rats demonstrated that 45 days of diabetes resulted in an increase in albuminuria, urinary TGF-β1 and GLUT1 protein. [90] The treatment of TGF- b can regulate GLUT1 mRNA and protein levels and significant increase in glucose uptake in rat mesangial cells. [91] The cultured mesangial cells transducted with the human GLUT 1 gene and thus over-expressed the GLUT1 protein showed a marked increase in glucose uptake and synthesis of extracellular matrix molecules. The simultaneous presence of long-term high glucose concentration and TGF- β1 over-expression could enhance GLUT 1 up-regulation in the mesangial cells. [9, 12] With those evidences, hyperglycemia can induce the development of diabetic nephropathy via GLUT1 over-expression. The abundance of GLUT 1 results in the enhancement of glucose uptake and turns to stimulate TGF-β1 production in mesangial cells. Both proteins of TGF-β and GLUT 1 can influent the expression of one another.

This diabetic complication occurs in the first few years after diagnosis and could be detected early. Hyperglycemia is the most common cause of diabetic complications. Therefore, good control of blood glucose concentration is the first and pivot treatment in the prevention of diabetic complications such as nephropathy and other microvascular complications. In addition, a good management of hyperglycemia may delay the progression to the end stage renal disease. Early detection of excessive glucose in the body may be useful for those who tend to develop diabetes mellitus. The determination of glycosylated hemoglobin (Hb_{A1c}) level is a tool for early detection of excessive blood glucose.

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