ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2015 June 10; 6(5): 673-773

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

A peer-reviewed, online, open-access journal of diabetes

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WJD World Journal of Diabetes

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Biweekly Volume 6 Number 5 June 10, 2015

World Journal of Diabetes
Volume 6 Number 5 June 10, 2015

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NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
March 15, 2010

FREQUENCY
Biweekly

Xiu-Xia Song, Vice Director
World Journal of Diabetes
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLISHER
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8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
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PUBLICATION DATE
June 10, 2015

World Journal of Diabetes

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DOI: 10.4239/wjd.v6.i5.673

World J Diabetes 2015 June 10; 6(5): 673-678
ISSN 1948-9358 (online)

EDITORIAL

Update on type 2 diabetes-related osteoporosis

Kannikar Wongdee, Narattaphol Charoenphandhu

Kannikar Wongdee, Narattaphol Charoenphandhu, Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Bangkok 10400, Thailand
Kannikar Wongdee, Office of Academic Management, Faculty of Allied Health Sciences, Burapha University, Chonburi 20131, Thailand
Narattaphol Charoenphandhu, Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Author contributions: Wongdee K and Charoenphandhu N contributed equally for literature review, data analysis and preparation of the manuscript.

Supported by Grants from the Cluster and Program Management Office (CPMO), National Science and Technology Development Agency (P-11-00639); the Thailand Research Fund (TRF)-Mahidol University through the TRF Senior Research Scholar Grant (RTA5780001 to NC); the Faculty of Allied Health Sciences, Burapha University and Thailand Research Fund through TRF Research Career Development Grant (RSA5780041 to KW); the Research and Development Fund Burapha University (05/2557 to KW); the Faculty of Allied Health Sciences, Burapha University Research Grant of Fiscal Year 2015 (AHS05/2558 to KW).

Conflict-of-interest: Kannikar Wongdee and Narattaphol Charoenphandhu declare no conflicts of interest.

Correspondence to: Narattaphol Charoenphandhu, MD, PhD, Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand. naratt@narattsys.com
Telephone: +66-2-3547154
Fax: +66-2-3547154

Received: January 11, 2015
Peer-review started: January 15, 2015
First decision: February 7, 2015
Revised: February 12, 2015
Accepted: March 5, 2015
Article in press: March 9, 2015
Published online: June 10, 2015

Abstract

It was previously understood that body weight gain and obesity observed in type 2 diabetes mellitus (T2DM) could be beneficial since body weight increase elevated bone mineral density and thus helped maintain the skeletal framework. However, a number of recent findings in humans and rodents have revealed that T2DM is not only associated with trabecular defects but also increases cortical porosity, and compromised bone cell function and bone mechanical properties. Hyperglycemia and insulin resistance in T2DM may further induce osteoblast apoptosis and uncoupling bone turnover. Prolonged accumulation of advanced glycation end products and diminished activity of lysyl oxidase, an essential enzyme for collagen cross-link, can lead to structural abnormalities of bone collagen fibrils, brittle matrix, and fragility fractures. Our studies in T2DM rats showed that dyslipidemia, which often occurs in T2DM, could obscure the T2DM-associated changes in bone microstructure and osteopenia. Longitudinal bone growth regulated by the growth plate chondrocytes is also impaired by T2DM since differentiation of growth plate chondrocytes is arrested and retained in the resting state while only a small number of cells undergo hypertrophic differentiation. Such a delayed chondrocyte differentiation may have also resulted from premature apoptosis of the growth plate chondrocytes. Nevertheless, the underlying cellular and molecular mechanisms of insulin resistance in osteoblasts, osteoclasts, osteocytes, and growth plate chondrocytes remain to be investigated.

Key words: Advanced glycation end products; Chondrocyte apoptosis; Collagen; Dyslipidemia; Fracture; Growth plate; Type 2 diabetes mellitus; Osteoporosis

Core tip: Type 2 diabetes mellitus (T2DM) negatively affects bone density and strength by inducing cellular and extracellular matrix failures. Insulin resistance in T2DM deteriorates osteoblast proliferation and activity, but enhances osteoclast activity, leading to uncoupled bone remodeling. Hyperglycemia also aggravates osteoblast dysfunction, thus contributing to cellular failure. Extracellular matrix failure is caused by abnormal collagen synthesis and aberrant collagen structure and alignment, the latter of which results, in part, from advanced glycation end products (AGEs). With hyperglycemia and AGEs, impaired bone strength may occur despite high bone mineral density. It is, therefore, concluded that T2DM can be considered a cause of osteoporosis and/or poor bone mechanical properties.

Wongdee K, Charoenphandhu N. Update on type 2 diabetes-related osteoporosis. *World J Diabetes* 2015; 6(5): 673-678 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/673.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.673

INTRODUCTION

Although type 1 diabetes mellitus (T1DM) is known to compromise bone microstructure[1,2], how type 2 diabetes mellitus (T2DM) affects bone metabolism has long been debate for decades. It was previously believed that T2DM could be protective against osteoporosis since a number of clinical studies and meta-analyses revealed an increase in bone mineral density (BMD) in T2DM patients[1,2]. However, several recent lines of evidence in both humans and rodents have corroborated that T2DM is indeed detrimental to bone, leading to impaired osteoblast-mediated bone formation, accelerated bone resorption, microstructural defect, and poor bone quality. The previous controversial data stem from the use of low-resolution X-ray-based techniques, including measurement of areal BMD by dual energy X-ray absorptiometry (DXA), rather than evaluation of high-resolution bone microstructure or bone mechanical properties. This article has updated the recent findings of T2DM-related osteopenia and osteoporosis, as summarized in Table 1.

T2DM INCREASES BONE POROSITY AND DECREASES MECHANICAL BONE STRENGTH

Previous investigations in human mostly focus on trabecular bone changes in T2DM, but recent investigations have moved to the study of cortical changes and mechanical properties. By using more advanced techniques, such as high-resolution 3-dimensional computed tomography and microindentation, it was found that T2DM negatively affected bone strength despite the presence of relatively high BMD. Several cross-sectional studies in T2DM patients using high-resolution peripheral quantitative computed tomography (HR-pQCT) and magnetic resonance imaging (MRI) consistently revealed quality defects in both cortical and trabecular networks that would increase fracture risk[3-6]. For instance, Farr *et al*[3] by assessing bone quality with HR-pQCT in 30 postmenopausal T2DM patients at distal radius and distal tibia, found lower cortical thickness in T2DM was lower than normal non-diabetic controls, while bone microindentation testing showed lower bone material strength (BMS) in T2DM patients. Moreover, the radius quality evaluated by MRI, showed trabecular network holes being approximately 10% larger in postmenopausal T2DM patients than normal controls[5]. The cortical part was similarly affected by T2DM[4]. Patsch *et al*[4] investigated changes in bone microarchitecture in postmenopausal T2DM patients with or without fractures at radius and tibia by using DXA and HR-pQCT. Interestingly, they found that T2DM patients with fractures had higher pore-related deficits, *i.e.*, greater cortical pore volume, cortical porosity, and endocortical bone surface, than diabetic patients without fractures[4], consistent with the previous report in the radii of T2DM patients that having greater cortical pore volume (approximately 150%) and cortical porosity (approximately 125%) than normal individuals[6]. These cortical defects were often accompanied by impaired mechanical properties, such as increased failure load and low bone bending strength, that led to reduction in overall bone strength and increase in fracture risk[4,7].

DYSLIPIDEMIA MIGHT OBSCURE T2DM-INDUCED OSTEOPOROSIS

Previously it was believed that greater body weight or obesity associated with T2DM could be beneficial to the skeletal system through increasing BMD and bone mass[7,8]. However, our group recently reported the possible masking effects of dyslipidemia on diabetic bone in rats[9]. In our study, we determined the effects of dyslipidemia on bone microstructure were determined in Goto-Kakizaki (GK) diabetic rats treated with high cholesterol diet compared those fed with normal diet. The GK rats-a non-obese T2DM rat model without obesity-induced bone gain-were found to manifest stable fasting hyperglycemia and insulin resistance, while cholesterol-fed GK rats exhibited hypercholesterolemia, hypertriglyceridemia and hyperglycemia without significant weight gain[10]. Bone histomorphometry revealed that GK rats with T2DM manifested several signs of suppressed osteoblast function, such as decreases in osteoblast surface and bone formation rate, whereas the osteoclast-mediated

Table 1 Recent studies on the type 2 diabetes mellitus-related osteoporosis in humans (2010-2014)

Site of bone	Technique	Subjects	Main findings in T2DM group	Ref.
Distal radius	HR-pQCT	60 postmenopausal women	Low cortical thickness and low trabecular number	[3]
Distal tibia	HR-pQCT		Lower cortical thickness	
Mid-shaft tibia	Microindentation		Poor bone quality (low BMS)	
Distal radius	DXA, HR-pQCT	80 postmenopausal women with or without fractures	Higher cortical porosity in T2DM with fractures	[4]
Ultradistal radius	DXA, HR-pQCT		Higher cortical porosity in T2DM with fractures	
Distal tibia	DXA, HR-pQCT		Lower cortical porosity in T2DM with fractures	
Ultradistal tibia	DXA, HR-pQCT		Lower BMD and cortical porosity in T2DM with fractures	
Distal radius	MRI	60 postmenopausal women	Greater trabecular network holes	[5]
Distal radius	HR-pQCT	38 postmenopausal women	Higher cortical porosity	[6]
Tibia	HR-pQCT		Higher volumetric BMD and trabecular thickness	
Mid-shaft radius	pQCT	1171 men (≥ 65 yr)	Lower bone bending strength	[7]
Mid-shaft tibia	pQCT		Lower bone bending strength	

BMD: Bone mineral density; BMS: Bone material strength; DXA: Dual energy X-ray absorptiometry; HR-pQCT: High resolution-peripheral quantitative computed tomography; MRI: Magnetic resonance imaging; T2DM: Type 2 diabetes mellitus.

bone resorption was markedly enhanced. It was noted that, these microstructural changes disappeared after 16-wk of high cholesterol consumption, suggesting that high cholesterol diet and perhaps the resultant dyslipidemia could obscure the T2DM-associated osteopenia and changes in bone microstructural defect[9]. Thus, the difficulty in detecting bone deterioration in T2DM rats with dyslipidemia could explain, in part, why osteopenia was not observed in some T2DM studies.

T2DM AND LONGITUDINAL BONE GROWTH

Up until now, few studies have investigated relationship between T2DM and longitudinal bone growth. Generally, longitudinal bone growth is controlled by proliferation and differentiation of chondrocytes in the growth plate, which is histologically divided into 3 zones, *i.e.*, resting zone (RZ), proliferative zone (PZ) and hypertrophic zone (HZ). The RZ consists of low mitotic activity stem-like cells that gradually migrate to the PZ where chondrocytes proliferate and align into vertical columns and eventually reach the mature state in HZ. Thereafter, the hypertrophic chondrocytes in HZ undergo apoptosis and are replaced by capillaries and osteoblasts, which later use cartilaginous scaffold as a template for bone formation and bone elongation[11,12]. Since several investigations reported reduced bone length in diabetic rats compared with normal rats[9,13], T2DM may be a cause of aberrant growth plate function. Lapmanee *et al*[9] examined changes in the growth plate of diabetic GK rats and found impairment of chondrocyte differentiation as indicated by increased RZ height and decreased HZ height. It was possible that differentiation of chondrocyte precursors in T2DM rats were arrested and cells remained in the resting state, with only a small number of proliferating cells undergoing differentiation into hypertrophic chondrocytes[9].

Aiemlapa *et al*[14] further demonstrated the underlying mechanism of delayed growth plate chondrocyte differentiation. By using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay for apoptosis, they found premature apoptosis of chondrocytes in the HZ and chondro-osseous junction of GK rats. The massive loss of growth plate chondrocytes was accompanied by an increase in serum IGF-1 level, and overexpression of parathyroid hormone related protein (PTHrP), *runt*-related transcription factor (Runx2) and vascular endothelial growth factor (VEGF) in the growth plate, all of which might have been compensatory responses to mitigate excessive loss of chondrocytes due to premature apoptosis[14]. However, it was possible that the effects of T2DM on the growth plate might be dependent on animal strain and model of DM induction. For instance, Wu *et al*[15] found acceleration of longitudinal bone growth as indicated by bone elongation and increased heights of PZ and HZ in insulin resistant mice induced by high fat diet. In this model, insulin might remain to have a stimulatory effect on bone growth *in vivo* similar to its reported stimulatory effect on metatarsal linear growth *in vitro*[15]. Furthermore, high fat diet-induced dyslipidemia could complicate the matter since 7α-hydroxycholesterol and oxidized low-density lipoprotein (LDL) have been shown to modulate osteoblast and osteoclast functions, which, in turn, could have effects on bone elongation[16,17].

POSSIBLE CELLULAR MECHANISMS OF T2DM-RELATED FRAGILITY FRACTURES

The pathogenesis of T2DM-related fragility fracture can be looked upon from 2 aspects, *i.e.*, cellular failure and extracellular matrix failure. At cellular level, T2DM was associated with diminished activities of osteoblasts, osteoclasts and osteocytes, and increased apoptosis of bone cells[9,18-21]. A decrease in osteocyte density (number of osteocyte-occupied lacunae per unit

area) was conspicuously observed in streptozotocin-induced diabetic rats[21]. Hyperglycemia-induced insulin resistance is another important factor that cause both osteoblast and osteoclast malfunctions[22]. Since insulin is a suppressor of osteoclast-mediated bone resorption[23], T2DM-associated insulin resistance could enhance bone resorption. Moreover, high plasma glucose concentration can induce glucotoxicity in cells including osteoblasts, leading to osteoblast apoptosis[24]. *In vivo* experiment in transgenic liver-specific S503A CEACAM1 mutant (L-SACC1) mice, a model of impaired insulin clearance in the liver causing hyperinsulinemia and insulin resistance, suggested that the abnormally high bone mass in these mice might have resulted from low bone turnover as indicated by decreases in double-labeled surface (as determined by bone histomorphometry) and TRAP-positive osteoclasts, which represent activities of osteoblast-mediated bone formation and osteoclast-mediated bone resorption, respectively[22]. In other words, insulin resistance in this model was associated with a slowdown in bone turnover, which could eventually result in inadequate healing of microcracks, poor bone quality and increased fracture risk[22]. In addition, the experiment in high fat diet-fed Zucker diabetic fatty (ZDF) rats also showed impaired osteoblast function as indicated by downregulation of the expression of osteoblast-specific genes, *e.g.*, bone morphogenetic protein-2 (BMP-2), Runx2, osteocalcin and osteopontin. Suppression of osteoblastogenesis in these ZDF rats possibly compromised bone regeneration capacity. Subcritical bone defect regeneration study further showed that nondiabetic rats filled the defect by 57%, whereas diabetic rats could fill only 21% of bone defect in 12 wk[18].

T2DM not only caused deterioration of bone cell functions (cellular failure), but it also damaged bone extracellular matrix. Most studies suggested that T2DM caused abnormality in the structure of collagen, which is the most abundant protein in organic bone matrix. García-Hernández *et al*[25] reported that high glucose concentration indeed increased biomineralization in human alveolar bone-derived osteoblasts, but the mineral quality was lower than that in low glucose-exposed group. Determination of mineral quality in term of calcium/phosphate (Ca/Pi) ratio in the mineralized extracellular matrix nodules by energy-dispersive X-ray microanalysis (EDX) showed that high concentration of glucose significantly decreased Ca/Pi ratio on day 7 and 14 of treatment. Hammond *et al*[26] further studied nanoscale morphology of type Ⅰ collagen in tibiae of ZDF rats by Raman spectroscopy and reference point indentation (RPI), the latter of which applied a force to determine bone mechanical properties by measuring the relative displacement reference position[26,27]. RPI analysis revealed that bone matrix of ZDF diabetic rats was more resistant to plastic deformation, which might have resulted from abnormal formation of non-enzymatic collagen cross-link, toughening of the matrix, or the presence of advanced glycation end products (AGEs).

AGEs are non-enzymatic carbohydrate modifications of extracellular and intracellular proteins accumulated in long-lived tissues, such as skin and bone, and are often present in the plasma proteins of patients with DM and renal failure[28,29]. A number of investigations have revealed that AGEs are considered a factor that provokes fragility fractures in T2DM by inducing abnormal arrangement of collagen[26,28,30]. By using scanning electron microscope (SEM) and transmission electron microscope (TEM), Aoki *et al*[28] provided evidence that the rats subjected to adenine-induced renal failure exhibited AGEs accumulation and suppression of osteoblast function, similar to that observed in T2DM. SEM showed irregularity in collagen fibril alignment, while TEM revealed a wider diameter of collagen fibril in adenine-treated rats with renal osteodystrophy[28]. Immunohistochemistry also showed greater accumulation of AGEs in peritrabecular osteoblasts of adenine-treated rats than control rats. Further *in vitro* study in AGEs-treated MC3T3-E1 osteoblast-like cells showed a decrease in protein expression of secreted phosphoprotein 1 and lysyl oxidase, a mature osteoblast marker and essential enzyme for collagen cross-link, respectively. It was thus suggested that suppressed osteoblast differentiation and decreased lysyl oxidase production caused structural abnormalities of bone collagen fibrils leading to bone fragility[28].

Collagen is the most abundant protein in bone organic matrix, and it undergoes intra- and extracellular post-translational modifications[31]. To stabilize collagen fibrils, lysyl oxidase catalyzes intra- and intermolecular cross-link between collagen molecules essential for bone strength[31]. It was reported that glycation of collagen caused abnormal arrangement of collagen leading to brittle matrix and fragile bone[26,28,30], but little is known whether a decrease in lysyl oxidase-dependent collagen cross-link contributes to diabetic bone fragility and osteoporosis. The underlying mechanism of AGEs-attenuated lysyl oxidase activity was explored in mouse and rat primary osteoblasts and it was found that the carboxymethylated collagen, a form of AGEs, was not able to promote lysyl oxidase-mediated cross-linking due to failure of binding between abnormal collagen and discoidin domain receptor-2[30].

CONCLUSION

Currently, it can be concluded that T2DM compromises bone microstructure by inducing aberrant bone cell function (cellular failure) and abnormal matrix structure (matrix failure). Regarding the cellular effect, T2DM is associated with increased osteoblast apoptosis, diminished osteoblast differentiation, and enhanced osteoclast-mediated bone resorption, which, in part,

resulted from hyperglycemia and insulin resistance. Prolonged accumulation of AGEs coexisting with a decrease in lysyl oxidase activity causes abnormal structure and alignment of collagen, leading to bone fragility. Several confounding factors in T2DM, particularly body weight gain, obesity, and dyslipidemia, are able to mask the detrimental effects of T2DM, and may delay diagnosis of diabetic osteoporosis. In other words, bone is already damaged in T2DM despite a relatively high BMD. Although deleterious effects of T2DM on bone have been elucidated, the underlying cellular and molecular mechanisms remain unclear. For example, how does insulin resistance occur in osteoblasts and how do phosphorylation of insulin-receptor substrate isoforms (IRSs) and resultant insulin resistance in osteoblasts, osteoclasts and perhaps osteocytes contribute to diabetic bone loss? Indeed, osteocytes residing inside lacunae play an important role in bone remodeling in health and disease since they are responsible for inducing bone loss under certain conditions, such as during lactation[32,33]. Further investigation is required to demonstrate whether osteocytic dysfunction does exist in T2DM.

ACKNOWLEDGMENTS

The authors thank Professor Nateetip Krishnamra for valuable comments on the manuscript.

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P- Reviewer: Grieco A, Hiwatashi A **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Zhang DN

World Journal of Diabetes

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i5.679

World J Diabetes 2015 June 10; 6(5): 679-692
ISSN 1948-9358 (online)

REVIEW

Endothelial dysfunction as a predictor of cardiovascular disease in type 1 diabetes

Marcello C Bertoluci, Gislaine V Cé, Antônio MV da Silva, Marco V Wainstein, Winston Boff, Marcia Puñales

Marcello C Bertoluci, Marco V Wainstein, Departamento de Medicina Interna, Universidade Federal do Rio Grande do Sul, Porto Alegre RS 90035-003, Brazil
Marcello C Bertoluci, Serviço de Medicina Interna do Hospital de Clinicas de Porto Alegre, Porto Alegre RS 90035-903, Brazil
Marcello C Bertoluci, Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul, Porto Alegre RS 90035-003, Brazil
Gislaine V Cé, Winston Boff, Marcia Puñales, Instituto da Criança com Diabetes - Grupo Hospitalar Conceição, Porto Alegre RS 91350-250, Brasil
Antônio MV da Silva, Departamento de Fisioterapia e Reabilitação, Universidade Federal de Santa Maria, Santa Maria RS 97105-900, Brasil
Marco V Wainstein, Serviço de Cardiologia do Hospital de Clínicas de Porto Alegre, Porto Alegre RS 90035-903, Brazil

Author contributions: Bertoluci MC, Puñales M, Cé GV, da Silva AMV, Wainstein MV and Boff W contributed to this paper.

Conflict-of-interest: Dr. Wainstein reports grants and other from Biotronik, grants and other from Abbott Vascular, other from BBraun, outside the submitted work. The other authors declare not to have any type of conflict-of-interest related to the present review.

Correspondence to: Marcello C Bertoluci, MD, DMSc, Serviço de Medicina Interna do Hospital de Clinicas de Porto Alegre, Rua Ramiro Barcellos 2350- Sala 700, Santa Cecília, Porto Alegre 90035-003, Brazil. mbertoluci@uol.com.br
Telephone: +55-51-33598000
Fax: +55-51-33598000

Received: November 6, 2014
Peer-review started: November 10, 2014
First decision: February 7, 2015
Revised: March 11, 2015
Accepted: April 1, 2015
Article in press: April 7, 2015
Published online: June 10, 2015

Abstract

Macro and microvascular disease are the main cause of morbi-mortality in type 1 diabetes (T1DM). Although there is a clear association between endothelial dysfunction and atherosclerosis in type 2 diabetes, a cause-effect relationship is less clear in T1DM. Although endothelial dysfunction (ED) precedes atherosclerosis, it is not clear weather, in recent onset T1DM, it may progress to clinical macrovascular disease. Moreover, endothelial dysfunction may either be reversed spontaneously or in response to intensive glycemic control, long-term exercise training and use of statins. Acute, long-term and post-prandial hyperglycemia as well as duration of diabetes and microalbuminuria are all conditions associated with ED in T1DM. The pathogenesis of endothelial dysfunction is closely related to oxidative-stress. NAD(P)H oxidase over activity induces excessive superoxide production inside the mitochondrial oxidative chain of endothelial cells, thus reducing nitric oxide bioavailability and resulting in peroxynitrite formation, a potent oxidant agent. Moreover, oxidative stress also uncouples endothelial nitric oxide synthase, which becomes dysfunctional, inducing formation of superoxide. Other important mechanisms are the activation of both the polyol and protein kinase C pathways as well as the presence of advanced glycation end-products. Future studies are needed to evaluate the potential clinical applicability of endothelial dysfunction as a marker for early vascular complications in T1DM.

Key words: Endothelial dysfunction; Type 1 diabetes; Cardiovascular disease

Core tip: Endothelial dysfunction is an early finding in the natural history of type 1 diabetes and is predictive for microvascular disease and premature atherosclerosis. Decreased nitric oxide due oxidative stress is the central pathogenetic mechanism. Polyol pathway activation, protein kinase C (PKC) activation and advanced glycation product formation are also important. Long-term hyperglycemia, repeated hypoglycemia and microalbuminuria are factors associated. Intensive glycemic control and exercise training ameliorate endothelial dysfunction. Statins and renin-angiotensin system blockers are partially effective and may be influenced by hyperglycemia. There is a possible clinical benefit for the use of vitamin E and vitamin C that are still to be confirmed. PKC inhibitors are still investigative.

Bertoluci MC, Cé GV, da Silva AMV, Wainstein MV, Boff W, Puñales M. Endothelial dysfunction as a predictor of cardiovascular disease in type 1 diabetes. *World J Diabetes* 2015; 6(5): 679-692 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/679.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.679

INTRODUCTION

Micro and macrovascular complications are leading causes of morbidity and mortality in patients with type 1 diabetes mellitus (T1DM)[1,2]. Subjects with T1DM are prone to accelerated atherosclerosis[3] and have 3 to 6 times more risk of cardiovascular death than individuals without diabetes. Endothelial dysfunction (ED) is an early event along the natural history of T1DM, indicating a phenotype in risk for accelerated atherosclerosis, that may be independent of the classical cardiovascular risk factors[4,5]. Interestingly, traditional cardiovascular risk factors altogether can not explain the totality of the cardiovascular risk in T1DM[6,7]. Chronic hyperglycemia *per se*, although an important predictor of microvascular disease, is a weak predictor of macrovascular complications in both T1DM and T2DM[8,9]. Thus, much of the residual cardiovascular risk still remains unexplained. In this context, ED becomes a new important risk factor that should be debated in the cardiovascular scenario. In the present review, we discuss recent evidences in the pathogenesis of endothelial dysfunction, its role as a risk factor for cardiovascular disease and the potential interventions for reducing endothelial dysfunction in T1DM.

THE NORMAL ENDOTHELIUM

The vascular endothelium forms the cell layer that is directly in contact with the vascular lumen, separated from the smooth muscle layer of the basement membrane. Its role is to maintain the homeostasis between the blood and the arterial wall through the synthesis of substances that modulate vascular tone, inhibit platelet aggregation and control the proliferation of vascular smooth muscle cells[10].

In endothelial cells, nitric oxide (NO) is essential for the maintenance of integrity and homeostasis of endothelium[11]. NO is synthesized from L-arginine by the action of endothelial nitric oxide synthase (eNOS) in the presence of oxygen, NADP(H) and the NOS co-factor, tetrahydrobiopterin (BH4)[12]. The synthesized NO diffuses itself quickly into the smooth muscle cell layer and into platelets where it activates guanylate cyclase (GCA), with consequent production of cyclic GMP (cGMP). The presence of cGMP, in turn, promotes vascular relaxation and inhibition of platelet aggregation, keeping the equilibrium between pro and anti-thrombotic factors in the blood and arterial wall. However, as the half-life of NO is very brief, rapidly oxidizing into nitrate, the continuous activation of eNOS becomes the key determinant of NO synthesis and tissue bioavailability[11]. Normally, eNOs is activated by the turbulent blood flow against the luminal endothelial wall (shear-stress) as well as by the stretch of vascular wall cells and changes in the oxygen tension, promoting vascular muscle relaxation, an effect known as "endothelial-dependent vasodilation"[13,14].

The stability of the endothelium is also dependent on endothelial repair and regeneration, which are determined by migration and proliferation of surrounding mature endothelial cell resident the vascular wall[15]. Recently it has been demonstrated that circulating endothelial progenitor cells (EPCs) are important in the endothelial regeneration. EPCs are circulating bone-marrow-derived cells characterized by the expression of varying surface markers that adhere to the damaged endothelium promoting tissue repair[15]. Circulating EPCs are considered biomarkers of endothelial function and prognostic indicators of cardiovascular morbi-mortality. Endothelial dysfunction represents the breakdown of this endothelium homeostasis, leading to a pro-thrombotic and pro-inflammatory that may lead to progressive atherosclerosis.

METHODS FOR ASSESSMENT OF ENDOTHELIAL FUNCTION

Endothelial function can be investigated through invasively and non-invasively techniques. Coronary arteries can be evaluated invasively through angiography with quantitative measurement of changes in the vascular diameter in response to infusion of acetylcholine[16] and also through invasive venous occlusion plethysmography[17], which measures forearm blood flow in response to acetylcholine infusion in the brachial artery. The invasive nature of

these techniques, involving artery cannulation and infusion of vasoactive drugs[14], make them unfeasible to widespread use in clinical practice.

Non-invasive techniques, on the other hand, are being increasingly used in clinical settings. The flow mediated dilation (FMD) is the most popular technique currently used[18-20]. The rationale in FMD is based on reactive hyperemia responsive to shear-stress caused by turbulent blood flow, causing NO to be released and promoting endothelium-dependent vasodilation[14,21]. The measurement of vascular dilation can be done by capturing images of the brachial artery using high-resolution ultrasound[18]. Reactive hyperemia occurs after a period of ischemia, induced by occlusion of the brachial artery, with a sphygmomanometer cuff inflated with progressive release of vasodilator mediators such as adenosine and $^{+}$H ions from ischemic tissue. When the release of blood flow occurs, sudden shear-stress is produced in the brachial vein endothelium, which is a strong stimulus for releasing NO[19]. This mechanism depends on the integrity of eNOs. The lesser the dilation the more severe the dysfunction. Definition of ED is than considered arbitrarily when the increase in dilation is less than 8%[20].

Other non-invasive techniques are used less frequently. Peripheral artery tonometry induced by reactive hyperemia[22] assesses endothelial function by a combination of flow-mediated dilation and measurement of the amplitude of the arterial pulse wave expansion through a pneumatic sensor placed on the index finger. The microvascular reactivity on the forearm skin is evaluated with laser Doppler flowmetry, being the the iontophoresis of acetylcholine the endothelium-dependent vasodilator stimulus. The determination of the complacency of the dorsal hand vein is a minimally invasive method described by Aellig[23] in 1981 which has been used by our group[24]. It consists in a infusion of vasoactive drugs into the vein surface of the dorsum of the hand to measure endothelium-dependent vasodilation in response to acetylcholine, bradykinin or isoproterenol. The venous occlusion plethysmography can also be used to measure changes in forearm blood flow in response to reactive hyperemia. Finally, the measurement of the thickness of the intima-media layer (IMT) of the common carotid by ultrasound is a structural marker of atherosclerosis and correlates inversely with FMD in the brachial artery[25]. Increases in the IMT are indicative of early atherosclerosis.

ENDOTHELIAL CHANGES IN DIABETES

Chronic sustained hyperglycemia in diabetes promotes important structural and functional modifications in the endothelium, as reported in both experimental and clinical studies[26-29]. In the aorta of rabbits with alloxan-induced diabetes, endothelial changes are visible after 2 wk from the onset of hyperglycemia and become more severe after 6 wk of the diabetes onset[26]. The findings include adhesion of leukocytes, platelets and fibrin material on the endothelial surface. In mice, 6 wk after the onset of diabetes induced by streptozotocin (STZ), it is possible to observe increased endothelial permeability and endothelial cell apoptosis[27].

In a classic study using samples of human skin and subcutaneous tissue obtained from autopsies and biopsies of 24 patients with T1DM, which were compared to 9 non-diabetic controls, the most important finding was the increase in the thickness of the basement membrane in T1DM patients compared with non-diabetic subjects[28]. In another study[29], the increased thickness of the capillary basement membrane of skeletal muscle from patients with 12 years of T1DM, could be reversed by intensive glycemic control during one year[29]. In studies using electron microscopy, endothelial cells obtained from umbilical cord blood from pregnant women with T1DM show increased mitochondrial area when compared to pregnant women without diabetes[28]. The clinical significance of these structural changes, however, is still not clear to predict future atherosclerosis.

Important functional changes occur in the endothelium of T1DM. Hyperglycemia induces excess of electrons that leak from the oxidative chain and are captured by oxygen, generating superoxide excess and oxidative stress. Excessive superoxide production uncouples eNOS, impairing NO production[29]. The net effect is a reduction of NO production in response to shear stress in the inner vascular wall. By this way, the main determinant of ED is the preponderance of vasoconstrictor factors released by the endothelium in detriment of vasodilators factors due to the decreased availability of NO[30]. The dysfunctional endothelium leads to a migration of blood cells into the arterial wall, inducing proliferation of smooth muscle cells, platelet aggregation, LDLc oxidation, monocyte adhesion and synthesis of inflammatory cytokines, all factors contributing to atherogenesis[31].

In patients with T1DM, functional changes in endothelium occurs very early in the natural history of diabetes[32]. The duration of diabetes is a major determinant for the presence of endothelial dysfunction in T1DM, being inversely correlated with the endothelium-dependent dilation. ED generally occurs in the first decade of T1DM, earlier than increases in the carotid intima-media layer thickness (Table 1).

ED is a common finding in T1DM, generally seen after 4 years of disease. In the study by Singh *et al*[33], 31 adolescents with 6.8 years of T1DM and poor glycemic control presented both ED and increased intima-media layer thickness of carotid artery, compared with individuals without diabetes. The duration of diabetes was inversely correlated with the endothelium-dependent dilation[33]. These results were confirmed by other authors[34-38] and are in accordance

Table 1 Studies on Flow-Mediated Dilatation and Nitroglycerin Mediated Dilation in patients with type 1 diabetes

Ref.	*n*	Age	Time	Micro-albuminuria	HbA1c	FMD		NTG	
						Type 1 diabetes	Non-diabetes	Type 1 diabetes	Non-diabetes
Clarkson *et al*[36]	80	15-40	13	6%	9.5	5.8 ± 3.7[a]	9.3 ± 3.8	15.6 ± 5.6[a]	19.7 ± 6.6
Lambert *et al*[37]	52	32	15	No	7.9	12.9 ± 9.8	17.3 ± 9.9	14.3 ± 8.0	17.7 ± 8.7
Enderle *et al*[38]	17	41.5	21	No	8.0	8.2 ± 4.6	7.6 ± 4.2	16.3 ± 4.9	18.4 ± 6.4
Lekakis *et al*[35]	31	32	NO: 13	Yes	N: 6.5	NO: 5.8 ± 7[a]	11.0 ± 7	NO: 19.0 ± 6.9[a]	24.0 ± 9.0
			MA: 20		M: 7.1	MA: 0.7 ± 2.5[a]	-	MA: 15.0 ± 2.9[a]	
Dogra *et al*[34]	34	NO: 44	NO: 20	Yes	8.5-8.7	NO: 5.4 ± 0.6[a]	7.9 ± 0.6	MA: 11.9 ± 1.1[a]	20.0 ± 1.2
		MA: 48	MA: 25			MA: 3.2 ± 0.3[a]	-		
Singh *et al*[33]	31	15	7	No	8.6	4.2 ± 3.8[a]	8.2 ± 4.2	17.0 ± 6.0	18.0 ± 6.0
Järvisalo *et al*[4]	45	11	4	No	8.9	4.4 ± 3.4[a]	8.7 ± 3.3	-	-
Ladeia *et al*[39]	18	13	3	80%	9.3	10.9 ± 2.0	11.2 ± 2.4	-	-
Cé *et al*[5]	57	17	7	No	8.6	9.5 ± 6.5[a]	14.6 ± 5.6	22.3 ± 9.8[a]	29.3 ± 4.2

Age: Mean age in years; Time: Time of T1DM (in years); HbA1c: Hemoglobin A1c (%); NO: Normoalbuminuria; MA: Microalbuminuria; FMD: Flow-Mediated Dilatation; NTG: Nitroglycerin Mediated Dilation. [a]$P < 0.05$ *vs* controls.

to the concept that endothelial dysfunction is predictive of early atherosclerosis in T1DM.

More recent data indicate that ED can occur even before 4 years of onset of T1DM[4,39], preceding the onset of microalbuminuria. Järvisalo *et al*[4] compared non-obese, poor-controlled, recent onset T1DM children with age-matched children without diabetes, with respect to FMD and the thickness of intima-media carotid. They observed the presence of endothelial dysfunction in 36% of cases, a lower peak of flow mediated dilation response and increased intimal-media thickness compared with controls. The authors concluded that ED is a common finding in children in the early years of T1DM and may be a predictor for the development of premature atherosclerosis.

The presence of ED, however, is not uncommon before 4 years of T1DM[32]. We found a prevalence of 35.7% of ED in a sub-group of T1DM patients with less than 5 years of diabetes[5]. The data from the above studies indicates that it ED may begin to occur 3 to 5 years from the onset of T1DM.

FACTORS ASSOCIATED WITH ED IN T1DM

Gender

The impact of gender in ED is still undefined, but, in one study, boys with T1DM seemed to be at increased risk. Bruzzi *et al*[40] studied 39 children with T1DM and 45 healthy age-matched controls, evaluated longitudinally with FMD at baseline and 1 year of follow-up[40]. At baseline, T1DM boys and girls had similar FMD values, however, after 1 year, boys had more endothelial dysfunction than girls. The rationale of this difference is still unknown since multivariate analysis did not identify important predictors of endothelial dysfunction[40].

Acute hyperglycemia

Acute hyperglycemia is capable to induce reversible endothelial dysfunction in normal individuals. When non-diabetic subjects are acutely exposed to high concentrations of glucose during dextrose infusion for 6 h, there is an attenuation of the arterial endothelium-dependent vasodilation induced by methacholine (endothelium-dependent vasodilation) while preserving the vasodilator response to nitroprusside (non-endothelium dependent vasodilation)[41]. This indicates that acute rises in blood glucose in contact to a previous normal endothelium can cause acute endothelial dysfunction, but it is not sufficient to promote vascular smooth muscle dysfunction. In another study in normal subjects[42], it was also demonstrated that acute hyperglycemia can cause significant hemodynamic and rheological changes such as increases in systolic and diastolic blood pressure, heart rate and plasma catecholamines, while decreasing arterial blood flow to the leg. Platelet aggregation to ADP and blood viscosity also showed increments. When the authors infused the natural precursor of NO formation, L-arginine, blood pressure and artery flow changes were reversed. When they infused the inhibitor of endogenous NO synthesis, N^G-monomethyl-L-arginine (L-NMMA), hemodynamic effects of hyperglycemia were reproduced, indicating that acute hyperglycemia reduces NO availability even in normal subjects[42].

The effect of acute high glucose in normal endothelium, however, is not observed in all studies. Houben *et al*[43], studied the effect of acute glucose infusion for 24 h in normal individuals and did not observed changes in vascular dilatation of skin microcirculation induced by acetylcholine (Ach), nitroprusside, norepinephrine or nitric oxide synthase antagonist (L-NMA). The differences between studies may be due to methodological differences. It is accepted, however, that insulin can attenuate acute endothelial dysfunction, promoting compensatory vasodilation which may have biased the studies. In other studies, where the action of insulin was blocked

by octreotide, the effect of acute hyperglycemia alone was evident[41].

Long-term hyperglycemia

The association between HbA1c and flow-mediated dilation (FMD) is seen in some cross-sectional studies with patients with T1DM. In the study of Ladeia *et al*[39], with 19 normo and microalbuminuric T1DM patients, there was a moderate positive correlation between FMD and HbA1c. In another study[44], patients with T1DM with HbA1c above 6.0% had significant impairment of endothelial function compared to patients with HbA1c below 6%, indicating that chronic mild increases in mean hyperglycemia are also associated to ED in T1DM.

We studied the impact of chronic glycemic control in endothelial function of T1DM in a historical cohort study[5]. T1DM adolescents under 5 year of disease were evaluated for ED and had their mean HbA1c obtained from medical records in the same institution since their diagnosis. Considering as a whole, the mean historical HbA1c was clearly higher in T1DM patients with endothelial dysfunction compared with T1DM without ED. Interestingly, we observed a moderate inverse correlation between FMD and the historical mean of HbA1c in the first 2 years after the diagnosis of T1DM but not with the more recent HbA1c. The plausible explanation was that endothelial function could be more affected by the long-term than by the short-term glycemic control, supporting the concept of metabolic memory[45]. Glycation of the endothelium in the first years of T1DM seems, by this way, decisive to determine future endothelial dysfunction in T1DM[32].

Post-prandial hyperglycemia

The effect of postprandial hyperglycemia in endothelial function was studied by Giugliano *et al*[42] in individuals with type 2 diabetes. The combined effect of postprandial glucose and hypertriglyceridemia was associated with increased serum concentrations of adhesion molecules such as ICAM-1, E-selectin and VCAM-1. Markers of oxidative stress such as nitrotyrosine increased sharply after ingestion of 75 g of oral glucose. This effect was greater when an additional lipid overload was used, indicating that both acute hyperglycemia and hyperlipemia can affect endothelial function in diabetes[46].

In another study, the same authors[47] observed that, after a glucose overload, ED accentuates until the second hour, but returns to basal level after 4 h from the beginning of the overload. Though, lipid overload did not change FMD until the fourth hour. These data suggests that the effect of postprandial glycaemia in endothelial function is independent of the postprandial lipemia, although both might be mediated by increased oxidative stress.

Hypoglycemia

Recently, it was demonstrated that repeated episodes of hypoglycemia in subjects with T1DM may be associated with endothelial dysfunction and could be an aggravating factor for preclinical atherosclerosis. In a case-control study[48], T1DM with repeated hypoglycemia episodes were compared with age and sex-matched T1DM controls who did not have frequent hypoglycemias. Vascular function was assessed by FMD, intimal-media carotid artery thickness (IMT) and endothelial dysfunction markers such as von Willebrand factor (vWf). The group with increased hypoglycemia episodes presented lower percentages of FMD in response to ischemia, increased IMT measures and higher endothelial function markers. In another study[49], with cross-sectional design, T1DM children and age and gender-matched healthy children were evaluated for vascular function and continuous glucose monitoring system (CGMS) in order to compare the impact of glucose variability and hypoglycemia episodes in vascular function. Subjects with T1DM had significantly lower FMD compared to healthy children. However, when comparing CGMS parameters, the authors found significant inverse relationship between FMD with hypoglycemia indexes but not with variability indexes.

The biological rationale for hypoglycemia inducing endothelial dysfunction in T1DM is that acute hypoglycemia can induce rapid pro-inflammatory, platelet aggregatory, anti-fibrinolytic response, and recurrent hypoglycemia may induce changes in hemostatic factors and viscosity which may decrease perfusion in diabetic microangiopathy[50].

Glycemic variability

Glycemic variability (GV) is a term exclusively related to blood glucose fluctuations and must be differentiated from post-prandial glucose (PPG). GV is related to glucose variability along the day, while PPG is related to the precise time of glucose rise after a meal. PPG effect in vascular function can also be influenced by other confounders such as hypertriglyceridemia.

Whether GV is an important factor to cause ED is still a matter of debate. The effect of glycemic variability in ED was studied in T1DM with normal urinary albumin excretion[51]. Patients were exposed to 48 h of good (mean 113 mg/dL) or poor metabolic control (mean 286 mg/dL) and evaluated with FMD and serological markers of endothelial function. Both endothelium-dependent and endothelium-independent vasodilation were significantly impaired after the poor control period in relation to good glycemic control. There was also a significant increase in vWf levels after the deterioration of control. These results indicate that endothelial function may suffer significant impact of acute variability of blood glucose in T1DM, although it

can be reversed with improvement of glycemic control. However this effect was not seen in other studies. In the DCCT study, the GV in glycemic data using 7-point self monitoring blood glucose (SMBG) obtained every 3 mo did not correlated to macrovascular complications[52]. We were also not able to detect a relationship between FMD and the standard deviation (SD) of glycemia in T1DM, using day 7-point SMBG for 30 d preceding FMD (data unpublished). Finally, in a cohort study of T1DM[53], the standard deviation of blood glucose, calculated from self-monitoring blood glucose data along 11 years of follow up, was predictive for incident peripheral neuropathy[53]. The influence of GV in vascular function and future micro or macro vascular complications of T1DM is not yet established.

Microalbuminuria

Microalbuminuria is strongly associated with ED in T1DM. Dogra *et al*[34] studied long-term T1DM patients with microalbuminuria with poor glycemic control who were compared to normoalbuminuric T1DM and to non-diabetic individuals. FMD was more severely impaired in T1DM patients with microalbuminuria being albuminuria an independent predictor of ED. In another study, Lekakis *et al*[35] observed lower FMD values in microalbuminuric compared with normoalbuminuric patients. In a similar study with children and adolescents with T1DM with less than 5 years of disease[39], there was a negative correlation between the percent of endothelium-mediated dilation and albuminuria. Mean FMD was also significant decreased in microalbuminuric. ED is also be present in type 2 diabetes patients with normal albuminuria with long duration T2DM during chronic poor glycemic control[54]. In T1DM patients with normoalbuminuria ED is less common[40].

MECHANISMS OF ED IN T1DM

Oxidative stress

Children with T1DM have increased oxidative stress and reduction of anti-oxidant defense compared to healthy children and to their parents[55,56] and these results are similar in adolescents with T1DM[57]. Moreover, endothelial progenitor cells are also reduced in children with T1DM compared to non-diabetic controls possibly related to oxidative stress[58].

Hyperglycemia can cause excessive production of superoxide in the mitochondria oxidative chain of endothelial cells. The excess of superoxide reacts rapidly with NO, reducing NO bioactivity and producing peroxynitrite (ONOO-). Peroxynitrite is a potent oxidant agent and an activator of the lipid peroxidation which may impair endothelial function by stimulating arachidonic acid metabolism[59]. The overproduction of superoxide and NO favors the formation of peroxynitrite by interfering with the production of the eNOS cofactor, tetrahydrobiopterin (BH4)[60].

NAD(P)H oxidase is a chief determinant enzyme of superoxide production in animal models of vascular diseases, including diabetes[32]. In arteries of patients with diabetes who were submitted to artery bypass surgery, it was demonstrated that the endothelium can produce superoxide induced by a dysfunctional eNOS. Dysfunctional eNOS is caused by the oxidation of the co-factor BH4 into BH2. The enzymatic uncoupling of eNOS in human endothelium turns eNOS into dysfunctional eNOS which promotes a transition from NO production to superoxide production[60,61].

Protein kinase C pathway activation

Protein kinase C (PKC) is a cytoplasmic family of enzymes with a wide variety of actions in intracellular signal transduction. The activation of PKC by decreases endothelium derived nitric oxide synthesis, whereas its inhibition increases NO release. The beta isoforms are activated in response to hyperglycemia[62]. The activation of PKC system is also associated with increased albuminuria in rats[63].

There are several mechanisms in which PKC may decrease the bioavailability of NO. PKC antagonizes activation of eNOS, decreases NO concentration, and induces NAD(P)H oxidase to produce superoxide, which, in turn, uncouples eNOS, inducing the production of even more superoxide. PKC is associated with various vascular disorders such as a decrease of Na^+/K^+ ATPase, increased extracellular matrix, increased vascular permeability, contractility and cell proliferation. The activation of PKC system is also associated with increased albuminuria in rats[63].

In a randomized double-blind placebo controlled clinical trial, in healthy individuals submitted to acute hyperglycemia with hyperglycemic clamp technique, FMD was attenuated by hyperglycemia and reversed after treatment for 7 d with the PKC beta inhibitor, LY333531, indicating that the PKC-B system is an important regulator of hyperglycemia-induced endothelial dysfunction[64].

Advanced glycation products

In the presence of sustained hyperglycemia, tissue proteins such as collagen undergo non-enzymatic glycation and formation of cross-links, resulting in advanced glycation end-products (AGEs). AGES promotes a permanent chemical modification of proteins, stimulating cellular responses through specific anti-proliferative receptors[65,66]. These receptors were first observed in experiments in mouse peritoneal macrophages[66], showing ability to remove modified glycated proteins. AGEs may reduce the availability of endothelial NO, and reactive AGE intermediates may compromise their anti-proliferative effect.

Polyol pathway activation

Chronic hyperglycemia increases the activity of aldose reductase enzyme and leads to activation of polyol pathway, transforming glucose into sorbitol and subsequently into fructose. It also induces the consumption of NADP(H), an important cofactor for NO synthesis[61]. As NADP(H) is an important cofactor for NOS to NO synthesis, its depletion leads to reduction of NO production. It remains uncertain, however, the magnitude of importance in the prevention of human atherosclerosis.

ED AS A MARKER OF CARDIOVASCULAR RISK IN T1DM

Although endothelial dysfunction and chronic low-grade inflammation have been associated with atherothrombotic cardiovascular disease, independently of traditional cardiovascular risk factors in either individuals with or without diabetes, a clear cause-effect relationship with atherosclerosis is not yet established in the natural history of T1DM.

In non-diabetic patients with coronary disease, endothelial dysfunction is predictive for increasing risk of cardiovascular events[67]. In an observational study, 157 patients with mild coronary disease were classified according to the severity of ED, which was defined by intracoronary ultrasound with vascular reactivity after administration of acetylcholine, adenosine or nitroglycerin[67]. They were followed by a mean of 28 mo for the assessment of cardiovascular outcomes. At the end of follow up, patients with more severe ED presented 14% of cardiovascular events, while those with mild or no ED had no cardiovascular outcomes ($P < 0.05$)[67]. This study demonstrated, for the first time, that patients with mild coronary disease but with severe ED were at increased risk for cardiovascular events.

Serum markers of ED

The vWf and C-Reactive protein (CRP) are related to ED and inflammation. In the population-based cohort study, the HOORN study[68], the predictive value of the serum ED marker, vWf, was evaluated for cardiovascular mortality in T2DM patients. The cohort including 2.484 caucasian individuals with ages between 50-70 years, in which 27% had T2DM and 27% had impaired glucose tolerance, was followed by 5 years[68]. Patients with vWf levels in the upper tertile had a 3 fold increase in cardiovascular mortality compared to those in the lower tertiles, even after adjustments for age, sex and glucose tolerance status. The relative risk for all-cause mortality associated with vWf was 2.03 (95%CI: 1.19 to 3.47). The predictive value of vWf was not confirmed in ARIC study[69], however, vWF is also an independent predictor of cardiovascular mortality in specific populations[70].

CRP is an inflamatory marker and can be increased in T1DM patients without clinical macroangiopathy, compared with healthy subjects[71]. This increase is greater in the presence of micro or macroalbuminuria compared with normoalbuminuric patients indicating an association between endothelial dysfunction and vascular inflammation[71].

The mechanisms by which the cardiovascular risk is associated with elevated levels of vWf and CRP are not completely understood. It may reflect generalized endothelial dysfunction, increased prothrombotic state[71], inflammation and greater risk for developing atherosclerosis[72]. Von Willebrand factor in combination with *t*-PA measurement may also be an index for endothelial dysfunction[73].

Markers of endothelial function can also be determinants of inflammation. In the EURODIAB Prospective Complications Study[74], a nested case-control study of 543 T1DM participants, the levels of serum markers of ED such as E-selectin, vascular adhesion molecule-1 cell (VCAM-1) and inflammatory markers were determined. In this study, endothelial dysfunction was strongly associated with inflammatory activity suggesting that endothelial dysfunction may interact with vascular inflammation in T1DM which potential consequences in accelerating atherosclerosis[74].

Flow mediated dilation

Impaired flow mediated dilation (FMD) may also predispose to early atherosclerosis in T1DM patients, as seen by the development of increased carotid artery thickness (IMT). In a cross-sectional study, 45 children with T1DM and 30 healthy matched in age, gender and body size were evaluated for FMD and IMT[75]. Children with diabetes presented lower peak FMD response and increased IMT compared to non-diabetic children. In another cross-sectional study[75], T1DM adolescents without diabetes complications were compared with healthy age-matched controls in respect to FMD and the presence of diastolic dysfunction with pulse wave Doppler and tissue Doppler echocardiography measurements. ED was associated with segmental diastolic dysfunction. These studies suggest that ED is associated with indirect evidences of premature atherosclerosis, however, long-term prospective studies are still needed to conclude if FMD is predictive for cardiovasclular events in early T1DM.

CLINICAL MANAGEMENT OF ED IN T1DM

Intensive insulin therapy and glycemic control

There are compelling evidences indicating that optimizing glycemic control with intensive insulin therapy reduces the development and progression of microvascular complications[1]. Although endothelial

dysfunction and oxidative-stress are early changes in T1DM, both conditions are only partially reversed by insulin therapy alone[76]. In adults with 8 years of T1DM who are in poor glycemic control, acute intravenous insulin infusion can only partially reverse impaired FMD, even after completely normalizing glycaemia. It is likely that a more prolonged treatment is necessary for achieving a reversion to normal functioning of endothelium-dependent vasodilation in T1DM[76].

In a clinical trial, 92 children and adolescents with T1DM in conventional insulin therapy were randomized for either continuing in conventional insulin therapy or to switch to a more intensive insulin therapy, including insulin infusion pump and multiple insulin injections. After 1 year of intensive insulin therapy, the baseline vascular response to acetylcholine and the levels of E-selectin improved significantly in the intensive group, while no effect was seen in the conventional group. Interestingly, in this study the benefit was independent of HbA1c, suggesting that intensive insulin therapy may confer vascular protection in addition to improving glycemic control[76].

Exercise

Exercise has a great impact in the mitigation of ED in patients with cardiovascular risk factors both in T1DM and T2DM. In children with T1DM, 30 min of aerobic training, two times a week, for 18 wk can significantly increase flow mediated dilation in around 65%[77]. In adults with T1DM, 60 min of aerobic training, 2 times a week, significantly improves flow mediated dilation in more than 50% after 24 wk[78]. In a cross sectional study, T1DM adolescents who did more than 60 min daily of moderate-to-vigorous physical activity have higher flow mediated dilation than inactive patients with diabetes[79]. Many other studies also show improvement of vasodilator response in T2DM without coronary artery disease, with both aerobic and mixed aerobic/resistance training with 8 to 12 wk of duration[80-82].

The main mechanism underlying the amelioration of vasodilation in response to exercise is largely related to the increased in nitric oxide (NO) bioavailability, resulted from the increased activity and expression of the eNOS and the diminished degradation of NO due to the action of radical oxygen species (ROS). Cultured cells experiments[83] indicate that shear -tress can induce eNOS expression and activity due to stabilization eNOS mRNA or increasing its synthesis. In humans with coronary artery disease, it was also demonstrated a two-fold increase in eNOS expression and a 3.2 increase in the phosphorylation of eNOS after 4 wk of regular training[84]. The increase in antioxidant defenses such as the activity of superoxide dismutase and glutathione peroxidase is another important mechanism underlying the improvement of endothelial function by exercise seen in patients with heart failure[85].

Exercise training can increase the number of circulations EPCs in healthy subjects[86] and coronary artery disease patients[87] after 4 wk of regular training. EPCs are decreased in patients with T1DM compared to healthy subjects[83,88]. The effect of exercise impact in EPCs in type 1 patients, however, remains to be clarified.

Anti-oxidants

Experimental studies demonstrated that antioxidants can modulate the response of the endothelium dependent vasodilation, endothelium-leukocyte interactions and the balance of pro and anti-thrombotic factors[89-91].

In clinical studies, treatment with oral high-dose vitamin E during 6 mo have yielded conflicting results in ED improvement in patients with T1DM. In the first study, Skyrme-Jones *et al*[92] compared the effect of vitamin E 1000 UI/d with placebo in a double-blind randomized clinical trial in patients with T1DM in FMD. They found significant increases in FMD after 3 mo in the group receiving vitamin E. They considered that vitamin E decreased the LDLc oxidant capacity, thus reducing ED. In a double-blind randomized clinical trial in adults with both T1DM and T2DM, Beckman *et al*[93] compared the use of a combination of vitamin E (800 UI/d) with vitamin C (1000 mg/d) compared to placebo. After 6 mo, FMD increased significantly in the T1DM group but not in T2DM. These data are promising, however, neither all studies confirm these findings. In a randomized clinical trial, Economides *et al*[94] studied the effect of high-dose vitamin E (1800 UI) against placebo in T1DM and T2DM along 12 mo but failed to find improvements in ED[94]. The clinical effectiveness of vitamin E in improving ED and reducing the progression to atherosclerosis remains to be established in larger trials in T1DM.

Ascorbic acid infused together with intravenous insulin with near normalization of glycaemia, can rapidly normalize endothelial dysfunction in T1DM[75]. This effect is not completely attained, however, when either intensive glycemic control with insulin or ascorbic acid infusions are used alone, indicating that an additive effect of both treatments exist, an effect that may be limited to new-onset type 1 diabetes[89]. Ascorbic acid can also decrease transcapillary albumin escape[95] and urinary albumin excretion in T1DM adults[96]. In children with T1DM, the combined use of ascorbic acid and vitamin E can increase superoxide-dismutase levels[57].

Low ingestion of antioxidants, especially vitamins, is associated with increased risk of cardiovascular disease and atherosclerosis[97,98]. The inverse correlation between concentrations of antioxidant agents, vitamins and disease risk could be associated to higher requirement of antioxidant molecules during inflammatory diseases.

Insufficient supply with these compounds may further accelerate disease process[99]. On the other hand, these data are not yet been convincingly established in clinical trials and are still controversial[100-102].

Statins

Statins may have benefic effect on endothelial dysfunction in patients with T1DM. In a clinical trial with 204 long-term T1DM randomized to receive atorvastatin 40 mg plus hypolipemic diet or placebo plus hypolipemic diet for 6 mo, FMD increased 44% and PAI-1 was reduced in atorvastatin group compared to placebo[103]. Similar results were observed in a small cross-over trial including 16 T1DM with microalbuminuria[104] who received atorvastatin 40 mg or placebo for 6 wk with a 4-wk period of washout. FMD and non-endothelium dependent vasodilation increased significantly while using atorvastatin. In a meta-analysis of 10 studies including 845 patients with both T1DM and T2DM[105], statin therapy significantly ameliorates FMD in patients with diabetes, although heterogeneity among trials was found. Statins however, improved FMD only in patients with better endothelial function. Factors associated with improvements were: T1DM, younger age, lower baseline lipid levels and blood pressure. Mechanisms enrolled in this effect are not completely known but may be related to reductions in LDLc as well as pleiotropic anti-oxidant effects of statins.

ACE inhibitors

Experimental evidences suggest that ACE inhibition may have benefic effects to the endothelium *in vitro*[106,107]. In a clinical trial, quinapril showed benefit in coronary endothelial function of non-diabetic patients with CAD[108]. ACE inhibitors improve FMD in T1DM with microalbuminuria although not in T1DM with normoalbuminuria[109]. In another small trial with normotensive T1DM patients with microalbuminuria, ACE-I inhibitors improved both FMD and GTN in the femoral artery after 1 wk of treatment[110].

The direct renin blockade was studied in normoalbuminuric T1DM with the use of aliskiren during 4 wk of monotherapy, followed by 4 wk of a combination of aliskiren and ramipril. In both conditions of hyperglycemia and euglycemia of short duration, obtained through euglycemic clamp and hyperglycemic clamp techniques, ED ameliorated with aliskiren alone and improved further when in combination with ramipril. This effect only occurred in the euglycemic phase of the study. Effects were abolished when patients became hyperglycemic[111].

PKC inhibitors

Ruboxistaurin (RBX) is an orally administered isoform-selective inhibitor of PKC which was demonstrated to have beneficial effect in experimental models of diabetic retinopathy[112] and in hemodynamic retinal abnormalities of patients with diabetes[113]. The studies PKC-DRS[114] and PKC DRS2[115] showed a 50% reduction in vision loss of patients treated with RBX. The effect of RBX was than studied in 2 combined phase 3 trials: MBDL and MBCU. Both were randomized, double-blind, placebo-controlled, clinical trials, including T1DM and T2DM with ages above 18 years. Patients had HbA1c below 11%, blood pressure below 160/90 mmHg and diabetic retinopathy. Patients were submitted to pan-photocoagulation or focal photocoagulation after randomization. Patients were than randomized to RBX 32 mg or placebo and followed by 36 mo (in MBDL) and 48 mo (in MBCU). Altogether, 1040 patients were randomized. Sustained moderate visual loss occurred in 4.4% of placebo *vs* 2.3% of RBX treated patients (P = 0.045). The results were promising, indicating a potential reduction in visual loss of 50% above standard care[116]. RBX has also been studied in diabetic neuropathy in other smaller studies. In a 6 mo clinical trial, RBX *vs* placebo in patients with both T1DM and T2DM. Patients who were randomized for RBX presented improvement of neuropathic symptoms and ameliorated decreased skin microvascular blood flow[117]. Although promising, RBX is not available for clinical use.

CONCLUSION

ED should be a concern for clinicians as an early and common phenomenon in T1DM, which may be predictive for future microvascular disease and early atherosclerosis. The clinical use of endothelial function measurement in clinical practice, specially FMD, is a potential tool to enhance cardiovascular risk prediction. Long-term intensive insulin treatment with optimized glycemic control along with exercise training are essential to prevent ED in these patients. Drugs such as statins and ACE-inhibitors are partially effective and may be influenced by the degree of hyperglycemia, with better response in microalbuminuric patients. There is also a possible benefit in using anti-oxidants such as vitamin E and vitamin C, but there is a clear demand for long-term randomized clinical trials to define their role in ED treatment. New agents such as PKC inhibitors are still investigative, but hold promise for future treatment of ED in T1DM.

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P- Reviewer: Magni P **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Zhang DN

World Journal of Diabetes

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i5.693

World J Diabetes 2015 June 10; 6(5): 693-706
ISSN 1948-9358 (online)

REVIEW

Management of critically ill patients with type 2 diabetes: The need for personalised therapy

Palash Kar, Karen L Jones, Michael Horowitz, Adam M Deane

Palash Kar, Adam M Deane, Discipline of Acute Care Medicine, Level 5, Eleanor Harrald Building, University of Adelaide, South Australia 5000, Australia
Palash Kar, Adam M Deane, Intensive Care Unit, Level 4, Emergency Services Building, Royal Adelaide Hospital, South Australia 5000, Australia
Karen L Jones, Michael Horowitz, Adam M Deane, Centre for Research Excellence, University of Adelaide, South Australia 5000, Australia
Karen L Jones, Michael Horowitz, Discipline of Medicine, Level 6, Eleanor Harrald Building, University of Adelaide, South Australia 5000, Australia

Author contributions: Kar P was involved in conception and design of manuscript, acquiring and interpretation of data and drafting and revising the manuscript for final submission; Jones KL and Horowitz M co-supervised Kar P and were involved in conception, design and coordination of the manuscript along with drafting and revising the manuscript; Deane AM supervised Kar P, and was involved in conception and design of manuscript, acquiring data, analysis and interpretation of data, and drafting and revising the manuscript for final submission; all authors read and approved the final manuscript.

Conflict-of-interest: The authors declare there are no non-financial competing interests. Horowitz M has participated in advisory boards and/or symposia for Novo/Nordisk, Sanofi-aventis, Novartis, Eli-Lily, Boehringer Ingelheim, AstraZeneca, Satlogen and Meyer Nutraceuticals.

Correspondence to: Dr. Palash Kar, Intensive Care Unit, Level 4, Emergency Services Building, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia. p_kar@hotmail.com
Telephone: +61-8-82224624

Received: December 24, 2014
Peer-review started: December 26, 2014
First decision: February 7, 2015
Revised: February 20, 2015
Accepted: April 1, 2015
Article in press: April 7, 2015
Published online: June 10, 2015

Abstract

Critical illness in patients with pre-existing diabetes frequently causes deterioration in glycaemic control. Despite the prevalence of diabetes in patients admitted to hospital and intensive care units, the ideal management of hyperglycaemia in these groups is uncertain. There are data that suggest that acute hyperglycaemia in critically ill patients without diabetes is associated with increased mortality and morbidity. Exogenous insulin to keep blood glucose concentrations < 10 mmol/L is accepted as standard of care in this group. However, preliminary data have recently been reported that suggest that chronic hyperglycaemia may result in conditioning, which protects these patients against damage mediated by acute hyperglycaemia. Furthermore, acute glucose-lowering to < 10 mmol/L in patients with diabetes with inadequate glycaemic control prior to their critical illness appears to have the capacity to cause harm. This review focuses on glycaemic control in critically ill patients with type 2 diabetes, the potential for harm from glucose-lowering and the rationale for personalised therapy.

Key words: Diabetes; Critically ill; Intensive care; Management; Personalised therapy

Core tip: With diabetes increasing in prevalence, the optimal management of glycaemia in critically ill

patients with pre-existing diabetes remains unknown. Recent data has highlighted therapeutic uncertainties specific to these patients with suggestions that targeted blood glucose concentrations may benefit from consideration of a patient's premorbid glucose state. In patients with uncontrolled type 2 diabetes, it may be safer to target blood glucose concentrations between 10-14 mmol/L, however definitive studies of critically ill patients with poorly controlled diabetes are required. In contrast, in patients with CIAH, or those with well-controlled diabetes (HbA1c < 7.0) have data supporting a more conservative target (6-10 mmol/L).

Kar P, Jones KL, Horowitz M, Deane AM. Management of critically ill patients with type 2 diabetes: The need for personalised therapy. *World J Diabetes* 2015; 6(5): 693-706 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/693.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.693

INTRODUCTION

Patients with diabetes mellitus may develop an acute severe illness that necessitates a level of care that can only be provided within an intensive care unit (ICU)[1]. In the majority of critically ill patients with pre-existing diabetes, the pathophysiological response to the acute illness or injury, and/or the treatments involved, may lead to deterioration in glycaemic control. Despite the high and increasing prevalence of diabetes (both within the community and in the critically ill), the optimal management of glycaemia in critically ill patients with pre-existing diabetes remains unknown. However, recent data has highlighted the therapeutic uncertainties specific to these patients.

The majority of critically ill patients with diabetes have type 2 diabetes[2]. The limited information relating to patients with type 1 diabetes precludes speculation as to whether management of glycaemia in this group should be different from that in type 2 diabetes. Accordingly, this review focuses on critically ill patients with type 2 diabetes addressing issues including prevalence, potential rationale for harm and evidence for personalised therapy.

PREVALENCE

In the community type 2 diabetes occurs frequently with global health expenditure estimated at US $376 billion in 2010, which is expected to rise to US $490 billion by 2030 due to increasing prevalence[3,4]. In Australia it is estimated over the last 15 years, the prevalence has increased from 8.5% to 12.0%[5]. There is a substantial variation in the prevalence of diabetes between countries, peaking in Nauru (31%)[6]. Factors relating to the increase in prevalence include increasing obesity, increasing age and racial region. A limitation in estimating prevalence is that many patients remain unaware of their diagnosis. For example, the estimated prevalence in the United States is 13% of the population, of which 40% is unrecognised or undiagnosed[7].

Diagnosis of diabetes

The prevalence of recognised and unrecognised diabetes varies according to the definitions used, as well as the location and the populations studied. The current diagnostic criteria used by the American Diabetes Association (ADA) involves one of the following; an HbA1c $\geqslant$ 6.5, a fasting glucose $\geqslant$ 7 mmol/L, a 2 h post glucose tolerance test following a 75 g oral glucose load of $\geqslant$ 11.1 mmol/L, or a random blood glucose $\geqslant$ 11.1 mmol/L with symptoms of hyperglycaemia[8]. These criteria were ratified by the World Health Organization (WHO) in 2011[9].

Given each test (HbA1c, fasting, postprandial or random blood glucose) reflects different physiological phenomena, different populations may be diagnosed when using each criterion[10,11]. Each diagnostic test has advantages and disadvantages. Both the fasting glucose and 2 h post glucose tolerance test are established standards, relatively rapid and easy to perform, and predict microvascular complications. However, these tests are subject to day-to-day variability, require patients to fast and only reflect glucose homeostasis at a single point in time[12]. HbA1c is convenient (with no fasting required), can predict microvascular complications, is a better predictor of macrovascular disease (than fasting glucose or 2 h post glucose tolerance test) and has low day-to-day variability[8,12]. Additionally, as the physiological responses to acute illness cause deterioration in glycaemia, estimating glucose control prior to the acute illness - using markers such as HbA1c - to accurately determine which patients have unrecognised diabetes and which patients have "stress hyperglycaemia" is possible[13]. Weaknesses include variations amongst ethnic groups and age, it may be misrepresentative in certain medical conditions (such as certain forms of anaemia and haemoglobinopathies) and the need for a validated, standardised assay[12].

Prevalence of diabetes in hospitalised patients

Compared to the general population, the prevalence of diabetes in hospitalised adult patients (*i.e.*, admitted to general wards) is considered to be greater. Depending on the population, estimates range from between 11%-35% of all patients (Table 1).

Numerous studies in the critically ill have evaluated the prevalence of glucose intolerance (Table 1). However, a limitation of the studies reported is that investigators were unable to identify those patients who had so-called "stress hyperglycaemia" (or critical illness associated hyperglycaemia (CIAH) - the condition of acute glucose intolerance that is confined to the period of critical illness) and those who have unrecognised diabetes. Several studies use either fasting blood glucose ($\geqslant$ 7 mmol/L) and/or random

Table 1 Prevalence of diabetes in hospital population (chronological order)

Ref.	Year	R-D	UR-D	Total study patients	Location	Diabetes diagnosed by	Unrecognised diabetes diagnosed by
Umpierrez *et al*[14]	2002	495 (26%)	223[1] (12%)	1886	Atlanta, United States	Admission history	Fasting blood glucose ⩾ 7 mmol/L Random blood glucose ⩾ 11.1 mmol/L × 2
Wallymahmed *et al*[15]	2005	126 (11%)	13[1] (1%)	1129	Liverpool, United Kingdom	Admission history Hospital records	Random blood glucose ⩾ 11.1 mmol/L
Wexler *et al*[17]	2008	136 (19%)	33 (5%)	695	Boston, United States	Admission history Hospital records	HbA1c > 6.5
Mazurek *et al*[18]	2010	342 (35%)	152 (16%)	971	New York, United States	Admission history Hospital records Medication review	HbA1c ⩾ 6.5
Feldman-Billard *et al*[16]	2013	355 (17%)	156[1] (7%)	2141	Multicentre (France)	Admission history	Fasting blood glucose ⩾ 7 mmol/L

[1]May include patients with stress hyperglycaemia/critical illness associated hyperglycaemia. R-D: Recognised diabetes; UR-D: Unrecognised diabetes.

glucose concentrations (⩾ 11.1 mmol/L) for diagnosis of diabetes[14-16].

Investigators have also measured glycated haemoglobin (HbA1c) on admission to identify hospitalised patients with unrecognised diabetes. A prospective observational study of 695 patients in Boston, Massachusetts[17], selected a cutoff HbA1c of > 6.5% to diagnose diabetes, with 19% of patients having diabetes previously diagnosed and 5% having undiagnosed diabetes. Another study of 971 patients admitted to the general medical ward of an urban hospital located in the Bronx, New York[18] - which may be assumed to admit a larger cohort of lower-income patients - 35% were known to have diabetes, and 16% undiagnosed diabetes, using an HbA1c ⩾ 6.5.

In summary, the prevalence of diabetes in hospitalised patients varies according to geography. In the developed world, diabetes is more prevalent amongst lower socioeconomic groups[19-21]. Furthermore, diabetes is a risk factor for certain diseases (*e.g.*, cardiovascular disease) and prevalence will be greater if a specific population (*e.g.*, patients presenting with myocardial ischaemia) is studied[22].

Prevalence of diabetes in patients admitted to ICU

The prevalence of diabetes in patients admitted to the ICU is estimated to be between 12%-40% (Table 2). Similar to the prevalence in hospitalised patients, the wide range reflects the definitions used and the population studied. Multiple single centre observational studies from the United States[23-25] report prevalence between 13% and 21%, therefore it is likely that the true prevalence is close to this range. More recently, Falciglia *et al*[26] undertook a retrospective cohort study across 173 ICUs in the United States and reported that 30% of the 259040 patients had a history of diabetes according to ICD-9 codes[26].

A single centre, observational study from London, United Kingdom[27], found 16% of patients had a history of diabetes. A retrospective observational study of 4946 patients admitted to one of two hospitals in Melbourne and Sydney, Australia[28], reported 15% had diabetes. While a single, mixed medical/surgical ICU from Amsterdam, The Netherlands[29], found 12% of 5961 patients admitted had a history of diabetes. These data indicate that the prevalence in other developed countries may be similar to, or slightly less than, the United States.

Data from international studies are consistent with this concept. Stegenga *et al*[30] utilised data collected as part of a randomised interventional study[31] to evaluate whether diabetes affects the outcome of sepsis in patients admitted to one of 164 ICUs across 11 countries and reported that 23% had pre-existing diabetes. In retrospective observational data derived from 44964 patients admitted to one of 23 ICUs worldwide[32], 29% had a history of diabetes documented in their medical records, but the prevalence varied substantially according to geography. For example, in an ICU from Geelong, Australia, the prevalence was 14%, while in a hospital < 100 km away (Melbourne) it was 24%, whereas patients admitted to Tampa Bay, United States, the prevalence was 39%.

The prevalence of diabetes in the critically ill varies across studies. Multiple observational studies estimate the prevalence at 12%-30%[23-29,30,32-35]. However, these studies have significant limitations. Most importantly, the prevalence may be under represented due to diabetes that is either unrecognised or not documented.

A number of interventional studies have also reported diabetes prevalence in ICU patients (Table 2). Two prospective, randomised, controlled studies of surgical and medical ICU patients admitted into the ICU in Leuven, Belgium, compared an intensive insulin therapy (ITT, blood glucose level 4.4-6.1 mmol/L) *vs* conventional treatment (insulin started if the blood glucose was > 12 mmol/L and maintained between 10-11.1 mmol/L)[36,37]. These studies reported diabetes at 13% and 17% respectively.

Other interventional studies include single centre[38,39] and multicentre trials[40-42], with the largest being in 2009, the NICE-SUGAR (Normoglycaemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation) study. This was conducted across 42 ICUs

Table 2 Prevalence of diabetes in the intensive care unit population (chronological order)

Ref.	Year	Study type	R-D	UR-D	Total study patients	Location	Recognised DM diagnosis	Unrecognised diabetes diagnosed by
Van den Berghe *et al*[36]	2001	Interv	204 (13%)	N/A	1548	Leuven, Belgium	Admission history	N/A
Finney *et al*[27]	2003	Observ	86 (16%)	N/A	523	London, United Kingdom	Unknown	N/A
Whitcomb *et al*[23]	2005	Observ	574 (21%)	395[1] (15%)	2713	Baltimore, United States	Admission history	Hyperglycaemia without a history of DM
Van den Berghe *et al*[37]	2006	Interv	203 (17%)	N/A	1200	Leuven, Belgium	Admission history	N/A
Krinsely[24]	2006	Observ	1110 (21%)	N/A	5365	Stamford, United States	Hospital records (ICD-9 codes) for the first 2 yr then all available info	N/A
Egi *et al*[28]	2008	Observ	728 (15%)	N/A	4946	Multicentre (Australia)	Hospital records	N/A
Treggiari *et al*[25]	2008	Observ	1361 (13%)	N/A	10456	Seattle, United States	Hospital records	N/A
Arabi *et al*[39]	2008	Interv	208 (40%)	N/A	523	Riyadh, Saudi Arabia	Admission history Hospital records	N/A
Bronkhurst *et al*[38]	2008	Interv	163 (30%)	N/A	537	Multicentre (Germany)	Unknown	N/A
Del La Rosa *et al*[42]	2008	Interv	61 (12%)	N/A	504	Medellin, Colombia	Admission history	N/A
Finfer *et al*[41]	2009	Interv	1211 (20%)	N/A	6029	Multicentre (Australia, NZ, Canada)	Admission history	N/A
Preiser *et al*[40]	2009	Interv	203 (19%)	N/A	1078	Multicentre (Europe)	Admission history	N/A
Falciglia *et al*[26]	2009	Observ	77850 (30%)	N/A	259040	Multicentre (United States)	Hospital records (ICD-9 codes)	N/A
Stegenga *et al*[30]	2010	Observ	188 (23%)	N/A	830	Multicentre (Worldwide)	Admission history	N/A
Hermanides *et al*[29]	2010	Observ	699 (12%)	N/A	5961	Amsterdam, Netherlands	Hospital records (computerised system)	N/A
Krinsely *et al*[33]	2011	Observ	669 (21%)	N/A	3263	Multicentre (United States, Europe)	Hospital records (ICU clinical database)	N/A
Krinsley *et al*[32]	2013	Observ	12880 (29%)	N/A	44964	Multicentre (Worldwide)	Admission history	N/A
Plummer *et al*[34]	2014	Observ	220 (22%)	55 (6%)	1000	Adelaide, Australia	Admission history Phone call to GP HbA1c ⩾ 6.5	HbA1c ⩾ 6.5 without a history of DM

[1]May include patients with stress hyperglycaemia/critical illness associated hyperglycaemia. Interv: Interventional; Observ: Observational; R-D: Recognised diabetes; UR-D: Unrecognised diabetes; NZ: New Zealand; N/A: Not available.

throughout Australia, New Zealand and Canada[41], and noted 20% of its 6029 patients with a history of diabetes, with the majority (92%) having type 2 diabetes.

It should be recognised that there are limitations to using data from these interventional studies. Inclusion into these studies usually requires hyperglycaemia and therefore leads to selection bias, which artificially increases any estimate of prevalence. The interventional trials estimated ICU prevalence at 13%-40%[36-42].

Prevalence of unrecognised diabetes

Patients may have diabetes that is unrecognised prior to admission[2]. This may not represent "stress hyperglycaemia" or CIAH - as the hyperglycaemia is chronic rather than acute. Unrecognised diabetes is important as it not only impacts on estimations for the actual prevalence of the condition, but, as a growing body of evidence suggests, chronic glucose control may have implications on optimal acute glucose ranges in the critically ill.

Hospital and ICU prevalence of unrecognised diabetes can be estimated from the studies mentioned (Tables 1 and 2) along with other studies cited below (Table 3). Hospital prevalence is estimated to be between 5%-16%[16-18,43] and ICU prevalence between 6%-14%[34,44]. The prevalence in patients with ischaemic heart disease (*e.g.*, presenting with acute myocardial infarction) appears to be higher[45,46].

In two European studies, patients with an acute myocardial infarct and without a history of diabetes subsequently underwent an oral glucose tolerance test (OGTT) to diagnose diabetes[45,46]. The prevalence of diabetes was found to be over 30% at discharge, and between 25%-31% at 3 mo. In London (United Kingdom), Emergency Department patients were

Table 3 Prevalence of undiagnosed diabetes in the hospital population (chronological order)

Ref.	Year	Diagnosis	UR-D	Total study patients	Location	Patient population
Norhammer *et al*[45]	2002	OGTT	51 (31%) at discharge 36 (25%) at 3 mo	164 144	Multicentre (Sweden)	Post AMI, Hospital/ICU
George *et al*[47]	2005	Fasting blood glucose ⩾ 7 mmol/L	13 (3%)	427	London, United Kingdom	Emergency Department
Wexler *et al*[17]	2008	HbA1c > 6.5	33 (5%)	695	Boston, United States	Hospital
Lankisch *et al*[46]	2008	OGTT	31 (32%) at discharge 19 (31%) at 3 mo	96 62	Wuppertal, Germany	Post AMI, Hospital/ICU
Mazurek *et al*[18]	2010	HbA1c ⩾ 6.5	152 (16%)	971	New York, United States	Hospital
Feldman-Billard *et al*[16]	2013	Fasting blood glucose ⩾ 7 mmol/L	156 (7%)	2141	Multicentre (France)	Hospital
Plummer *et al*[34]	2014	HbA1c ⩾ 6.5	55 (6%)	1000	Adelaide, Australia	ICU
Hoang *et al*[44]	2014	HbA1c ⩾ 6.5	14 (14%)	102	New Haven, United States	Medical ICU
Ochoa *et al*[43]	2014	HbA1c ⩾ 6.5	8 (9%)	92	Abilene, United States	Hospital

UR-D: Unrecognised diabetes; OGTT: Oral Glucose Tolerance Test; AMI: Acute myocardial infarction.

screened for diabetes *via* fasting blood glucose[47] and it was reported that 3% patients had unrecognised diabetes.

We recently performed a single centre observational study in a mixed medical/surgical ICU in Adelaide, Australia, and separated patients with diabetes (either known or unrecognised) and CIAH using HbA1c to accurately estimate the prevalence of each condition[34]. Of 1000 consecutively admitted ICU patients, 22% had known diabetes (5% were type 1) and 6% had unrecognised diabetes (HbA1c ⩾ 6.5%). The absence of previously diagnosed diabetes was confirmed by a phone call to the patient's usual local medical officer (general practitioner).

Subsequently, Hoang *et al*[44] also estimated the prevalence of undiagnosed diabetes in a prospective, observational study in a single medical ICU[44]. All patients with hyperglycaemia and those with known diabetes underwent measurement of HbA1c with diabetes defined as an HbA1c ⩾ 6.5%. Sixty-six percent of the 299 patients enrolled into the study had a history of diabetes. Of the remaining 102 hyperglycaemic patients without diabetes, 14% had an HbA1c ⩾ 6.5%.

In summary the prevalence of undiagnosed diabetes is difficult to determine, and as previously noted, depends on the definitions used and the location of the patient population. Current "best estimate", albeit on limited data from single centres, suggest that the prevalence of undiagnosed diabetes is either similar to, or slightly greater than, the background prevalence in the community.

RATIONALE FOR HARM FROM HYPERGLYCAEMIA, HYPOGLYCAEMIA AND GLYCAEMIC VARIABILITY

Hyperglycaemia

Hyperglycaemia in type 2 diabetes reflects the outcome of factors affecting both insulin secretion, with β-cell dysfunction resulting in a relative insulin deficiency, and insulin resistance as a result of both environmental and genetic factors[48,49]. However, the pathogenesis of hyperglycaemia in the critically ill patient, either with CIAH, or in those with pre-existing diabetes and experiencing a deterioration in their glucose control, is complex and poorly understood[2]. Patient predisposition (including insulin resistance and β-cell function), the underlying illness (which can result in catecholamine release, stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, and the release of inflammatory cytokines) and the management involved (including glucocorticoids, vasopressors and nutrition) appear to be of major relevance[1].

The activation of the HPA axis and the sympathetic system cause the "stress" response. In the majority of patients "stress" hormones (including cortisol and catecholamines) markedly increase. In addition, the underlying illness may stimulate the production of cytokines (such as TNF-α, IL-1 and IL-6)[1,50]. These three components (HPA axis, sympathetic system and cytokine release) lead to excessive gluconeogenesis, glycogenolysis and insulin resistance, thereby augmenting stress hyperglycaemia[50]. Glucagon is the major modulator of gluconeogenesis and may be stimulated by TNF-α, however cortisol and adrenaline (epinephrine) are also likely to contribute[1,51,52].

Insulin resistance is thought to occur due to a number of pathways. Glucose enters cells *via* plasma membrane glucose transporters (GLUTs), which are down regulated in times of stress, possibly due to the presence of TNF-α and IL-1[50]. Diminished glucose uptake by peripheral tissue may occur due to high cortisol and adrenaline (epinephrine) concentrations[1,53]. As discussed, acute illness results in increased level of cytokines, which exacerbates hyperglycaemia and stimulates inflammation and oxidative stress[1].

It should be considered that acute hyperglycaemia may represent a "protective" physiological response of

Table 4 Observational studies (diabetes as a binary variable) and outcomes related to hyperglycaemia (chronological order)

Ref.	Year	Study pts	Study point	Patients without diabetes	Patients with diabetes	Overall message
Rady *et al*[35]	2005	7285	Glycaemia *vs* hospital mortality	Inc mortality with blood glucose > 8 mmol/L	Inc mortality with blood glucose > 11.1 mmol/L	Mortality inc in non diabetics (10%) compared to diabetics (6%), ($P < 0.01$)
Whitcomb *et al*[23]	2005	2713	Admission hyperglycaemia (> 11.1 mmol/L) *vs* in-hospital mortality	Admission hyperglycaemia associated with inc mortality in CICU, CTICU and NSICU	Admission hyperglycaemia not associated with mortality	Mortality inc in non diabetics (10%) compared to diabetics (5%), ($P < 0.05$)
Krinsely[24]	2006	5365	Pre ITT and post ITT *vs* hospital mortality	Dec mortality risk with mean blood glucose 3.9-6.7 mmol/L Inc mortality risk with mean blood glucose > 7.8 mmol/L Mortality drop 19% (pre-ITT) to 14% (post-ITT), $P < 0.01$	Dec mortality risk with mean blood glucose 3.9-5.5 mmol/L Inc mortality risk with mean blood glucose > 10.0 mmol/L No statistically significant change in mortality pre and post ITT	Non-diabetics: 4.5-fold inc in mortality from lowest mean blood glucose, 3.9-5.5 mmol/L (9%) to highest, > 10mmol/L (40%) Diabetics: 2-fold inc in mortality from lowest mean blood glucose, 3.9-5.5 mmol/L (13%) to highest, > 10mmol/L (26%)
Egi *et al*[28]	2008	4896	Glycaemia *vs* mortality	Inc risk of ICU mortality with hyperglycaemia - with non survivors spending more time with blood glucose > 8.0 mmol/L	No association with hyperglycaemia and ICU mortality Lower OR of death at all levels of hyperglycaemia	Diabetic patients: lower ICU mortality ($P = 0.02$) No difference in hospital mortality between groups ($P = 0.3$)
Falciglia *et al*[26]	2009	259040	Glycaemia *vs* mortality	5-fold inc in mortality from lowest mean blood glucose, 3.9-6.1 mmol/L (8%) to highest, > 16.7 mmol/L (41%)	2-fold inc in mortality from lowest mean blood glucose, 3.9-6.1 mmol/L (6%) to highest, > 16.7 mmol/L (11%)	Hyperglycaemia associated with inc mortality in diabetics and non diabetics Mortality greater for hyperglycemic non diabetics patients
Stegenga *et al*[30]	2010	830	DM *vs* outcomes of sepsis	Admission hyperglycaemia (> 11.1 mmol/L) associated with inc 28 and 90 d mortality ($P < 0.03$)	Admission hyperglycaemia had no effect on diabetic mortaltity	Diabetes did not influence mortality in sepsis
Krinsley *et al*[32]	2013	44964	Hyperglycaemia, hypoglycaemia, and glycemic variability *vs* mortality (and how DM effects this)	Inc mortality with higher mean blood glucose (≥ 7.8 mmol/L) Dec mortality with lower blood glucose (4.4-7.8 mmol/L)	Inc mortality with mean blood glucose between 4.4-6.1 mmol/L Dec mortality when blood glucose were higher (6.2-10 mmol/L)	Hyperglycaemia, hypoglycaemia, and increased glycemic variability are independently associated with mortality in ICU patients Diabetic status tempers these relations

Inc: Increased; Dec: Decreased; CICU: Cardiac Intensive Care Unit; CTICU: Cardiothoracic Intensive Care Unit; NSICU: Neurosurgical Intensive Care Unit; ITT: Intensive insulin therapy.

the host during periods of stress[50]. An acute rise in glycaemia may facilitate glucose delivery at critical times and promote anti-apoptotic pathways, protecting against cell death[50]. While uncontrolled acute hyperglycaemia is clearly harmful, the threshold at which harm occurs in the critically ill patient remains to be determined[2]. The majority of studies that have evaluated this issue have enrolled heterogenous cohorts - and patients with diabetes only comprised a small proportion of the sample evaluated. Based on recent data it is increasingly likely that the glucose threshold in a patient with diabetes, particularly those with chronic hyperglycaemia, will differ from that in a patient who is naïve to hyperglycaemia. A patient with poorly controlled diabetes, i.e., with a history of high blood glucose levels and consequently high HbA1c, will be more tolerant of hyperglycaemia but susceptible to the adverse effects of hypoglycaemia (see below), such that the thresholds for both variables are greater than a patient who is naïve to hyperglycaemia - either those with well controlled diabetes or those with CIAH.

Multiple studies have examined the effects of hyperglycaemia on morbidity and mortality in the ICU population with inconsistent and controversial outcomes. Moreover, the majority of these studies have not categorised patients into those with chronic hyperglycaemia or acute glucose intolerance.

There are numerous observational studies (Table 4). In 2005, a case controlled study of 7285 ICU patients reported that in individuals without known diabetes, mortality was increased when blood glucose levels were > 8 mmol/L but this signal was absent in patients with diabetes[35]. Overall, mortality was significantly greater in patients without diabetes when compared to patients with diabetes. A retrospective study of 2713 patients admitted into ICU[23] reported an association between mortality and hyperglycaemia in patients without a history of diabetes in the cardiac, cardiothoracic, and neurosurgical intensive care units. In an audit of 5365 ICU

patients evaluated before and after implementation of an intensive glucose control policy[24], mortality was increased in patients with hyperglycaemia who were not known to have diabetes when compared to those with diabetes. In 2008, Egi *et al*[28] reported a retrospective study of 4946 patients in which ICU mortality increased with increasing mean blood glucose level in patients without diabetes but this signal of harm was absent in those with pre-existing diabetes[28].

A retrospective cohort study of 259040 ICU admissions also reported an association between mortality and hyperglycaemia, with the relationship far stronger in patients without a diagnosis of diabetes when compared to those with pre-existing diabetes[26]. A retrospective analysis of a previous study[31] included 830 patients admitted with severe sepsis (defined as sepsis associated with acute organ dysfunction)[30], and reported that hyperglycaemia was predictive of subsequent death in those patients not known to have diabetes. Additionally, a multicentre retrospective study of 44964 patients divided into 2 cohorts (with and without known diabetes)[32], reported increased mortality with higher mean blood glucose concentrations (≥ 7.8 mmol/L) when compared to blood glucose concentrations 4.4-7.8 mmol/L in patients without diabetes. In contrast, patients with diabetes were more likely to die when mean blood glucose concentrations were between 4.4-6.1 mmol/L when compared to patients with greater blood glucose concentrations (6.2-10 mmol/L).

A number of interventional studies have evaluated the relationship between chronic and acute hyperglycaemia and outcomes (Table 5). In a pooled analysis of studies conducted in a single centre in Leuven, intensive insulin therapy (ITT, aiming for blood glucose concentrations between 4.4-6.1 mmol/L) was reported to reduce mortality and morbidity in patients without a diagnosis of diabetes, but this was not the case in patients with diabetes, if anything, there was a trend for harm with intensive insulin therapy in patients with diabetes such that mortality was non-significantly greater at a lower mean blood glucose range (6.1-8.3 mmol/L, 21.2% *vs* < 6.1 mmol/L, 26.2%, $P = 0.4$ and > 8.3 mmol/L, 21.6%, $P = 0.9$)[54].

Subsequently, a number of interventional, randomised, controlled trials, containing patients with diabetes, comparing ITT to more conventional glucose targets have been published[38-42]. A trial of 523 mixed (medical and surgical) ICU patients[39] reported no survival benefit in patients with diabetes with ITT, but ITT was associated with an increased prevalence of hypoglycaemia. The Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) study assigned 537 ICU patients with severe sepsis to either ITT or more conventional glucose targets while receiving either 10% pentastarch or a modified Ringers lactate in a two-by-two factorial study[38]. The study was suspended at interim analysis for safety reasons with ITT being associated with increases in episodes of severe hypoglycaemia and adverse events. De La Rosa *et al*[42] also evaluated ITT in 504 ICU patients (61 with diabetes) and there was no mortality or morbidity benefit observed, but an associated increased risk of hypoglycaemia, when administering ITT.

In 2009, the NICE-SUGAR study compared ITT with conventional glucose control in 6029 ICU patients and established that the observations from the initial Leuven studies regarding ITT were not generalisable outside that specialised institution[41]. However, amongst the 1211 patients with pre-existing diabetes in the NICE-SUGAR study the administration of ITT did not appear more harmful than in patients without diabetes. The Glucontrol study[40], an international, multicentre trial involving over 1000 ICU patients-was stopped early due to protocol violations, and it was, accordingly, underpowered. However, there was no evidence to suggest any benefit with ITT and data in patients with diabetes were not specifically described.

Recently a number of studies have attempted to measure chronic glycaemia as a dynamic (HbA1c), rather than a binary, variable (*i.e.*, presence of diabetes - yes/no) (Table 6). Egi *et al*[55] performed a retrospective observational study of 415 patients with diabetes (from two Australian ICUs) in whom glycated haemoglobin (HbA1c) had been measured within 3 mo of their critical illness and evaluated how this measure of pre-existing glycaemia impacted on the interaction between acute glycaemia and mortality[55]. It was reported that in patients with elevated preadmission HbA1c levels (> 7%) the number of deaths were significantly fewer when blood glucose concentrations were > 10 mmol/L.

Consistent with this observation, we recently measured HbA1c on admission and glucose concentrations for the first 48 h of ICU admission[34] and observed that acute peak glucose concentrations were associated with increased mortality only in patients with adequate premorbid glycaemic control (defined as HbA1c < 7%), but not in patients with chronic hyperglycaemia (defined as an HbA1c ≥ 7%). This finding was also supported by Hoang *et al*[44] who assessed the prevalence of undiagnosed diabetes (*i.e.*, HbA1c ≥ 6.5%) among those with hyperglycaemia in a medical ICU. Patients with an HbA1c ≥ 6.5% were found to have significantly lower mortality compared to those with an HbA1c < 6.5% (11.7% *vs* 19.3%, $P = 0.038$), despite having greater glucose concentrations.

In summary the outcomes of the largest and most generalisable randomised study are consistent with the concept that the optimal glucose concentrations in unselected critically ill patients are between 6-10 mmol/L[41]. However, observational data, post-hoc analysis of interventional studies and studies measuring chronic glycaemia as a dynamic variable suggest that patients with pre-existing diabetes may warrant higher targets. Indeed, there is increasing data suggesting that targets should be personalised depending on both

Table 5 Interventional studies (diabetes as a binary variable) and outcomes related to hyperglycaemia (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Van den Berghe *et al*[54][1]	2006	2748	ITT (blood glucose 4.4-6.1 mmol/L) *vs* CIT (insulin if blood glucose > 12 then target 10-11.1 mmol/L) on mortality	Reduced mortality and morbidity with ITT	No survival benefit with ITT Higher rates of hypoglycaemia	Hosp mortality 20% (40/200) of the DM patients in conventional arm Hosp mortality 22% (46/207) of the DM patients in the ITT arm
Arabi *et al*[39]	2008	523	ITT (blood glucose 4.4-6.1 mmol/L) *vs* CIT (blood glucose 10-11.1 mmol/L) on ICU mortality	Mortality: ITT (14%) *vs* CIT (14%) - no significant difference (P = 0.2)	Mortality: ITT (13%) *vs* CIT (20%) - no significant difference (P = 0.3)	No significant difference in ICU mortality between IIT and CIT (P = 0.3)
Brunkhorst *et al*[38]	2008	537	ITT (blood glucose 4.4-6.1 mmol/L) *vs* CIT (blood glucose 10-11.1 mmol/L) on mortality	28 d mortality: ITT 25% *vs* CIT 23% (P = 0.8) 90 d mortality: ITT 40% *vs* CIT 32% (P = 0.2)	28 d mortality: ITT 25% *vs* CIT 32% (P = 0.3) 90 d mortality: ITT 40% *vs* CIT 42% (P = 0.9)	No mortality benefit with ITT *vs* CIT Stopped early due to safety risk
Del La Rosa *et al*[42]	2008	504	ITT (blood glucose 4.4-6.1 mmol/L) *vs* CIT (blood glucose 10-11.1 mmol/L) on morbidity and mortality	ICU mortality ITT 37% *vs* CIT 32% (no significance)[2] In-hospital mortality: ITT 40% *vs* CIT 39% (no significance)[2]	Mortality: ITT (38%) *vs* CIT (31%) - no significant difference	No difference in ICU mortality, 28 d mortality or ICU infections Increased hypoglycaemia in ITT
Finfer *et al*[41]	2009	6029	ITT (blood glucose 4.4-6.1 mmol/L) *vs* CIT (blood glucose < 10 mmol/L) on mortality	Mortality: ITT (27%) *vs* CIT (24%) - no significant difference	Mortality: ITT (32%) *vs* CIT (28%) - no significant difference	ITT arm - inc 90 d mortality No difference in those with and without DM (P = 0.60)
Preiser *et al*[40]	2009	1078	ITT (blood glucose 4.4-6.1 mmol/L) *vs* CIT (blood glucose 7.8-10 mmol/L) on mortality	ICU mortality ITT 17% *vs* CIT 15% (P = 0.4)[2] Hospital mortality: ITT 23% *vs* CIT 19% (P = 0.1)[2]	Not described	Stopped early due to protocol violations

[1]Contains pooled data from the 2001 (surgical) and 2006 (medical) study; [2]Mortality of total patients (includes non-diabetic and diabetic patients). ITT: Intensive insulin therapy; CIT: Conventional insulin therapy; Inc: Increased; Dec: Decrease.

diabetic status and recent glycaemic control.

Hypoglycaemia

In most cases, treatment of hyperglycaemia in the critically ill involves the use of insulin, which is associated with increased risks of both hypoglycaemia and glycaemic variability[56]. The severity of illness may also result in a hypoglycaemia and therefore it is important to be circumspect when attributing mortality to hypoglycaemia[57]. Additionally, hypoglycaemia may have adverse biological effects including an increase in systemic inflammatory response, impairment of the sympathetic nervous system, inhibition of the biological response to stress, along with cerebral vasodilation and neural damage[2,58]. Experimentally, the use of insulin and consequent hypoglycaemia may be associated with hypotension, vasodilation, and reduced autonomic responses to subsequent hypoglycaemic episodes[58]. Furthermore, critically ill patients may be more prone to the effects of hypoglycaemia itself, which may include cardiac arrest, seizure and coma[59].

Studies examining the effects of hypoglycaemia in critically ill patients with pre-existing diabetes are limited. Interventional studies describing this relationship have been summarised (Table 6). Of note, post hoc analysis of the NICE-SUGAR data indicate that intensive insulin therapy increases episodes of moderate (2.3-3.9 mmol/L) and severe ($\leqslant$ 2.2 mmol/L) hypoglycaemia, both of which are associated with increased risk of death[56]. This relationship was similar among patients with and without a diagnosis of diabetes.

In addition to these studies, there are a number of observational studies that have evaluated this association (Table 7). A retrospective database review of 408 ICU patients (102 index cases, 306 controls) published in 2007[60] reported that a history of diabetes was associated with severe hypoglycaemia and that a single

Table 6 Observational studies that have recorded chronic glycaemia as a dynamic variable (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Egi *et al*[55]	2011	415	Does preexisting hyperglycaemia modulate the association between glycemia and outcome in ICU patients with DM	N/A	Patients with elevated preadmission HbA1c levels (> 7%) showed a mortality benefit when mean ICU glucose concentrations were > 10 mmol/L	Relationship between HbA1c and mortality changed according to the levels of time-weighted average of blood glucose concentrations
Plummer *et al*[34]	2014	1000	Prevalence of CIAH and recognized/unrecognized DM in ICU and to evaluate the premorbid glycaemia on the association between acute hyperglycaemia and mortality	50% had CIAH Risk of death inc by 20% for each increase in acute glycaemia of 1 mmol/L	Well controlled DM (HbA1c < 6%) and adequately controlled (DM 6%-7%) - risk of death as per non diabetic patient HbA1c ⩾ 7% (insufficiently controlled DM) had no significance between mortality and acute glycaemia	22% had recognised DM 6% had unrecognised diabetes
Hoang *et al*[44]	2014	299	Prevalance of unrecognized DM amongst those with CIAH and the association between baseline glycaemia and mortality	102 (34%) had no history of DM 14/102 (14%) had unrecognized DM (diagnosed with HbA1c ⩾ 6.5)	197 (66%) had a history of DM	Lower HbA1c had inc mortality (in this population of CIAH patients) despite lower median glucose values and less glucose variability Mortality in HbA1c < 6.5 (19%) *vs* HbA1c ⩾ 6.5 (12%), $P = 0.04$

Inc: Increased; Dec: Decreased; N/A: Not available.

hypoglycaemic episode was associated with an increased risk of mortality (compared with those without an episode of severe hypoglycaemia). Egi *et al*[61] reported mild or moderate hypoglycaemia was associated with mortality in critically ill patients - with mortality substantially increasing according to severity of hypoglycaemia - and patients with diabetes were more likely to suffer from insulin-associated hypoglycaemia.

The blood glucose threshold that adverse events occur may be greater in patients with pre-existing diabetes. In a retrospective multi-centre observational study[32] increased mortality was reported in 12880 patients with pre-existing diabetes who had mean glucose concentrations between 4.4-6.2 mmol/L. While the investigators were not able to differentiate between patients with well-controlled or poorly-controlled diabetes, these data support the concept that the threshold for "hypoglycaemia" may be increased in critically ill patients with diabetes when compared to non diabetic patients. For example, if a patient typically has blood glucose concentrations above 10 mmol/L, and, in hospital, insulin is administered to achieve blood glucose concentration of about 6 mmol/L, this may result in a "relative" hypoglycaemia.

Glycaemic variability

Glycaemic variability (GV) describes the fluctuations in blood glucose concentrations, as marked fluctuations may be associated with multiple adverse effects such as apoptosis, cytokine production and increased markers of oxidative stress[59]. Oxidative stress markers have been shown to increase with glucose fluctuations[62,63]. GV may be assessed by a number of methods. Techniques to quantify variability are reviewed elsewhere[64].

Multiple studies in the critically ill have established ~~as~~ an association with poor outcomes and GV[44,65-71], however the evidence in patients with pre-existing diabetes is limited and inconsistent (Table 8). In 2006, Egi *et al*[65] published a retrospective, electronic database analysis of 7049 ICU patients in 4 centres around Australia, using standard deviation as a marker of glucose variability, and focusing on the association of blood glucose variability and mortality[65]. Both mean and standard deviation of blood glucose were independently associated with mortality.

A retrospective, single center cohort study of patients admitted with sepsis reported that GV was also independently associated with increased mortality and importantly, that this was independent of hypoglycaemia and the presence of diabetes[66]. Another retrospective study of 3252 patients reported that increased GV was associated with mortality[67] and diabetes was associated with greater GV. A prospective, observational study of 42 patients used non-lineal dynamics to measure glycaemia in time series[69]. Patients underwent continuous glucose monitoring system measuring interstitial glucose concentrations every 5 min for 48 h. The authors reported greater variability was associated with increasing mortality, even in patients with diabetes. However, given the small cohort, these results must be treated with caution.

Other studies have reported no relationship between mortality and GV in patients with diabetes. A retrospective, observational study of 4084 critically ill patients (942 with known diabetes)[68] reported that GV was associated with mortality in patients without diabetes, but not in patients with diabetes. More

Table 7 Observational studies and outcomes related to hypoglycaemia (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Krinsley and Grover[60]	2007	408	Risk factors for developing hypoglycaemia in ICU and outcomes	Severe hypoglycaemia associated with septic shock. Renal insufficiency, mechanical ventilation, illness severity and use of ITT	Associated with inc risk of severe hypoglycaemia ($P < 0.01$) DM had no association with mortality	Mortality in severe hypoglycaemia cohort 56% *vs* control cohort 40%, $P < 0.01$
Egi *et al*[61]	2010	4946	Hypoglycaemia *vs* risk of death in critically ill patients	Mild or moderate hypoglycaemia was associated with mortality in critically ill patients Mortality increases as severity of hypoglycaemia increases	Diabetic patients more likely to suffer from insulin-associated hypoglycaemia	22% of total patients had one episode of hypoglycaemia Hospital mortality: hypoglycaemic cohort 37% *vs* control cohort 20%, $P < 0.01$
Krinsely *et al*[33]	2011	6240[1]	Mild hypoglycaemia (blood glucose level < 3.9 mmol/L) *vs* risk of mortality in critically ill patients.	Mild hypoglycaemia was associated with a significantly increased risk of mortality	The association between hypoglycaemia and mortality was independent of diabetic status	Inc severity of hypoglycaemia was associated with inc risk of mortality Hypoglycemic patients had higher mortality regardless of diagnostic category and ICU LOS
Krinsley *et al*[32]	2013	44964	Hyperglycaemia, hypoglycaemia, and glycemic variability *vs* mortality (and how DM effects this)	Inc mortality with higher mean blood glucose (≥ 7.8 mmol/L) Dec mortality with lower blood glucose (4.4-7.8 mmol/L)	Inc mortality with mean blood glucose between 4.4-6.1 mmol/L Dec mortality when blood glucose were higher (6.2-10 mmol/L)	Hyperglycaemia, hypoglycaemia, and increased glycemic variability are independently associated with mortality in ICU patients Diabetic status tempers these relations

[1]Contains partial data from one prospective RCT (Glucontrol trial) and complete data from two observational cohorts (United States and The Netherlands). Inc: Increased; Dec: Decrease; LOS: Length of stay.

recently in the study by Hoang *et al*[44] of 299 patients there was no association between GV and mortality in their entire cohort, however the group with diabetes (128 patients) had a lower rate of mortality despite having a higher GV. Additionally, a retrospective analysis of 2782 ICU patients, comparing different GV indices and mean glucose concentrations to predict mortality and ICU acquired infections[70] reported that while GV was associated with infections and mortality in patients without pre-existing diabetes, in those with diabetes GV was greater but was not associated with either mortality or infection.

In summary, there is a strong relationship between GV and mortality in critically ill patients that has been confirmed in multiple studies. However, with respect to patients with diabetes, data are inconsistent. This may be due a number of factors, including small numbers studied resulting in lack of power, or that patients with chronic hyperglycaemia are protected somewhat by glycaemic excursions during acute illness. Research is warranted to further understand whether GV is harmful in patients with pre-existing diabetes.

RATIONALE FOR PERSONALISED THERAPY AND THAT THE HARM FROM EACH OF THESE DOMAINS MAY VARY ACCORDING TO PRE EXISTING PHYSIOLOGY

Diabetes is known to be associated with a large burden of illness in the outpatient setting and is associated with increased mortality[72]. Paradoxically, as discussed, multiple studies exist suggesting that acute hyperglycaema in critically ill patients without diabetes (*i.e.*, patients with CIAH) is associated with increased mortality and morbidity when compared to those with known diabetes[73]. There is growing evidence that chronic hyperglycaemia may lead to cellular conditioning, and that in fact, may be protective against acute hyperglycaemia mediated damage during an episode critical illness[1]. These outcomes suggest that current target glucose levels in patients naïve to hyperglycaemia, or those suffering from CIAH, may be harmful to those with chronic hyperglycaemia or poorly controlled diabetes.

CONCLUSION

This review articulates the need for further research to be done to identify the ideal glucose targets in critically ill patient with pre-existing diabetes. Not only does hyperglycaemia occur frequently in this group, but, recent data suggests that targeted blood glucose concentrations may benefit from consideration of a patient's premorbid glucose state.

Our recommendations are to avoid treating patients with diabetes as a homogenous group. Treatment of the critically ill patient with type 2 diabetes should be personalised to their internal milieu. There is preliminary evidence suggesting that higher blood glucose concentrations (*e.g.*, up to 14 mmol/L) in patients with uncontrolled type 2 diabetes may not be

Table 8 Observational and interventional studies and outcomes related to glycaemic variability (chronological order)

Ref.	Year	Study pts	Study point	Non diabetic patients	Diabetic patients	Overall message
Egi *et al*[65]	2006	7049	GV (measured by SD and %CV) *vs* mortality (hospital and ICU)	Both mean and GV of blood glucose were significantly and independently associated with ICU and hospital mortality GV was a stronger predictor of ICU mortality than mean glucose concentration	Inc mortality when comparing highest and lowest glucose SD No other significant relation with blood glucose (SD and mean) and ICU/hospital mortality Logistic regression: DM associated with decrease OR for ICU mortality	The mean ± SD of blood glucose: Survivors 1.7 ± 1.3 mmol/L *vs* Non survivors 2.3 ± 1.6 mmol/L ($P < 0.001$) Post logistic regression analysis, both mean and SD of blood glucose were significantly associated with ICU and hospital
Ali *et al*[66]	2008	1246	GV *vs* hospital mortality in septic ICU patients	GV is independently associated with hospital mortality in sepsis	Mortality rise remained even after adjusting for a diagnosis of diabetes	Higher observed mortality with increasing levels of variability Higher odds of hospital mortality with lower mean blood glucose + high GV or higher mean blood glucose + lower GV
Krinsely[67]	2008	3252	GV *vs* mortality in ICU patients	Inc GV conferred a strong independent risk of mortality	Multivariable regression analysis demonstrated that diabetes had an independent positive correlation to SD	Amount of GV had a significant effect on mortality - *e.g.*, patients with mean blood glucose 3.9-5.5 mmol/L mortality: Lowest GV 6% while high GV 30%
Krinsely[68]	2009	4084	Impact of DM or its absence on GV as a risk factor for mortality	Low GV was associated with increased survival High GV was associated with increased mortality	Higher measures of GV No association between GV and mortality among diabetics	Attempts to minimize GV may have a significant beneficial impact on outcomes of critically ill patients without diabetes
Lundelin *et al*[69]	2010	42	Glycemic dynamics (measured *via* non-lineal dynamics) *vs* mortality in ICU patients	Loss of complexity (therefore higher variability) in glycaemia time series is associated with higher mortality	This association persisted in diabetics No difference in DFA (detrended fluctuation analysis a measure of complexity) between DM and nondiabetics	In critically ill patients, there is a difference in the complexity of the glycaemic profile between survivors and nonsurvivors Loss of complexity correlates with higher mortality
Meyfroidt *et al*[71]1	2010	2 748	Blood glucose signal characteristics *vs* hospital mortality,	GV was independently associated with hospital mortality	Increased mortality was seen in both diabetics and non diabetic patients.	Increased glucose amplitude variation was associated with mortality, irrespective of blood glucose level
Hoang *et al*[44]	2014	299	Prevalance of unrecognized DM amongst those with CIAH and the association between baseline glycaemia and mortality	102 (34%) had no history of DM 14/102 (14%) had unrecognized DM (diagnosed with HbA1c ⩾ 6.5)	197 (66%) had a history of DM	Lower HbA1c had inc mortality (in this population of CIAH patients) despite lower median glucose values and less glucose variability Mortality in HbA1c < 6.5 (19%) *vs* HbA1c ⩾ 6.5 (12%), $P = 0.04$
Donati *et al*[70]	2014	2 782	GV and mean BGLs *vs* mortality and intensive care unit-acquired infections	High GV is associated with higher risk of ICU acquired infection and mortality	Diabetic patients had higher mean BGL and GV No change in mortality or infections	Mean BGL was not associated with infections and mortality

[1]Interventional study data - pooled from the Leuven trials. GV: Glycaemic variability; SD: Standard deviation; %CV: Coefficient of variation; Inc: Increased; Dec: Decreased; OR: Odds rat.

harmful. For this reason it may be safer to target blood glucose concentrations between 10-14 mmol/L in this group. However, definitive studies of critically ill patients with poorly controlled diabetes are required before this approach is incorporated into clinical practice. In contrast, in patients with CIAH, or those with well-controlled diabetes (HbA1c < 7.0), a more conservative target (6-10 mmol/L) is supported by considerable data.

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P- Reviewer: Bestas A, Casadesus D **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Zhang DN

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DOI: 10.4239/wjd.v6.i5.707

World J Diabetes 2015 June 10; 6(5): 707-714
ISSN 1948-9358 (online)

REVIEW

Co-occurrence of type 1 diabetes mellitus and celiac disease

Amit Akirov, Orit Pinhas-Hamiel

Amit Akirov, Beilinson Campus, Rabin Medical Center, Petach-Tikva 49100, Israel
Amit Akirov, Orit Pinhas-Hamiel, Sackler School of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel
Orit Pinhas-Hamiel, Pediatric Endocrine and Diabetes Unit, Safra Children's Hospital, Sheba Medical Center, Ramat-Gan 52621, Israel

Author contributions: Akirov A reviewed the literature and drafted the manuscript; Pinhas-Hamiel O helped with the review of the literature and the retrieval of the data from the articles and helped draft the paper; all authors read and approved the final manuscript.

Conflict-of-interest: The authors report no conflict of interest.

Correspondence to: Amit Akirov, MD, Beilinson Campus, Rabin Medical Center, 1 Kaplan Street, Petach-Tikva 49100, Israel. amit.akirov@gmail.com
Telephone: +972-3-524650760
Fax: +972-3-9221605

Received: August 28, 2014
Peer-review started: August 30, 2014
First decision: December 17, 2014
Revised: December 30, 2014
Accepted: March 16, 2015
Article in press: March 18, 2015
Published online: June 10, 2015

Abstract

The co-occurrence of celiac disease (CD) and type 1 diabetes (T1DM) has been reported as 5-7 times more prevalent than CD alone. The clinical presentation and natural history of CD in patients with T1DM may vary considerably. Less than 10% of patients with T1DM and CD show gastrointestinal symptoms. Therefore, experts support screening for CD in T1DM patients, though there is no consensus as to the recommended frequency of screening. When stratified by time since CD diagnosis, longer follow-up and coexistence of CD are associated with significant increased risk of diabetic associated morbidity and mortality. Early CD diagnosis and treatment with a gluten-free diet are essential.

Key words: Type 1 diabetes mellitus; Celiac disease; Glycemic control; Gluten free diet; Pediatrics

Core tip: Increased prevalence rates of celiac disease (CD) are described among individuals with type 1 diabetes mellitus (T1DM). Specifically celiac disease is more prevalent in females with T1DM. Less than 10% of patients with T1DM and CD show gastrointestinal symptoms therefore screening is necessary. The significant increase of diabetic associated morbidity and mortality, emphasize the importance of early diagnosis of CD and appropriate treatment with gluten-free diet.

Akirov A, Pinhas-Hamiel O. Co-occurrence of type 1 diabetes mellitus and celiac disease. *World J Diabetes* 2015; 6(5): 707-714 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/707.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.707

INTRODUCTION

Celiac disease

Celiac disease (CD), previously known as celiac sprue, affects 0.6%-1.0% of the population worldwide, with wide geographic variation, for unknown reasons[1,2]. The autoimmune disorder is triggered in genetically

predisposed patients by gluten ingestion[1-4]. Symptoms of CD include malabsorption and malnutrition, vitamin deficiencies, iron deficiency anemia, failure to thrive, short stature, diarrhea, anorexia, constipation, vomiting, and abdominal distension. Other complications associated with untreated CD include osteoporosis, obstetric complications, and neurologic disorders, as well as enteropathy-associated T-cell lymphoma and adenocarcinoma of the jejunum[5,6]. However, several reports in the literature state that many cases of CD are asymptomatic or associated with mild symptoms[7-9]. Diagnosis of CD is based on intestinal biopsy and the presence of specific antibodies; however, most cases of CD remain undiagnosed[10,11]. Currently, the only effective treatment for CD is a lifelong gluten-free diet (GFD), which results in resolution or improvement for most individuals[12].

Epidemiology of T1DM and CD

The association between CD and T1DM was first described in the late 1960s[13]. Studies published during the last few years have demonstrated elevated prevalence rates of CD among individuals with T1DM: 4.4% in the United Kingdom, 3.7% in Israel, 4.8% in Greece, and 6.4% in Germany; and as high as 10.5% in Brazil and 11.1% in India[14-19].

The incidence of T1DM is rapidly increasing in children and adolescents, with a reported increase of 3% annually[20,21]. Similarly, a longitudinal study documented an increase in the prevalence of CD in the mid 1990s, from 3.3% to 10.6%, most probably due to changes in environmental factors[22].

CD is a female predominant disease, and is 2-3 times more common among females[23]. Although there is no gender difference in the prevalence rates of T1DM, CD is also more prevalent in females than in males with T1DM. The etiological risk factors for developing antibodies against the small bowel are thought to be different from those for T1DM[24-26].

Genetics

Genetic background plays a key role in the predisposition to CD, as suggested by higher prevalence among family members and higher concordance rates in monozygotic than dizygotic twins (over 80% compared with 11%)[27].

The human leukocyte antigen (HLA) plays a key role in the genetic predisposition to CD, as there is a strong association between both *HLA-DQ2* and *HLA-DQ8*, and between CD. The negative predictive value of HLA typing is high, as CD is extremely rare in patients carrying neither *DQ2* nor *DQ8* alleles[28,29].

An overlap in the genetic susceptibility conferred by *HLA-DQ2* is the basis for the increased prevalence of CD in patients with T1DM. Over 90% of those with CD express HLA-DR3/DQ2 haplotype, as well as 55% of those with T1DM, compared with less than 25% of the general population[30]. Bakker *et al*[31] confirmed the high prevalence of HLA-DQ2 haplotypes in patients with both T1DM and CD, and reported that HLA-DQ2 homozygosity confers the highest risk for CD among patients with CD. DQ2 has been cited by a number of studies as the major susceptibility factor for CD. *HLA-DQ8*, another important allele for CD, is considered a stronger susceptibility factor for T1DM. DQ8 heterozygosity is claimed to be the strongest risk factor for the development of T1DM[32,33]. Trynka *et al*[34] reported 57 independent CD association signals from 39 non-HLA genes that confer a predisposition to CD. However, although genetic predisposition is essential, it is not sufficient for the development of CD, as the pathogenesis of CD involves an external trigger, namely gluten.

SCREENING AND DIAGNOSIS

In the general population

According to the modified guidelines of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[10], testing for CD is recommended in children and adolescents with otherwise unexplained signs and symptoms suggestive of CD, and among asymptomatic children and adolescents with an increased risk for CD, such as patients with T1DM or first-degree relatives with CD.

Diagnosis of CD is based on the presence of villous atrophy and crypt hyperplasia by intestinal biopsy and the presence of antibodies against tissue transglutaminase (TTG) or endomysium (EMA). The diagnosis is confirmed by an antibody decline to a GFD. According to ESPGHAN guidelines, in patients with suspected CD and certain conditions (typical symptoms, high titer of TTG antibodies and predisposing HLA genotypes), there is no obligation to complete duodenal biopsy and histology[10].

Finally, potential CD, a term coined by Ferguson in the 1990s, refers to patients with positive CD-associated antibodies, but with normal, or almost normal, jejuna mucosa[35]. These patients usually have mild symptoms, if any, of CD. The number of patients diagnosed with potential CD is increasing, following the raised attention for CD and screening tests of high-risk populations[36]. Numerous studies in the general population demonstrate that CD is often only diagnosed after several years of CD related complaints[37-39]. Some reported that diagnosis of irritable bowel syndrome preceded the correct diagnosis of CD in many patients[40,41].

CD in T1DM patients

Less than 10% of patients with T1DM and CD show gastrointestinal symptoms[42]. Therefore, most professional societies recommend screening of patients with T1DM for CD. However, there is no consensus regarding the recommended screening tests and the frequency of screening[43].

Recommended screening test: Most guidelines support screening based on TTG IgA (confirmed by EMA), or TTG IgG in patients with IgA deficiency, because of its high sensitivity and specificity[44,45]. Most experts argue that in patients with CD-associated antibodies, it is mandatory to perform esophagoduodenoscopy with small bowel biopsies to confirm the diagnosis[10].

Timing and frequency of screening: Neither in the guidelines issued by ESPGHAN nor those issued by the National Institute for Health and Clinical Excellence, is the timing of screening specified. As for the frequency of screening, ESPGHAN guidelines recommend retesting at intervals, with no firm evidence, but opinion is every 2-3 years[10]. NICE guidelines state that the evidence is insufficient to make a recommendation regarding the frequency of screening for CD in patients with T1DM[10,46]. The International Society for Pediatric and Adolescent Diabetes recommends screening for CD at diagnosis of T1DM, every year in the first five years of follow-up, and less frequently in successive years[4,47,48].

Bakker *et al*[31] reported that almost 50% of T1DM patients diagnosed with CD in adulthood had CD related complaints for over 5 years prior to the diagnosis of CD. Furthermore, their findings demonstrated a bimodal distribution of the age of diagnosis of CD in patients with T1DM, with peak incidence rates at the ages of 10 and 45 years.

CLINICAL PRESENTATION OF CD IN PATIENTS WITH T1DM

CD diagnosis most often follows the diagnosis of T1DM, and only a minority of patients were diagnosed first with CD[7,31,49].

Age of onset

The mean age at onset of T1DM is younger in those with both T1DM and CD, than in those with only T1DM[25,50]. In an observational cohort study of 4379 people aged $\leqslant$ 18 years from Australia, the mean age at T1DM onset was 6.6 ± 4.0 years in those with T1DM and CD, compared with 8.4 ± 4.1 years in those without CD[51].

Signs and symptoms

The natural history of CD in patients with T1DM may vary considerably, as the diagnosis of CD can precede the diagnosis of T1DM, or be established at the onset of T1DM, during routine screening tests at follow-up. Accordingly, the presentation of CD varies greatly, from asymptomatic or mild symptoms to poor growth and considerable morbidity[7-9,44]. In individuals with diabetes, symptoms of CD may be divided into two main categories, those directly associated with CD and those related to the impact of CD on diabetes.

Signs and symptoms directly associated with CD

These include malabsorption and malnutrition, vitamin deficiencies, iron deficiency anemia, failure to thrive, short stature, diarrhea, anorexia, constipation, vomiting, and abdominal distension.

Growth in children with CD and T1DM compared to children with T1DM alone: As stated above, differences between reports may be due to whether CD diagnosis results from routine screening or is prompted from signs and symptoms.

Body weight was found to be significantly lower among children with T1DM with screening-identified CD compared to those with T1DM only; however, there was no difference in height[52]. Of 41951 children and adolescents surveyed in Germany, only 22273 (53%) had been screened for CD. Those with both T1DM and CD had a significantly lower weight standard deviation and height standard deviation score (SDS)[50]. In a subgroup of 183 patients, those with both diseases had significantly lower height and weight SDS after 5-year follow-up[53]. Previously, we demonstrated a higher prevalence of growth impairment among patients with both CD and T1DM, compared to patients with T1DM alone. Patients with CD were, on average, significantly shorter than their genetic target height potential, compared to patients with T1DM alone. Furthermore, poor adherence to GFD resulted in continuous growth impairment, compared to steady improvement among those with good adherence to a GFD[16]. Of note, patients with CD who do not improve their growth velocity after GFD should be evaluated for growth hormone deficiency secondary to autoimmune hypophysitis[54].

Signs related to the impact of CD on diabetes at diagnosis of CD

Glycemic control: Data remain inconsistent regarding glycemic control in patients with dual diagnosis of CD and T1DM. Data may differ based on the points of time HbA1c levels were assessed (at diagnosis *vs* at follow-up), whether diagnosis was based on routine screening or on symptoms, and in longitudinal studies whether adherence to GFD was assessed in parallel.

(1) HbA1c levels at diagnosis of CD. Malabsorption of nutrients may cause a reduction in HbA1c levels. In a controlled study in children mean age 10 years with T1DM duration of about 4 years, HbA1c levels at baseline did not differ significantly between patients with T1DM and CD, and between those with T1DM alone[55]. Yet, among adult T1DM patients who were newly diagnosed with CD, glycemic control was significantly worse than for those with T1DM alone, 8.2% *vs* 7.5%, $P = 0.05$[56]. The difference between these studies may reflect the impact of delayed diagnosis of CD.

(2) HbA1c levels at follow-up. In a controlled prospective 2-year follow-up study, mean HbA1c

levels did not differ significantly between patients with both T1DM and CD and between those with T1DM alone[57]. Similarly, in a large cohort from 297 centers in Germany and Austria, no statistically significant differences were found in mean HbA1c levels, between children with and without CD, mean age of 13.7 after 5 years of follow-up[50].

Acute events-hypoglycemic and diabetes keto-acidosis: CD is associated with mucosal changes that may interfere with the absorption of carbohydrates, even without leading to true malabsorption. An increased risk for symptomatic hypoglycemia was reported in the 6 mo before and after diagnosis of CD[58]. However, during long-term follow-up and under GFD, no differences were found in the numbers of severe hypoglycemic episodes[50]. There are no reports of increased risk of DKA episodes in individuals with both T1DM and CD[50].

Insulin requirements: One study reported significantly lower insulin requirements among patients with T1DM and CD than among those with T1DM alone[52]; yet the mean insulin requirement increased significantly from 0.88 to 1.1 units/kg per day after 12 mo GFD. In another study, there was no difference in insulin dosage per kilogram per day between patients with both T1DM and CD, mean CD duration of 3 years, and those without CD[59].

Other autoimmune diseases: Patients with CD are at increased risk for other autoimmune diseases, such as autoimmune thyroid disorders. Thyroid disorders have been reported to be an important risk factor for the development of CD among patients with T1DM[49,60].

COMPLICATIONS IN PATIENTS WITH T1DM AND CD

Complications may be divided into two main categories, those directly associated with CD and those related to the impact of CD on diabetes.

The long term complications associated with untreated CD include osteoporosis, obstetric complications, and neurologic disorders, as well as enteropathy-associated T-cell lymphoma and adenocarcinoma of the jejunum[2,6].

Diabetes associated complications in patients with CD

As for long-term complications among patients with T1DM and CD, the data are conflicting: some report that CD increases rates of complications[56], some show no difference, and others suggest lower incidence of complications[61]. These discrepancies may be due to differences in duration of undiagnosed CD.

Prevalence of complications in patients with T1DM and newly diagnosed CD

Among adults with T1DM duration of over 20 years, those with newly diagnosed CD had worse glycemic control and a significantly higher prevalence of retinopathy (58.3% *vs* 25%), nephropathy (41.6% *vs* 4.2%), and peripheral neuropathy (41.6% *vs* 16.6%)[56]. In contrast, Picarelli *et al*[61] reported significantly lower prevalence of nephropathy and retinopathy among adult T1DM aged about 50 years, with T1DM duration of about 18 years and newly diagnosed CD. The difference between these studies may be due to the unknown duration of undiagnosed CD, and to the difference in HbA1c levels between studies. In the latter, only those with HbA1c levels < 7.5% were included.

The prevalence of complications in patients with T1DM and CD: Long-term follow-up

A lower prevalence of retinopathy was reported in individuals with median durations of T1DM and CD of 27 and 3 years respectively, compared with controls (38.7% *vs* 67.4%). However, no difference in the prevalence of nephropathy was found between the groups[59]. The duration of CD was found to be correlated with the risk of diabetic retinopathy. When stratified by time since CD diagnosis, individuals with T1DM and CD were at a lower risk of retinopathy in the first 5 years after CD diagnosis, followed by a neutral risk in years 5 to 10. However, with longer follow-up, coexisting CD was a 2.83 increased risk factor for diabetic retinopathy at 10 to 15 years of follow-up, and a three-fold risk after 15 years of follow-up[62].

Patients with both T1DM and CD were reported to have more severe subclinical atherosclerosis than those presenting with only T1DM or CD. Among patients with both T1DM and CD, mean age of 39 years, mean T1DM duration of 18 years and CD duration of 8.5 years, carotid intima-media thickness was significantly greater in those with both T1DM and CD than in those with either T1DM or CD, suggesting that the association of these autoimmune diseases might accelerate the atherosclerotic process[63].

Finally, mortality in patients with both T1DM and CD was studied in 960 individuals aged 30 years, compared with 4608 with T1DM alone, matched for sex, age and disease duration. CD was not a risk factor for death in patients with T1DM during the first 5 years after CD diagnosis, but thereafter the hazard ratio for mortality increased as a function of follow-up time. Having a CD diagnosis for > 15 years was associated with a 2.80-fold increased risk of death in individuals with T1DM[64].

TREATMENT

The standard therapy for CD is GFD, which requires

avoiding wheat, rye, barley and oats. Patients with CD must follow this strict diet for their entire life. Delay in starting GFD increases the risk of osteoporosis, gastrointestinal malignancies, iron deficiency anemia, infertility, and other autoimmune disorders. Adherence to GFD augments the restrictions required by a diabetic dietary regimen.

GFD impact on glycemic control

Good glycemic control is essential to reduce the risks of T1DM related complications. However, many specially prepared gluten-free foods have high glycemic indices, and thus affect glucose levels, insulin requirements, lipid profiles and body mass indices (BMI). GFD may worsen glycemic control and can thus increase the difficulties of disease management for patients with T1DM and CD[42]. Numerous studies have evaluated the effect of CD and GFD on the metabolic control of patients with T1DM. Some reported better metabolic control with GFD among CD patients with T1DM[65,66]. Others did not show any change in HbA1c levels with GFD[67-72], and some reported worse glycemic control with GFD[73].

GFD impact on weight and height

Data on weight gain in patients with CD are inconsistent. Some studies report that treatment with GFD promotes a significant catch-up growth while others show no difference. The time of follow-up, age and stage of puberty of patients in different studies may explain the discrepancies. Twelve months after commencement of GFD, one study showed no statistically significant change in the SDS for height, weight and BMI of the 23 children assessed[74]. In a separate study, children with T1DM and CD had lower SDS for height and weight at CD diagnosis. After 2 years of follow-up, SDS was significantly increased for weight, and for height in prepubertal children[57].

Adherence to GFD

The compliance rates to GFD among patients with CD and T1DM is less than 60%, compared with about 80% among those with CD only[75]. The more severe problems of GFD adherence usually occur during adolescence[44].

QUALITY OF LIFE IN CHILDREN WITH T1DM

Families of children with both CD and T1DM report a higher burden than those affected by T1DM only. Similarly, health care providers perceived family burden to increase over time[76]. Yet, among children aged 8-18 years, no significant differences in quality of life were observed. However, parents of children with both CD and T1D did express greater concern about their children's social functioning. Adults (mean age 49 years) with both CD and T1DM scored lower in general health perception, social functioning and role limitation, as a result of physical health and emotional problems. In addition, concerns about diabetes related and social problems were significantly higher in those with both diagnoses[77].

ACKNOWLEDGMENTS

The authors are in debts to Mrs Cindy Cohen for her editorial assistance.

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P- Reviewer: Chang HY **S- Editor**: Gong XM **L- Editor**: A
E- Editor: Zhang DN

World Journal of Diabetes

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i5.715

World J Diabetes 2015 June 10; 6(5): 715-725
ISSN 1948-9358 (online)

REVIEW

Recent progress in the genetics of diabetic microvascular complications

Yi-Cheng Chang, Emily Yun-Chia Chang, Lee-Ming Chuang

Yi-Cheng Chang, Graduate Institute of Medical Genomics and Proteomics, National Taiwan University, Taipei 100, Taiwan
Yi-Cheng Chang, Department of Medicine, National Taiwan University Hospital, HsinChu branch, HsinChu 300, Taiwan
Yi-Cheng Chang, Lee-Ming Chuang, Department of Medicine, College of Medicine, National Taiwan University, Taipei 100, Taiwan
Emily Yun-Chia Chang, Lee-Ming Chuang, Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan
Lee-Ming Chuang, Institute of Preventive Medicine, College of Public Health, National Taiwan University, Taipei 100, Taiwan

Author contributions: Chang YC and Chang EYC drafted the manuscript and drew figures; Chuang LM conceived the outlines and approved the final manuscript.

Conflict-of-interest: None.

Correspondence to: Dr. Lee-Ming Chuang, MD, PhD, Department of Internal Medicine, National Taiwan University Hospital, 7, Chung Shan S. Rd, Taipei 100, Taiwan. leeming@ntu.edu.tw
Telephone: +886-2-23123456
Fax: +886-2-23938859

Received: September 9, 2014
Peer-review started: September 9, 2014
First decision: December 17, 2014
Revised: March 2, 2015
Accepted: March 16, 2015
Article in press: March 18, 2015
Published online: June 10, 2015

Abstract

Diabetic complications including diabetic nephropathy, retinopathy, and neuropathy are as major causes of morbidity and mortality in diabetes individuals worldwide and current therapies are still unsatisfactory. One of the reasons for failure to develop effective treatment is the lack of fundamental understanding for underlying mechanisms. Genetic studies are powerful tools to dissect disease mechanism. The heritability (h^2) was estimated to be 0.3-0.44 for diabetic nephropathy and 0.25-0.50 for diabetic retinopathy respectively. Previous linkage studies for diabetic nephropathy have identified overlapped linkage regions in 1q43-44, 3q21-23, 3q26, 10p12-15, 18q22-23, 19q13, 22q11-12.3 in multiple ethnic groups. Genome-wide association studies (GWAS) of diabetic nephropathy have been conducted in several populations. However, most of the identified risk loci could not be replicated by independent studies with a few exceptions including those in *ELMO1*, *FRMD3*, *CARS*, *MYO16/IRS2*, and *APOL3-MYH9* genes. Functional studies of these genes revealed the involvement of cytoskeleton reorganization (especially non-muscle type myosin), phagocytosis of apoptotic cells, fibroblast migration, insulin signaling, and epithelial clonal expansion in the pathogenesis of diabetic nephropathy. Linkage analyses of diabetic retinopathy overlapped only in 1q36 region and current results from GWAS for diabetic retinopathy are inconsistent. Conclusive results from genetic studies for diabetic neuropathy are lacking. For now, small sample sizes, confounding by population stratification, different phenotype definitions between studies, ethnic-specific associations, the influence of environmental factors, and the possible contribution of rare variants may explain the inconsistencies between studies.

Key words: Microvascular complications; Nephropathy; Retionopathy; Neuropathy; Diabetes

Core tip: Most risk genetic loci identified by genome-wide association studies (GWAS) for diabetic nephropathy could not be replicated by independent studies with a few exceptions including those in *ELMO1, FRMD3, CARS, MYO16/IRS2*, and *APOL3-MYH9* genes. These findings highlighted the importance of cytoskeleton reorganization, phagocytosis of apoptotic cells, fibroblast migration, insulin signaling, and epithelial clonal expansion in the pathogenesis of diabetic nephropathy. Conclusive results from GWAS for diabetic retinopathy and diabetic neuropathy are currently lacking.

Chang YC, Chang EYC, Chuang LM. Recent progress in the genetics of diabetic microvascular complications. *World J Diabetes* 2015; 6(5): 715-725 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/715.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.715

INTRODUCTION

The prevalence of diabetes mellitus is increasing globally, especially in developing countries[1]. This surge of diabetes mellitus prevalence poses a serious threat to the public and diabetic complications are ranked as major causes of morbidity and mortality worldwide. Several common mechanisms underlying these microvascular complications including the polyol pathway, advanced glycation end products pathway, protein kinase C pathway, the hexosamine pathway, and cytokines such as nuclear factor-κB, tumor growth factor-β, and vascular endothelial growth factor are well described and the unifying mechanism of superoxide production have been proposed[2]. Nevertheless, therapies targeting these pathways have not been very successful[3-5]. One of the reasons is the lack of fundamental understanding for underlying mechanisms.

Genetic studies provide a powerful tool to the understanding of disease mechanism. Previous family linkage analyses have successfully identified mutations responsible for high-penetrating monogenetic disease. Some discoveries, for example, the identification of *PCSK9* mutation through linkage analyses in hypercholesterolemic families, have resulted in major breakthroughs in therapy[6,7]. However, family linkage analysis is generally not adequately powered to detect genetic loci of complex disease. Over the last few years, the advent of genome-wide association studies (GWAS) have launched a great leap toward the genetic basis of complex diseases such type 2 diabetes mellitus, cancers, and psychiatric diseases. Intriguingly, many of the identified genetic loci were not previously considered to be related to these diseases and the discoveries indeed illuminated important pathophysiological pathways to these complex diseases. Diabetic microvascular complications are complex traits influenced by both environmental and genetic factors, and compelling evidences indicate that diabetic microvascular complications are heritable[8-12]. Here in this review, we only summarized the progress in the genetics for diabetic microvascular complications.

GENETICS STUDIES OF DIABETIC NEPHROPATHY

Linkage studies of diabetic nephropathy

The heritability (h^2) of diabetic nephropathy (DN) defined by reduced glomerular filtration rate (GFR) or albuminuria was estimated to be 0.3-0.44 in multiple Caucasian diabetic populations[8-10]. Previous linkage studies have repeatedly identified linkage region in 1q43-44, 3q21-23, 3q26, 10p12-15, 18q22-23, 19q13, 22q11-12.3 in multiple ethnic groups (Figure 1, Table 1)[13-24]. However, these linkage regions usually spanned over megabases and therefore exact locus or risk gene is unclear. In contrast, the resolution of linkage disequilibrium mapping (also called association mapping) is much higher than linkage studies. The distinction between linkage and association mapping is that family linkage mapping use the small amount of recombination events that occurs in each generation within a pedigree to localize a chromosomal region, which usually contains hundreds of genes; while population-based case-control association mapping uses large amount of recombinations that occurred during the evolutional history of a population to locate the risk loci, which generally did not extend over a few genes. However, population-based case-control association studies are susceptible to the population stratification and independent replication is essential to confirm the result of association studies.

Association studies of DN in type 2 diabetic patients

Several GWAS of DN have been conducted in several ethnic populations (Table 1, Figure 1). *ELMO1* (the engulfment and cell motility 1 gene) was first found to be associated with diabetic nephropathy in a GWAS in Japanese 2 diabetic patients (546 DN cases and 334 type 2 diabetic controls)[25]. Replication studies in the GoKinD collection (558 DN cases and 820 type 2 diabetes controls)[26], two African American cohorts [1136 end-stage renal diseae (ESRD) diabetes cases and type 2 diabetic 1160 controls][27], a Chinese population (123 DN cases and 77 type 2 diabetic controls)[28], and a Caucasian GWAS (547 ESRD and 549 type 1 diabetic controls)[29] confirmed this finding although the risk SNPs are not exactly the same with those reported in the original Japanese population (intron 16-20 in original Japanese GWAS, intron 16-20 in GoKinD, intron 13 in African Americans, intron 18 in Chinese). In a large meta-analysis of the GENIE

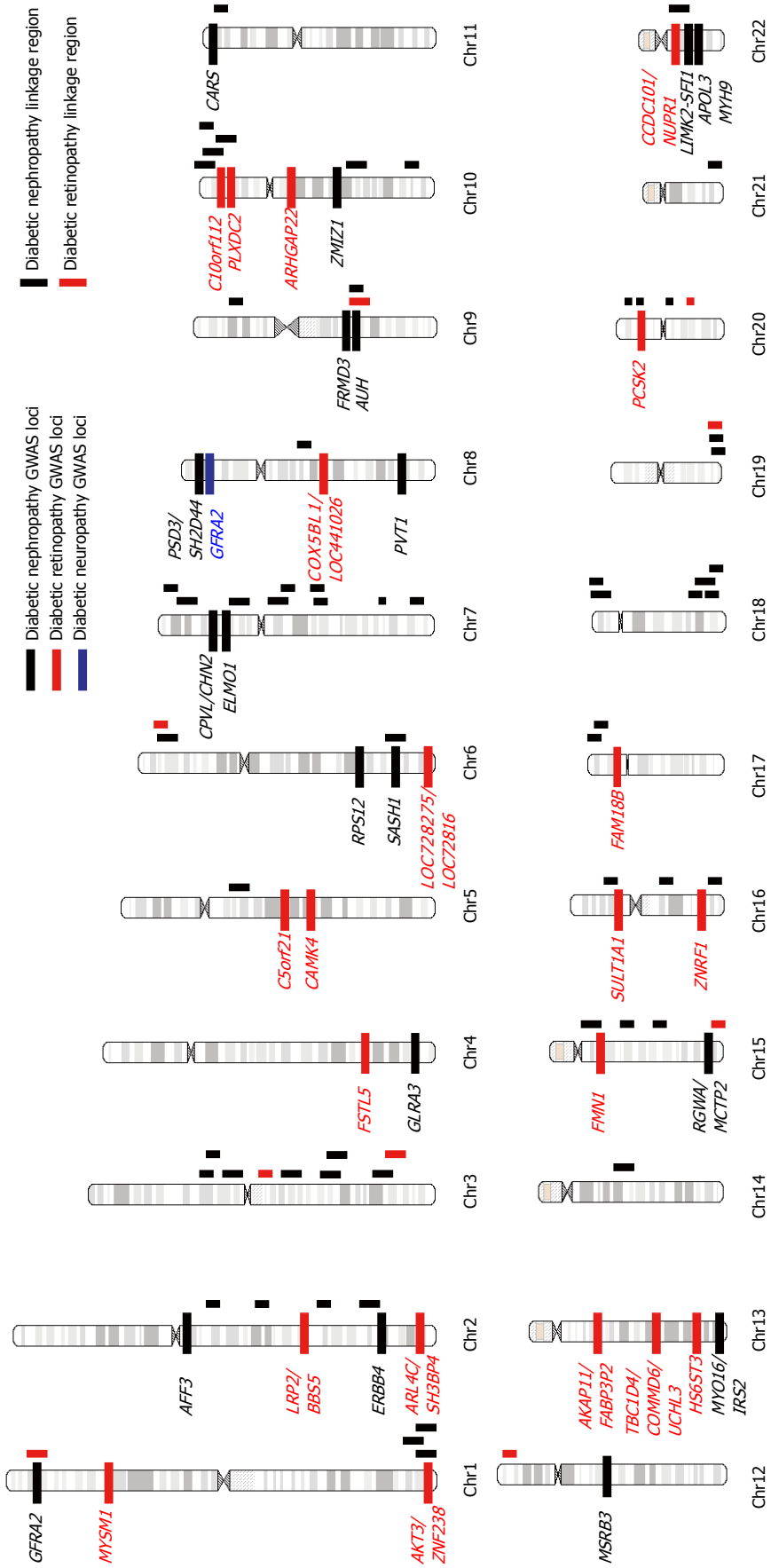


Figure 1 Genetic risk loci for diabetic nephropathy, retinopathy, and neuropathy indentified through genome-wide linkage (vertical bar) and association scans (horizontal bar). GWAS: Genome-wide association study; Chr: Chromosome.

consortium (including UK-ROI, FinnDiane, and GoKinD US) involving 2966 DN cases and 3399 type 1 diabetic controls, an expanded investigation of the *ELMO1* locus yielded only nominal associations with DN[30]. Furthermore, another replication studies in Pima Indians of Arizona involving 248 DN cases and 524 diabetic controls found significant association of SNPs in intron 13 but the associations were in the opposite direction from those observed in African Americans[31], and another study in 455 Mexican-American patients with DN and 437 controls failed to replicate the association[32].

A GWAS in Pima Indians comparing 105 diabetic ESRD and 103 controls identified plasmacytoma variant translocation (*PVT1*), an lncRNA gene, was associated with DN in type 2 diabetic patients[33]. Another large GWAS in an initial set of 965 African American type 2 diabetic patients with ESRD and 1029 controls without type 2 diabetes or kidney disease and further replication studies in 1246 type 2 diabetic patients indentified *SASH1* (SH3 Domain Containing 1), *RPS12* (ribosomal protein S12), *AUH* (AU RNA binding protein/enoyl-CoA hydratase), *MSRB3* (methionine sulfoxide reductase B3), *LIMK2* (LIM domain kinase 2)-*SFI1* (Sfi1 homolog, spindle assembly associated),

Table 1 Genome-wide linkage studies for diabetic nephropathy and retinopathy

Ethnicity and sample size	Type of diabetes	Phenotype definition	Linkage region (LOD score or *P*-value or MLS)	Ref.
Diabetic nephropathy				
954 African American, 781 American Indians, 614 European American, 1611 Mexican Americans (FIND)	1 + 2	Estimated GFR	10p12.31[1] (LOD: 2.16), 1q43[1] (2.26), 2q31.3 (1.91), 3p12.1 (2.19), 7q11.22 (2.19), 10p14[1] (2.16), 15q12 (2.84), 20q11.11[1] (3.34)	[13]
218 African American, 335 American Indians, 119 European American, 469 Mexican Americans (FIND)	1 + 2	Urine ACR	7q21.3 ($P = 8.6 \times 10^{-5}$), 10p15.3[1] (1.29×10^{-5}), 14q23.1 (7.8×10^{-4}), 18q22.3[1] (2.17×10^{-3})	[14]
3972 Americans (African American, American Indians, European American, Mexican Americans) (FIND)	1 + 2	DN defined by macroalbuminuria or ESRD, ACR	DN: 1q43[1] (LOD: 2.00), 6p24.3 (2.84), 7p21.3 (2.81), 10p15.1[1] (2.10), 11p15.3 (2.28), 15q21.1 (2.04) ACR: 2q22.3 (2.04), 3p13 (2.76), 7q21.2 (2.96), 16q13 (2.31), 22q12.3[1] (2.29)	[23]
882 American (African American, American Indians, European American, Mexican Americans) (FIND)	1 + 2	eGFR	1q43[1] (LOD: 1.87), 7q36.1 (4.23), 8q13.3 (2.75), 15q22.3 (2.08), 18q23.3 (1.40)	[24]
100 United States sibling pairs (Joslin Study on Genetics of Diabetic Nephropathy)	1	Proteinuria or ESRD	1q44[1] (MLS: 1.6), 2q14.1 (2.1), 3p13 (0.6), 5q14.2 (2.7), 10q26.1 (2.4), 17p13.1 (1.9), 19q13.43[1] (3.1), 20p12.1 (1.8)	[15]
63 extended United States families (Joslin Study on Genetics of Diabetic Nephropathy)	2	GFR	2q33.3 (LOD: 4.1), 10q23.31 (3.1), 18p11.22 (2.2)	[19]
556 Finnish, Danish, and French (FinnDiane)	1	Macroalbuminuria or ESRD	3q21-25[1] (LOD: 0.76), 6p21 (2.31), 9p21.2, 16p12, 19q13[1] (1.61), 22q11 (2.78)	[16]
83 Finnish sibling pairs	1	Macroalbuminuria or ESRD	3q21.3-23[1] (MLS: 2.67)	[21]
18 Turkish family + 101 sibling pairs of Pima India	2	Macroalbuminuria	18q22.3-23[1] (max LOD:6.14)	[17]
201 Pima India sibling pairs	2	Macroalbuminuria or ESRD	3q26.1[1] (LOD: 1.48), 7q32.3 (2.04), 20p12.3 (1.83)	[18]
206 African American sibling pairs	2	ESRD	3q13.3[1] (LOD: 4.55), 7p21.1 (3.59), 18q22.1[1] (3.72)	[22]
691 West African	2	GFR	7p12.2 (LOD: 1.84), 16q24.1 (3.56), 17p13.2 (2.08)	[20]
Diabetic retinopathy				
282 Mexican American sibling pairs	2	Non-proliferative DR and proliferative DR	3q12.3 (LOD: 2.41), 12p13.31 (2.47), 20q13.12 (4.47), 6p24.1 (2.28), 15q26.3 (2.53), 19q13.42 (2.21)	[45]
725 Pima Indian sibling pairs	2	Worse eye score	1p36.13 (LOD: 3.1)	[46]
210 Pima Indian sibling pairs	2	Hemorrhage, microaneurysm, and proliferative DR	3q26.31 (LOD: 1.36), 9q22.33 (1.46)	[18]

[1]Overlapped region. MLS: Maximum LOD score; DN: Diabetic nephropathy; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; ACR: Albumin-to-creatinine ratio; DR: Diabetic retinopathy.

APOL3 (apolipoprotein L, 3), and *MYH9* (myosin, heavy chain 9, non-muscle) genes as risk loci[34]. Among them, the association of *MYH9* risk variants has been replicated in another study involving 1963 European Americans diabetic patients[35]. Compelling evidence demonstrated that *APOL3-MYH9* gene clusters are also associated with non-diabetic nephropathy including focal segmental glomerulosclerosis and hypertensive nephropathy in African American as well as other ethnic populations[36-38].

Association studies of DN in type 1 diabetic patients

A large GWAS in a initial set of 820 DN cases and 885 type 1 diabetic controls in the GoKinD study and a replication set of 1304 participants in the Diabetes Control and Complication Trial/Epidemiology of Diabetes Control and Complication (EDIC) identified *FRMD3* (FERM domain containing 3), cysteinyl-tRNA synthase (*CARS*), carboxypeptidase, vitellogenic-like (*CPVL*)/chimerin 2, and intergenic region at 13q33.3 between *MYO16* and insulin receptor substrate 2 (*IRS2*) associated with DN[39]. Interestingly, another genome-wide linkage analysis and regional association fine mapping in 1007 general Mongolian also identified SNPs in the *FRMD3*, glycine amidinotransferase, and spermatogenesis associated 5-like 1 genes associated with estimated glomerular filtration rate[40]. A family-based candidate-gene association study involving 798 type 2 diabetic members in the Joslin Study of Genetics of Nephropathy replicated the association of SNPs in the *FRMD3*, *CARS*, and 13q33.3 between *MYO16* and *IRS2* genes[41]. Another GWAS in 547 Caucasian ESRD cases and 549 type 1 diabetic controls identified *ZMIZ1* (zinc finger, MIZ-type containing 1) gene is associated with DN[29]. This study also observed significant association of 13q33 variant near the *MYO16/IRS2* genes[29]. However, in a large replication study of 1535 Japanese type 1 and 2 diabetic patients, only variants in 13q33.3 between *MYO16/IRS2* gene but not those in *FRMD3*, *CPVL/CHN2*, or *CARS* are significantly associated with DN[42]. Furthermore, a large meta-analysis of the GENIE consortium (UK-ROI, FinnDiane, and GoKinD US) involving 2966 type 1 diabetic cases with DN and 3399 type 1 diabetes controls failed to replicate the association between SNPs in the *FRMD3*, *CARS*, and 13q33 loci near *MYO16*

Table 2 Genome-wide association studies for diabetic nephropathy, retinopathy, and neuropathy

Patients	Ethnic	Case	Control	Gene	Ref.	Replication studies	Non-replication studies
Diabetic nephropathy							
T2DM	Japanese	459 DN	242	*ELMO1*[1]	[25]	26, 27, 28, 29, 30	31, 32
T2DM	European	105 ESRD	102	*PVT1*	[33]		
T2DM	African American	965 ESRD	1029	*SASH1, RPS12,AUH, MSRB3, LIMK2-SKI1, APOL3-MYH9*[1]	[34]	35	
T1DM	Caucasian (GoKinD, DCCT/EDIC)	820 ESRD	885	*FRMD3*[1], *CARS, CPVL/CHN2*, 13q3 between *MYO16/IRS2*[1]	[39]	40, 41, 42	42, 30
T1DM	Caucasian	547 ESRD	549	*ZMIZ1*	[29]		
T1DM	GENIE (UK-ROI, FinnDiane, GoKinUS) + 9 follow-up studies	Stage 1: 4315 ESRD Stage 2: 1880 ESRD	Stage 1: 8568 Stage 2: 6656	*AFF3, RGMA/MCTP2, ERBB4*	[43]		
T1DM	Caucasian (FinnDiane + 7 follow-up studies)	5675 T1DM Urine albumin excretion rate		*PSD3, SH2D4A*	[44]		
Diabetic retinopathy							
T1DM	Caucasian (GoKinD and EDIC)	2829 PDR and macular edema	1856	*AKT3/ZNF238, LEKR1/CCNL1, KRT18P34/VEPH1, A2BP1*	[47]		
T2DM	Taiwanese	174 NPDR and PDR	575	*MYSM1, FSTL5, C5orfF21, PLXD2, ARHGAP22, HS6ST3*	[48]		
T2DM	Taiwanese	437 PDR	570	*TBC1D4-COMMD6-UCHL3, LRP2-BBS5*, and *ARL4C-SH3BP4*	[49]		
T2DM	Mexican-American	103 severe DR	183	*CAMK4, FMN1* genes	[50]		
Diabetic neuropathy							
United Kingdom	United Kingdom (GoDART)	572 diabetic neuropathic pain	2491	*GFRA2*	[51]		

[1]Loci that could be replicated in independent studies. T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; DN: Diabetic nephropathy; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; DR: Diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy.

and *IRS2* genes[30].

A recent huge meta-analysis involving 4315 type 1 diabetic nephropathy and ESRD cases and 8568 type 1 diabetic controls of the GENIE consortium and subsequent replication analyses in 9 independent cohorts (1880 cases and 6656 controls) revealed risk SNPs in the *AFF3* (AF4/FMR2 family, member 3) and *ERBB4* (v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4) genes and an intergenic SNP between *RGMA* (repulsive guidance molecule family member a)/*MCTP2* (multiple C2 domains, transmembrane 2) genes[43]. Another large GWAS for 24-h urine albumin excretion rare in type 1 diabetic patients including an initial set of 1925 patients (FinnDiane) and 3750 additional patients from 7 follow-up studies (Steno Diabetes Center, Italian individuals from the Milano region, Umea Diabetes Study from Sweden, Scania Diabetes Registry, NFS-ORPQ, UK-ROI) identified the strongest signal from the *PSD3* (pleckstrin and Sec7 domain containing 3)/*SH2D4A* (SH2 domain containing 4A) genes[44].

Collectively, current data from GWAS are not very consistent and only genetic loci in the *ELMO1*, *FRMD3*, *APOL3-MYH9*, *CARS*, and 13q33 between *MYO16* and *IRS2* genes have been successfully replicated in independent studies.

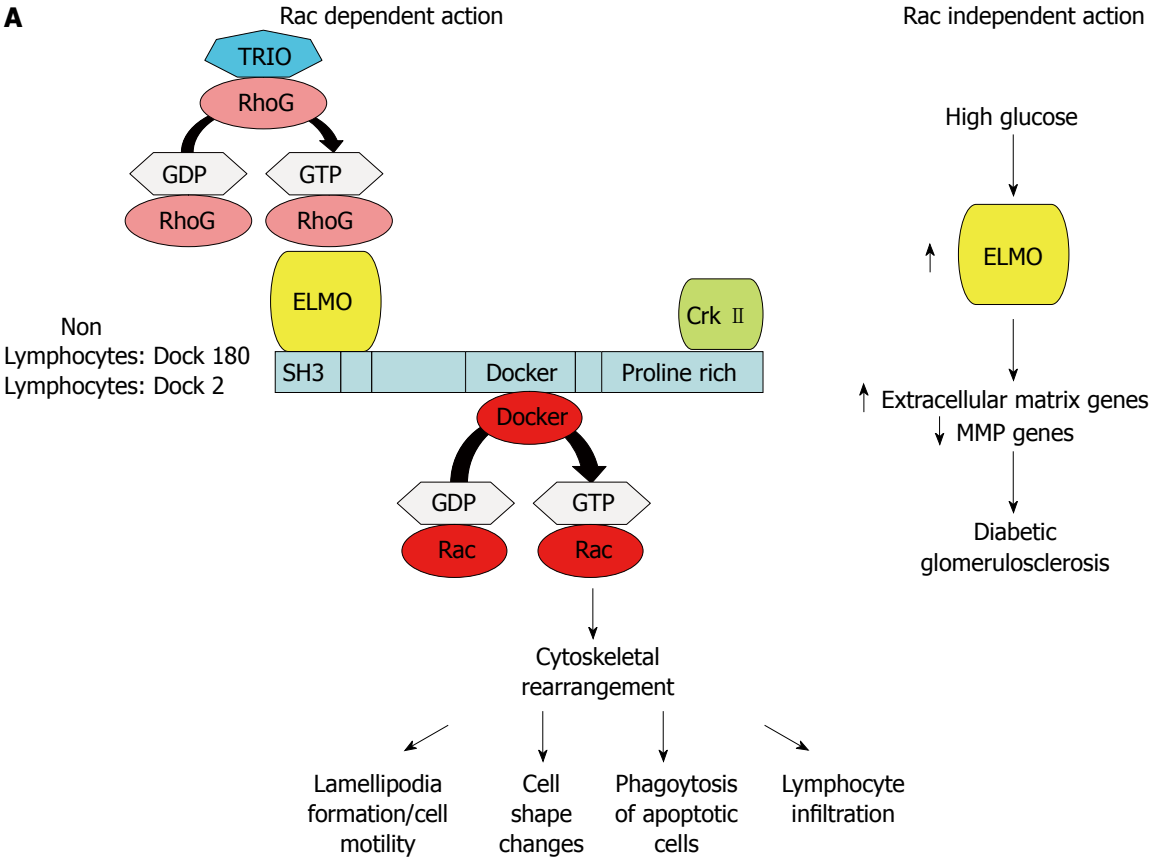
GENETIC STUDIES OF DIABETIC RETINOPATHY

Linkage studies of diabetic retinopathy

The heritability of diabetic proliferative retinopathy is estimated to be 0.25-0.50 in Caucasian populations[11,12]. Previous results of three family linkage analyses for diabetic retinopathy (DR) are summarized in Table 1 and Figure 1[18,45,46]. However, the only overlapped region is 1q36 between Pima Indians (LOD: 3.1) and Mexican Americans (LOD: 1.24) studies[45,46].

Association studies of DR

Four GWAS of DR have been published till now (Table 2, Figure 1). A large meta-analysis of GWAS in the GoKinD and EDIC cohorts involving 2829 cases of severe diabetic retinopathy defined by proliferative retinopathy and macular edema and 1856 type 1 diabetic controls identified several possible loci including intergenic SNPs between *AKT3/ZNF238, LEKR1/CCNL1, KRT18P34/VEPH1* and SNP in the *A2BP1* genes with *P*-value less than 10^{-6}[47]. After excluding cases with concomitant nephropathy to identify DR-specific genes, SNPs in the intergenic region between *LOC728275/LOC728316,* the *CCDC101/NUPR1/SULT1A2/SULT1A1* gene clusters, the *FAM18B*,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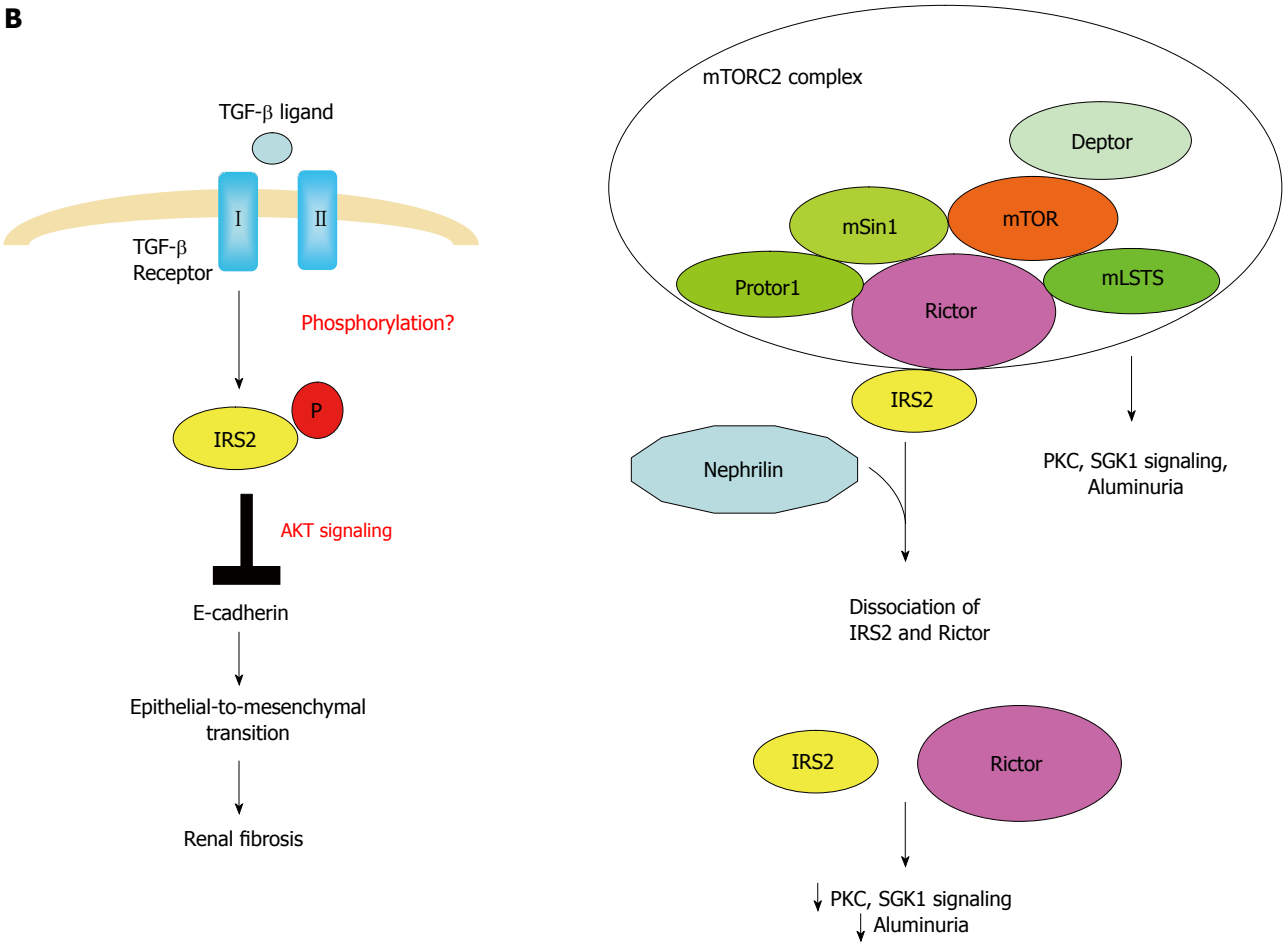
Lymphocyte infiltration
High glucose
ELMO
Extracellular matrix genes
MMP genes
Diabetic glomerulosclerosis


B
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AKT signaling
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Renal fibrosis
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Deptor
mSin1
mTOR
Protor1
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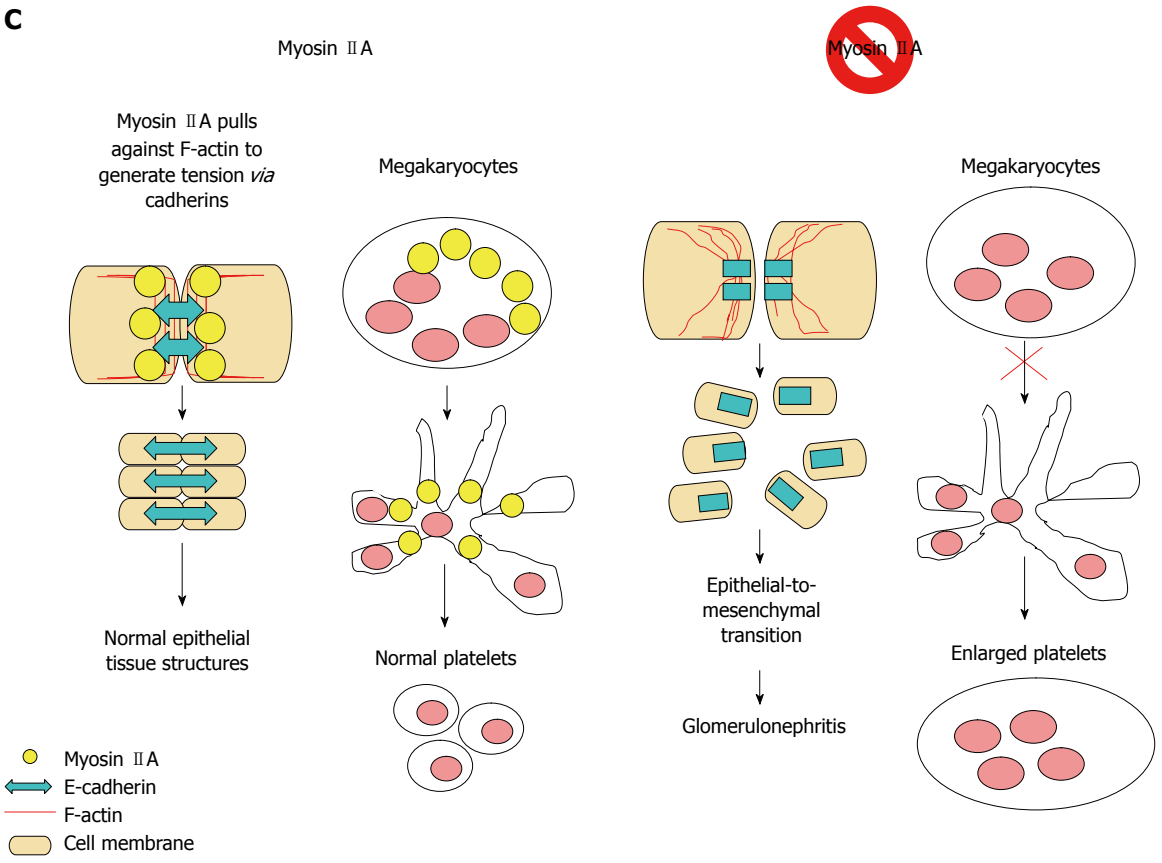

Figure 2 Possible molecular mechanisms. Possible molecular mechanisms by which ELMO1 (A), IRS2 (B), and MYH9 (C) regulate diabetic nephropathy. TRIO: Triple functional domain (PTPRF interacting); RhoG: Ras homolog family member G; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; MMP: Matrix metalloproteinases; Crk Ⅱ: V-Crk Avian Sarcoma Virus CT10 Oncogene Homolog Ⅱ; TGF-β: Transforming growth factor beta; AKT: Protein kinase B; mLSTS: Mammalian lethal with SEC13 protein 8; mTOR: Mammalian target of rapamycin; mTORC2: Mammalian target of rapamycin complex 2; mSin1: Mammalian SAPK interacting protein; PKC: Protein kinase C; SGK1: Serum- and glucocorticoid-induced kinase 1.

AKAP11/FABP3P2/TNFSF11 gene cluster, and intergenic region between *COX5BL1/LOC441026, ZNRF1, PCSK2, C10orf112* genes were found to be associated with DR[47]. A GWAS for DR involving 174 Taiwanese type 2 diabetic non-proliferative and proliferative retinopathy cases and 575 controls identified several genetic loci with *P*-value less than 10^{-6}, including *MYSM1, FSTL5, C5orfF21, PLXD2, ARHGAP22*, and *HS6ST3*[48]. Another GWAS in Taiwanese identified three risk loci in *TBC1D4-COMMD6-UCHL3*, *LRP2-BBS5*, and *ARL4C-SH3BP4* genes in the initial set of 437 cases of proliferative retinopathy and 570 type 2 diabetic controls. However, none of them were replicated in another 585 Hispanic diabetics[49]. A smaller GWAS comparing 103 Mexican-American type 2 diabetics with severe retinopathy and 183 type 2 diabetics identified suggestive signals in the *CAMK4* and *FMN1* genes[50]. However, the results from these 4 GWAS did not overlap with each other.

GENETIC STUDY OF DIABETIC NEUROPATHY

There was no heritability estimation for diabetic neuropathy in human and no family linkage study for diabetic neuropathy. Only GWAS comparing 572 diabetic neuropathic pain cases defined by treatment for diabetic neuropathic pain and positive monofilament test and 2491 diabetic controls in the Genetics of Diabetes Audit and Research Tayside (GoDARTS) identified potential signals from *GFRA2* gene[51] (Table 2, Figure 1).

PHYSIOLOGICAL INSIGHT FROM GENETIC STUDIES

The *ELMO1* gene encode for a signaling molecule involved in phagocytosis of apoptotic cells[52,53], fibroblast migration[52,54,55], cytoskeleton reorganization[56], and lymphocyte infiltration[57] through interaction with DOCK2 and DOCK180 (Figure 2A). *ELMO1* expression was found to be elevated in cells cultured under high glucose conditions and in the kidney of diabetic mice, but was weakly detectable in tubular and glomerular epithelial cells in normal kidney[25].

The *FRMD3* gene encodes for a member of the protein 4.1 superfamily. *FRMD3* has been demonstrated to be silenced in lung cancer tissue in genomic screening. *FRMD3* overexpression in different epithelial cell lines decreased clonal expansion, indicating *FRMD3*

as a potential tumor suppressor gene[58]. The *CARS* encodes for a cysteinyl-tRNA synthetase, which is a frequent gene fusion partner of anaplastic lymphoma kinase found in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor[59,60]. However, the link between *FRMD3* or *CARS* and diabetic nephropathy is currently poorly understood.

The 13q33 risk loci lie between the *MYO16* and *IRS2* genes. The *MYO16* gene encodes a novel unconventional myosin with divergent tails that is presumed to bind to membranous compartments and interact with actin filaments. *MYO16* has also been shown to be expressed during brain development and regulate neuronal morphogenesis through interaction with protein phosphatase and modulation of phosphoinositide 3-kinase signaling[61]. A GWAS for autism has identified risk loci within an intergenic region between the *MYO16* and *IRS2* genes[62]. A genome-wide linkage study and regional fine mapping for schizophrenia[63] and another GWAS of the Framingham Heart Study for pulse pressure[64] have identified *MYO16* as risk loci, indicating *MYO16* may play pleiotropic functions.

The *IRS2* gene encodes for an adaptor protein that interacts directly with the insulin receptors and the insulin-like growth factor Ⅰ receptor and is a key mediator of insulin signaling. IRS2 was expressed in renal epithelial and tubular cells. Deletion of *Irs2* causes reduced kidney size and reduced glomerular number in mice[65]. A study of transcriptome and metabolome profiles of the primary cultured inner medullary collecting duct cells grown in hyperosmolar culture medium identified IRS2 levels to be significantly altered[66]. IRS2 expression in kidney tubules has also been shown to be elevated nine fold in human diabetic nephropathy patients[67]. Transforming growth factor (TGF)-β1 is the primary cytokine shown to induce fibrosis. IRS2 has been shown to mediate TGF-β1 signals in kidney epithelial cells[68]. IRS2 has also been shown to interact with nuclear complex of rictor to regulate albuminuria in diabetic mice[69] (Figure 2B).

Mutations in MYH9 results in a familial autosomal dominant syndrome characterized by a variety of clinical features, including macrothrombocytopenia, deafness, nephritis, and cataract[70]. GWAS also identified common *MYH9* polymorphism as risk loci for non-diabetic nephropathy including focal segmental glomerulosclerosis and hypertensive nephropathy[36,27]. *MYH9* encodes the non-muscle myosin heavy chain 9, which, with other subunits, forms myosin Ⅱ. Myosin Ⅱ is a motor protein that binds actin to regulate cellular motility. *MYH9* is expressed in the podocytes, as well as in mesangial cells and arteriolar and peritubular capillaries in kidneys[71]. Classical deletion of *Myh9* in mice results in embryonic lethality due to loss of cell-cell adhesion and loss of cell movement during gastrulation. Podocyte-specfic deletion of *Myh9* in C57BL/6 mice results in susceptibility to experimental doxorubicin hydrochloride glomerulopathy[71]. Several strains of *Myh9* knockin mice showed macrothrombocytopenia, premature cataract formation, kidney abnormalities, including albuminuria, focal segmental glomerulosclerosis and progressive kidney disease, and mild hearing loss[72,73] (Figure 2C).

LIMITATIONS AND PROSPECTIVE

The major limitation of family linkage studies is their low resolution and power to detect variants with small effects, especially for complex genetic diseases. GWAS is a hypothesis-free and unbiased tool with finer resolution and greater power to detect risk loci. However, false positivity often results from population admixture or stratification in GWAS. Therefore, independent replications are essential for genetic association studies. However, current results from GWAS are not consistent since most identified loci are not reproducible except for a few genes such as *ELMO1*, *CARS*, *FRMD3*, *MYO16/IRS2*, and *APOL3/MYH9*. Small sample sizes, different phenotype definitions between studies, population-specific associations, and strong influence of environmental factors (medications, co-morbidities) may explain the failure of GWAS for diabetic complications. While GWAS are usually designed for common variants, rare variants with intermediate effects within should also be pursued with next-generation sequencing. The interaction with environmental factors should also be taken into account.

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P- Reviewer: Das UN, Ido Y, Kaya C, Uehara Y, Verrotti A
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Zhang DN

World Journal of Diabetes

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i5.726

World J Diabetes 2015 June 10; 6(5): 726-733
ISSN 1948-9358 (online)

Functions of Müller cell-derived vascular endothelial growth factor in diabetic retinopathy

Juan-Juan Wang, Meili Zhu, Yun-Zheng Le

Juan-Juan Wang, Meili Zhu, Yun-Zheng Le, Department of Medicine Endocrinology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States
Juan-Juan Wang, Department of Ophthalmology, Huizhou Municipal Central Hospital, Huizhou 516000, Guangdong Province, China
Yun-Zheng Le, Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States
Yun-Zheng Le, Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States
Yun-Zheng Le, Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States

Author contributions: All the authors equally contributed to this work.

Supported by The NIH grants, Nos. GM104934, EY020900 and EY021725 (NEI Core); Chinese National Natural Science Foundation grant, No. 81200699; grants from Presbyterian Health Foundation and Oklahoma Center for Adult Stem Cell Research, and an endowment from Choctaw Nation (to Le YZ).

Conflict-of-interest: None.

Correspondence to: Yun-Zheng Le, PhD, Department of Medicine Endocrinology, University of Oklahoma Health Sciences Center, 941 S. L. Young Blvd., BSEB 302G, Oklahoma City, OK 73104, United States. yun-le@ouhsc.edu
Telephone: +1-405-2711087
Fax: +1-405-2713973

Received: September 18, 2014
Peer-review started: September 18, 2014
First decision: January 8, 2015
Revised: February 21, 2015
Accepted: March 16, 2015
Article in press: March 18, 2015
Published online: June 10, 2015

Abstract

Müller cells are macroglia and play many essential roles as supporting cells in the retina. To respond to pathological changes in diabetic retinopathy (DR), a major complication in the eye of diabetic patients, retinal Müller glia produce a high level of vascular endothelial growth factor (VEGF or VEGF-A). As VEGF is expressed by multiple retinal cell-types and Müller glia comprise only a small portion of cells in the retina, it has been a great challenge to reveal the function of VEGF or other globally expressed proteins produced by Müller cells. With the development of conditional gene targeting tools, it is now possible to dissect the function of Müller cell-derived VEGF *in vivo*. By using conditional gene targeting approach, we demonstrate that Müller glia are a major source of retinal VEGF in diabetic mice and Müller cell-derived VEGF plays a significant role in the alteration of protein expression and peroxynitration, which leads to retinal inflammation, neovascularization, vascular leakage, and vascular lesion, key pathological changes in DR. Therefore, Müller glia are a potential cellular target for the treatment of DR, a leading cause of blindness.

Key words: Müller glia; Vascular endothelial growth factor; Protein modification; Inflammation; Blood-retina barriers; Diabetic retinopathy

Core tip: Diabetic retinopathy is a disorder of blood-retina barriers (BRBs) and neurons. Anti-vascular endothelial growth factor (VEGF) drugs are explored

for treating BRB breakdown in the disease. As VEGF is also potentially beneficial, it is essential to understand the cellular and molecular mechanisms of VEGF action in the retina. Discussion is centered on the usefulness of conditional gene targeting mice in dissecting the function of globally expressed VEGF and in identifying significant roles for Müller glia-derived VEGF in diabetes-induced changes in protein expression/modification, inflammation, and BRB lesions and leakage.

Wang JJ, Zhu M, Le YZ. Functions of Müller cell-derived vascular endothelial growth factor in diabetic retinopathy. *World J Diabetes* 2015; 6(5): 726-733 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/726.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.726

INTRODUCTION

Müller cells, the principal macroglia of mammalian retina, span from the vitreal surface to subretinal space and cover all retinal layers. This structural arrangement is ideal for them to serve as major supporting cells in regulating physiological and pathological responses in retinal vasculature and neurons. In the retina, Müller glia play many essential roles in metabolism, functions, maintenance, and protection by providing trophic factors, removing metabolic wastes, controlling extracellular space volumes and ion and water homeostasis, participating visual cycles, releasing neurotransmitters, regulating blood-retina barrier (BRB) function, and modulating innate immunity[1]. Müller glia is also a major respondent to various stresses by reactive gliosis which involves in morphological, biochemical, and physiological changes. Under some pathological conditions, uncontrollable alteration in growth factor production, such as overexpression of vascular endothelial growth factor (VEGF-A or VEGF) in diabetic retinopathy (DR), results in a detrimental effect and causes vision loss. In this mini-review, we will discuss the general role of VEGF in DR and the function of Müller cell-derived-VEGF (MCD-VEGF) in protein expression/modification, inflammation, BRB function, and vascular and neuronal integrity in diabetic animals.

DIABETIC RETINOPATHY

DR is a leading cause of blindness in working-age people in industrialized countries and is traditionally regarded as a microvascular complication in the eye of diabetic patients due to an apparent breakdown of the endothelial barrier, which is formed by orderly arranged tight junction proteins. The structural interactions between these tight junction proteins control the fluid flow through the barrier[2]. Patients with endothelial barrier breakdown demonstrate the following key clinical characteristics: retinal hemorrhages, microaneurysms, cotton-wool spots, lipid exudates, macular edema, capillary occlusion, and retinal neovascularization[2]. Studies with retinal pigment epithelium (RPE) barrier by others and us suggest that the breakdown of the tight junctions in the RPE barrier may contribute substantially to diabetes-induced blood-content leakage[3-5], which is responsible for at least some form of macular edema[6]. Therefore, DR is not just a microvascular disease, but rather a disorder of BRB. Macular edema resulted from BRB breakdown and retinal neovascularization are two most devastating causes of vision loss in diabetic patients. On the other hand, it is increasingly recognized that the loss of retinal neuronal function and viability occurs before the onset of BRB abnormalities in diabetic patients and in experimental animals[7-11]. Perhaps neuronal and BRB disorder is a more appropriate description for DR.

VEGF IN REGULATING BLOOD-RETINA BARRIER FUNCTION

VEGF-A or VEGF, a heparin-binding homodimeric glycoprotein[12,13], belongs to a family of seven members, including VEGF-A to -F and placental growth factor and (PlGF). Each of them may also have several isoforms due to alternative splicing, which affects their solubility, and thus is responsible for their cellular localization. The most intensively studied and predominant isoform of VEGF-A in humans is VEGF-A_{165}. VEGFs exert their function through complicated receptor- and co-receptor-mediated signaling cascades, which involves VEGF receptor-1 (VEGFR1), VEGFR2, VEGFR3, neuropilin-1, neuropilin-2, vascular endothelial cadherin, and integrin[14]. Much of the literature information regarding VEGFs in the eye is centered on the pathobiology of VEGF-A due to its high relevance to the pathogenesis of DR, retinopathy of prematurity (ROP), and age-related macular degeneration (AMD), leading causes of blindness.

VEGF is a potent mitogenic factor for endothelial proliferation and migration and tube formation during vessel development and is a major stimulator for embryogenesis, vasculogenesis, and angiogenesis[13,15]. Disruption of a single VEGF allele is lethal in mice at embryonic day 11-12[16,17]. VEGF is a major regulator of pathological neovascularization in proliferative DR[18]. VEGF blockade has been shown to inhibit hypoxia-induced retinal angiogenesis[19-21]. Due to its potent activity in inducing blood barrier hyperpermeability[22-25], VEGF is regarded as a major contributor to the high level of blood-content leakage in DR[26,27]. Overexpression of VEGF or its receptors, which causes disorganization of endothelial and RPE tight junctions[25,28,29], is associated with diabetic macular edema[30].

A major regulator for VEGF signaling is oxygen tension and VEGF expression is induced by hypoxia,

a pathological condition occurrs after early stages of DR[31]. While hypoxia-inducible factor-1 (HIF-1) is a critical regulator in this response[32], its degradation is controlled by von-Hippel-Lindhal (VHL) suppressor protein[33]. Therefore, HIF-1 (perhaps other HIFs) and VHL are key upstream regulators for VEGF-induced BRB breakdown in DR through VEGFR2[13,15,34]. Although VEGF signaling may be a major pathogenic mechanism for DR, various growth factors, inflammatory cytokines, and prostaglandins may also affect the disease through VEGF signaling-dependent or independent mechanisms[35-38]. As a result of intensive effort in VEGF pathobiology and pharmacology, anti-VEGF agents are utilized as a major strategy for the treatment of retinal neovascularization, BRB breakdown, and macula edema in DR.

CONDITIONAL VEGF DISRUPTION IN MÜLLER GLIA

VEGF is produced by several retinal cell-types. Müller glia and RPE cells are thought to produce high levels of VEGF[39]. Indirect evidences obtained from *in vivo* localization and molecular biology approach with cell cultures suggest a significant role for VEGF in regulating BRB function in DR[40,41]. However, the importance of MCD-VEGF in DR remained unclear for a long time after the initial discovery of VEGF as a potential pathogenic factor in DR. A major reason for this is that Müller glia only comprise a small portion of retinal cells and VEGF is expressed by multiple cell-types, which makes it difficult to detect Müller cell-specific VEGF expression by immunohistochemistry (IHC) or immunoblotting (IB). Additionally, more and more "new" VEGF functions have been identified since the discovery of its involvement in BRB function in the mid-1990s[27,42]. While other retinal cells may not produce a high level of VEGF at a given time, it is almost impossible to pinpoint the local effect of VEGF produced by these cells, if VEGF action is blocked globally by genetic, immunological, biochemical, or pharmacological approaches. Therefore, cell-specific approach may be the "only" way to delineate the precise function of Müller cell-derived VEGF (MCD-VEGF). As Müller glia play such a critical role in general health and functions of the retina, a better understanding of their biology is paramount to the prevention and treatment of retinal diseases[43]. For this purpose, several laboratories developed cell-specific genetic tools for Müller glia[44], which is very helpful for dissecting the specific function of globally expressed proteins, such as VEGF, in Müller cells.

In a serendipity fashion while developing inducible Cre/*lox* system for the RPE using the promoter of human vitelliform macular dystrophy-2 (*Vmd2*) gene[45,46], we identified one transgenic founder mouse that was capable of carrying out productive Cre-mediated excisive recombination in Müller glia. This transgenic Cre-drive line provides an opportunity to generate conditional *VEGF* knockout (CVKO) mice that disrupt VEGF expression mainly in Müller glia. The CVKO mice were generated by breeding this Müller glial Cre-drive line with a mouse line carrying *loxP*-flanked *VEGF* gene (*VEGF*^{ff}), called floxed *VEGF* mice[45,47,48]. The degree of VEGF disruption in the Müller glia was assessed by IB with primary Müller cell cultures derived from the CVKO mice, which reduced VEGF production by 66%. This degree of VEGF knockout (KO) caused a near 50% reduction of total retinal VEGF in CVKO mice under normal conditions[48,49]. To ascertain whether the production of MCD-VEGF was substantially decreased in CVKO mice in disease models[49], we examined VEGF expression in diabetic and hypoxic models (see detail below). While diabetes and hypoxia doubled retinal VEGF in WT mice, the deletion of MCD-VEGF caused a near 50% decrease of VEGF overexpression in the retina under hypoxic or diabetic conditions (Table 1). These data were corroborated by IHC with CVKO mice[48,49]. Considering the fact that Müller cells only comprise a small portion of retinal cells, our data undisputedly suggest that Müller glia are a major cellular origin of VEGF in mouse retinas.

Table 1 Alteration in protein expression/modification in diabetic or hypoxic Müller cell-specific KO mice

Model/time	Proteins/modification	Alteration
STZ-induced diabetes/6 mo	Albumin	Decrease (59%)
STZ-induced diabetes/2 mo	ICAM1	Decrease (62%)
STZ-induced diabetes/2 mo	Nitrotyrosine	Decrease (19%)
STZ-induced diabetes/6 mo	Occludin	Increase (60%)
STZ-induced diabetes/2 mo	pNF-κB p65	Decrease (48%)
STZ-induced diabetes/2 mo	TNF1α	Decrease (53%)
STZ-induced diabetes/6 mo	VEGF	Decrease (51%)
STZ-induced diabetes/6 mo	ZO1	Increase (130%)
Oxygen-induced retinopathy	Albumin	Decrease (56%)
Oxygen-induced retinopathy	VEGF	Decrease (45%)
Oxygen-induced retinopathy	Occludin	Increase (35%)

STZ: Streptozotocin; VEGF: Vascular endothelial growth factor; ICAM1: Intercellular adhesion molecule-1; TNFα: Tumor necrosis factor-α; NF: Nuclear factor; ZO1: Zonula occludens-1.

MÜLLER CELL-DERIVED VEGF IN PROTEIN EXPRESSION/MODIFICATION AND RETINAL INFLAMMATION

To determine whether deletion of MCD-VEGF resulted in any significant changes in DR-associated gene expression, we performed IB analysis with retinal extracts from CVKO mice after diabetes was induced with streptozotocin (STZ). HIF1α is a major parameter for oxygen tension and the induction of HIF1α contributes to the increase in VEGF[50]. To delineate the regulatory mechanisms between HIF1α and VEGF, we examined the expression level of HIF1α in hypoxic and diabetic CVKO mice. Although HIF1α was up-regulated significantly in hypoxic retinas at P14 (see detail below) and diabetic retinas (two month post-STZ injection) in WT controls, there was no apparent

Table 2 Pathological changes in diabetic or hypoxic Müller cell-specific KO mice

Model	Pathological changes	Alteration
STZ-induced diabetes	Leukocytosis	Decrease (75%)
STZ-induced diabetes	Vascular leakage	Decrease (60%)
STZ-induced diabetes	Acellular capillaries	Decrease (45%)
STZ-induced diabetes	Vascular leakage	Decrease (60.0%)
Oxygen-induced retinopathy	Pre-retinal neovascularization	Decrease (34%)
Oxygen-induced retinopathy	Neovascularization area	Decrease (40%)
Oxygen-induced retinopathy	Vaso-obliteration area	Decrease (30%)

STZ: Streptozotocin.

difference in the levels of retinal HIF1α between the CVKO mice and WT controls under hypoxic and diabetic conditions[48,49], suggesting that hypoxia/HIF1α is an upstream regulator of VEGF produced by Müller glia.

Nuclear factor-kappa-B (NF-κB) is a transcription factor and a major player in inducing early pathological changes, such as inflammation, in DR[51,52]. To explore if MCD-VEGF regulated NF-κB in diabetic retina, we examined the expression/phosphorylation of NF-κB p65 subunit. While there was no detectable change in the total NF-κB p65 level between the CVKO mice and WT controls, the loss of MCD-VEGF caused a dramatic decrease (48%) of phosphorylated (activated) NF-κB p65 in the retina 2 mo after STZ-injection (Table 1). This result suggests that activated p65 is downstream of MCD-VEGF in DR[49]. Nitric oxide (NO) is an important inflammatory mediator and its level is a representation of oxidative stress in the retina of diabetic patients and animals[53-55]. Peroxynitrite is a highly reactive oxidant, which is formed by the rapid combination of NO with superoxide. Increased peroxynitrite formation may be directly linked to diabetes-induced VEGF overexpression and there is a possible loop effect of VEGF signaling and peroxynitrite formation[56,57]. To determine the role of MCD-VEGF in protein nitration, we examined the level of nitrotyrosine, a biomarker of peroxynitrite, in retinal protein extracts from CVKO and control mice 2 mo after STZ-injection. The retinal extracts from diabetic CVKO mice demonstrated a 19% decrease of proteins with nitrotyrosine (Table 1), indicating that the disruption of MCD-VEGF reduced oxidative stress.

Inflammation is an early pathological response in DR. To identify the role of MCD-VEGF in retinal inflammation in DR, we examined the levels of pro-inflammatory markers in CVKO mice by IB analysis for intercellular adhesion molecule-1 (ICAM1) and tumor necrosis factor-α (TNFα), 2 mo after STZ-injection. Compared with controls, the CVKO mice showed 62.3% and 52.9% reduction of ICAM1 and TNFα (Table 1), respectively. These results suggest that the deletion of MCD-VEGF substantially inhibits inflammation in diabetic retinas[49]. This notion is reinforced by the result from the leukostasis assay showing a 75.0% reduction of adherent leukocytes, a cardinal feature of retinal inflammation in DR, in the retinal microvasculature of the CVKO mice 2 mo after STZ injection (Table 2). Collectively, these data point to a major role for MCD-VEGF in developing inflammation in DR.

MÜLLER CELL-DERIVED VEGF IN BRB FUNCTION AND INTEGRITY

As diabetic rodents usually do not develop retinal neovascularization[58], the closest way to investigate this is to utilize oxygen induced retinopathy (OIR), a model mimicking an infant blinding disease, ROP. To examine the effect of MCD-VEGF on BRB function, the severity of retinal neovascularization was examined in CVKO mice subjected to OIR. The CVKO mice were placed in hyperoxia (75% oxygen) from postnatal day 7 (P7) to P12 and were then kept under normoxia for up to 5 d. In OIR model, the retina was relatively hypoxic at P14 (compared with that at P7-12), as judged by the abundance of HIF1α. The level of retinal VEGF in hypoxic CVKO mice was decreased by 45% (Table 1). At P17, fluorescein angiography was performed and OIR-treated CVKO mice demonstrated 40% and 30% reductions in areas of retinal neovascularization and of vaso-obliteration, respectively (Table 2). Retinal sections from OIR-treated CVKO mice also showed a 34% reduction in the number of pre-retinal neovascular endothelia (Table 2). As a consequence of decreasing retinal neovascularization and unhealthy microvasculature, we observed a 56% reduction of OIR-induced vascular leakage in CVKO mice (Table 1), judged by IB for albumin. This observation was supported by IHC data[48].

IB analysis with retinal and vitreous extracts prepared from PBS-perfused diabetic WT mice demonstrated a 1.5-fold increase in extravascular albumin 6 mo after STZ-injection. However, the disruption of MCD-VEGF caused a near 60% reduction of albumin leakage in age-matched diabetic mice (Table 1). Such a reduction can be visualized in the retinal flat-mounts of diabetic CVKO mice by intravenously injected fluorescein isothiocyanate-labeled albumin[49].

To delineate the mechanistic insights of MCD-VEGF in diabetes-induced vascular leakage, we analyzed the levels of occludin and Zonula occludens-1 (ZO1), two major tight junction proteins, in the retina[49]. While diabetes resulted in 39% and 58% decreases of occludin and ZO1, respectively, in WT animals, no alteration in occludin and ZO1 expression was observed in diabetic CVKO mice. As a result, the diabetic CVKO mice had 60% and 130% upregulation of occludin and ZO1 (Table 1), compared with that of diabetic WT controls. Our data indicated that the disruption of MCD-VEGF resulted in a significant reduction of diabetes-induced retinal vascular leakage by attenuating the depletion of occludin and ZO1. This result was supported by a 36% increase in the level

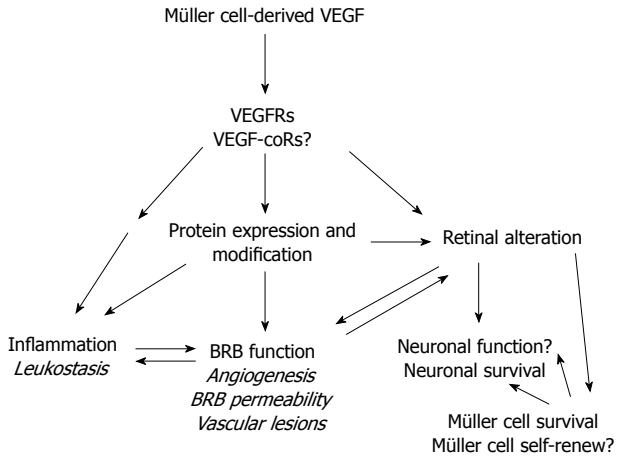

Figure 1 A simplified schematic diagram for the potential roles of Müller cell-derived-vascular endothelial growth factor in diabetic retinopathy. Alteration in pathological characteristics caused by MCD-VEGF is indicated in italic. Potential functions without direct proof by experimental and clinical data are indicated with a question marker. MCD-VEGF plays a significant role in causing retinal inflammation, neovascularization, vascular leakage, vascular lesion, and protein alteration and modification in the pathogenesis of DR. MCD-VEGF: Müller cell-derived-VEGF; DR: Diabetic retinopathy; VEGF: Vascular endothelial growth factor.

of occludin in the retina (Table 1) and by qualitative evidence from IHC in hypoxic CVKO mice generated with OIR[48]. Collectively, our data suggest that MCD-VEGF was a major inducer of vascular leakage in OIR-treated hypoxic mice and in diabetic mice through VEGF signaling-induced decrease of tight junction integrity.

To assess retinal vascular lesions in diabetic CVKO mice, the number of acellular capillaries in trypsin digested retinal flat-mounts was examined 6 mo after diabetes was induced[49]. The diabetic CVKO mice had 45% fewer acellular capillaries than that in controls (Table 2), suggesting that the loss of MCD-VEGF had a protective effect on retinal microvasculatures, which reduced vascular leakage through the endothelial barrier in DR.

MÜLLER CELL-DERIVED VEGF IN RETINAL DEVELOPMENT AND INTEGRITY

Disruption of MCD-VEGF in the CVKO mice did not affect the development of retinal and choroidal vasculatures and overall retina[48], as determined by morphological analysis in retinal sections with light microscopy, functional test with electroretinography, and retinal and choroidal vascular density and morphological examination in retinal and RPE/choroidal flat-mounts with IHC and fluorescein angiography. Although negative results from conditional gene KO studies are not conclusive, our observation is somewhat expected as Müller glia are one of the last few cell-types to develop and KO of VEGF in neural retina during embryonic development results in abnormal retinal vessels[59]. In our study, the loss of MCD-VEGF did not affect retinal integrity in the aging CVKO mice under normal and diabetic mice[49]. This result is seemly contradictory to the observation that VEGF is a survival factor for retinal ganglion cells, photoreceptors, and Müller glia[60,61]. The following may account for the "discrepancy": the disruption of MCD-VEGF in the CVKO mice did not remove VEGF completely as several types of retinal cells produce permeable VEGF and a basal level of VEGF may be sufficient for physiological VEGF function, such as neuronal function and integrity in the retina. In addition, our studies did not blocked VEGF signaling in any retinal cells. However, the result that MCD-VEGF had no apparent role in retinal development and integrity provides an advantage to investigating the role of MCD-VEGF in CVKO mice. Since there was no distinguishable defect in the animals under normal conditions, any phenotypical difference between the CVKO and control mice can be attributed to the defects from deleting MCD-VEGF.

CONCLUSION

Work from others and our laboratories demonstrated a major role for MCD-VEGF in DR, as summarized in Figure 1. Our data clearly pinpoint that MCD-VEGF plays a major role in protein alteration/modification, inflammation, neovascularization, vascular leakage, and vascular lesion in DR. Our study also suggests that MCD-VEGF may be a downstream regulator of DR-related master regulator, such as HIF1α/hypoxia, but upstream regulator for others, such as NF-κB and peroxynitrite. We also need to keep in mind that DR is a multifactorial disease. Other growth factors and pro-inflammatory mediators may be involved in developing DR in a VEGF-independent manner. A better understanding of VEGF-dependent and -independent pathways is the key to new therapeutic strategies for intervening multiple drug targets simultaneously, since anti-VEGF strategy alone cannot prevent DR completely. The CVKO mice provide an excellent animal model in this new endeavor.

VEGF is a neural protectant and it has been shown to modulate neuronal function in the brain[62]. Potentially, MCD-VEGF may also be involved in regulating neuronal integrity. With the development of tools in studying Müller glia and their relationship with neuronal survival in diabetes and hypoxia[44,63,64], the role of MCD-VEGF in neuroprotection in the retina should be sorted out shortly. Current literature suggests that MCD-VEGF may have a critical role in maintaining Müller glia through autocrine in hypoxia and diabetes. However, it is not clear whether MCD-VEGF acts solely as trophic factor in the maintenance of Müller glia. Other mechanism, such as proliferation (Müller cell self-renew), may also be potentially important to the maintenance of retinal integrity. In principles, mammalian Müller cells are capable of dedifferentiation, proliferation, and differentiation into various retinal neurons under various conditions and

are considered as a major retinal stem cell[65]. It will be fascinating if MCD-VEGF can act similarly as other growth factors in differentiating Müller cells to neurons for neuro-protection[66].

Although there are many publications on VEGF or MCD-VEGF in DR, little is known about its actually signaling pathways. As discussed earlier, the presence of at least seven VEGF receptors and co-receptors accounts for the difficulties in revealing their mechanisms. Additionally, VEGF is a secreted protein and loss of VEGF produced by a single cell-type can be compensated by others. Delineating detailed mechanisms may greatly enhance our understanding to the pathogenesis, treatment, and neuronal function in DR, which is critical to the improvement and safety of current anti-VEGF strategy and to the design of new treatments for the disease.

ACKNOWLEDGMENTS

We thank Dr. Gennadiy P Moiseyev for critical reading of this manuscript and members of our laboratories for providing data and helpful discussions.

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P- Reviewer: Das UN, García Mayor R, McNally RJQ, Saleh J, Swierczynski J **S- Editor**: Tian YL **L- Editor**: A **E- Editor**: Zhang DN

World Journal of Diabetes

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DOI: 10.4239/wjd.v6.i5.734

World J Diabetes 2015 June 10; 6(5): 734-743
ISSN 1948-9358 (online)

MINIREVIEWS

What neonatal complications should the pediatrician be aware of in case of maternal gestational diabetes?

Delphine Mitanchez, Catherine Yzydorczyk, Umberto Simeoni

Delphine Mitanchez, Division of Neonatology, Department of Perinatology, Armand Trousseau Hospital, 75012 Paris, France
Catherine Yzydorczyk, Umberto Simeoni, Division of Pediatrics and DOHaD Laboratory, CHUV University Hospital and UNIL, 1011 Lausanne, Switzerland

Author contributions: All the authors made substantial contributions to conception and design of the review, made critical revisions and final approval of the version of the article to be published.

Conflict-of-interest: None.

Correspondence to: Delphine Mitanchez, MD, PhD, Division of Neonatology, Department of Perinatology, Armand Trousseau Hospital, 26 Avenue du Docteur Arnold Netter, 75012 Paris, France. delphine.mitanchez@trs.aphp.fr
Telephone: +33-1-44736191
Fax: +33-1-44736892

Received: August 28, 2014
Peer-review started: August 28, 2014
First decision: December 17, 2014
Revised: January 29, 2015
Accepted: March 16, 2015
Article in press: March 18, 2015
Published online: June 10, 2015

Abstract

In the epidemiologic context of maternal obesity and type 2 diabetes (T2D), the incidence of gestational diabetes has significantly increased in the last decades. Infants of diabetic mothers are prone to various neonatal adverse outcomes, including metabolic and hematologic disorders, respiratory distress, cardiac disorders and neurologic impairment due to perinatal asphyxia and birth traumas, among others. Macrosomia is the most constant consequence of diabetes and its severity is mainly influenced by maternal blood glucose level. Neonatal hypoglycemia is the main metabolic disorder that should be prevented as soon as possible after birth. The severity of macrosomia and the maternal health condition have a strong impact on the frequency and the severity of adverse neonatal outcomes. Pregestational T2D and maternal obesity significantly increase the risk of perinatal death and birth defects. The high incidence of maternal hyperglycemia in developing countries, associated with the scarcity of maternal and neonatal care, seriously increase the burden of neonatal complications in these countries.

Key words: Birth defects; Hypoglycemia; Respiratory distress; Preterm; Perinatal mortality; Type 2 diabetes; Obesity

Core tip: Increased mortality and morbidity are historically attributed to neonates of diabetic mothers. A discerning analysis of the literature shows that these adverse outcomes are uncommon among infants born from "pure" gestational diabetes mellitus (GDM) mothers, well managed during pregnancy. Macrosomia is the predominant adverse outcome and the main factor linked to neonatal complications. Poor maternal glycemic control, especially in the context of maternal type 2 diabetes and obesity increases the risk of all adverse neonatal outcomes, most strikingly the risk of perinatal mortality and birth defects. Developing strategies for screening and managing women with GDM must be encouraged notably in middle and low income countries and, also to limit the adverse effects on global health population in the future.

Mitanchez D, Yzydorczyk C, Simeoni U. What neonatal complications should the pediatrician be aware of in case of maternal gestational diabetes? *World J Diabetes* 2015; 6(5): 734-743 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/734.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.734

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of any degree with onset or first recognition during pregnancy. In high income countries, but also in middle and low income countries, because of the spreading of industrialized lifestyle, the incidence of obesity and type 2 diabetes (T2D) has dramatically increased, and subsequently the incidence of GDM[1].

In high-resource countries, progress has been made during the past fifty years regarding preconceptional care, screening and management of GDM. However, in low and middle-income countries, quality of antenatal care to detect and manage GDM, are often poorly available. As a consequence, the prenatal and neonatal burden of GDM may be paradoxically higher in these countries, although this point is not well documented[2].

Much of the currently available knowledge on the consequences of maternal diabetes on the offspring has been provided by studies on type 1 diabetes (T1D), while the risks related to GDM, which is much more frequent, need to be clarified in order to improve and to adapt neonatal management[3]. Moreover, extensive data suggest that the offspring of diabetic mothers is furthermore exposed to an increased risk of developing chronic, non-communicable diseases at adulthood[4].

Neonatologists are facing first-line this new epidemiologic setting. This review addresses the currently available knowledge on short term consequences of GDM in neonates and focuses on situations with increased risks of neonatal adverse outcomes.

SHORT TERM OUTCOMES

Macrosomia

Macrosomia is the most constant complication in GDM. The concept of excessive fetal growth is expressed either by the word "macrosomia" or by the expression "large for gestational age" (LGA). Macrosomia is defined by a birth weight (BW) of 4000 or 4500 g and more, depending on the authors. However, in this definition, gestational age (GA) is not taken into account. The term LGA corresponds to a BW $\geqslant$ 90th percentile or > +2SD (> 97th percentile) for GA. This definition allows premature newborns with excessive fetal growth to be identified. Macrosomia in newborns of diabetic mothers is characterized by excess body fat, an increased muscle mass and organomegaly, without increase in brain size.

The Pedersen-Freinkel's hypothesis, expressed sixty years ago, suggested that fetal overgrowth is related to increased transplacental transfer of maternal glucose, which stimulates the release of insulin by fetal pancreatic beta cells[5]. Insulin is a major factor of fetal growth and it up-regulates the Insulin-like Growth Factor (IGF) system, subsequently leading to fetal macrosomia. According to this hypothesis, different studies have characterized the link between maternal glycemia and neonatal macrosomia or fat mass[6,7].

The HAPO study showed a continuous, positive association between maternal glycemia, fetal hyperinsulinism and BW[8]. A linear and continuous relationship between body fat percentage in newborns, maternal glycaemia and fetal insulin levels has been found in this study[9]. More recently, other mechanisms that may also contribute to fetal overgrowth were evoked, like maternal metabolic environment and placental modifications[10]. In particular, maternal lipids availability and transport to the fetus may be enhanced in case of maternal diabetes[11].

Hence, all types of maternal diabetes are risk factors for macrosomia. As discussed below, macrosomia is *per se* a cause increased neonatal adverse outcome and this point emphasizes the importance of recognizing the excess of growth, even in preterm infants. Treatment of GDM significantly reduces the rate of macrosomia[12,13].

Preterm birth

A number of studies have reported an increased risk of preterm births in case of diabetes. However, data are not always available on the respective proportion of induced and spontaneous births, considering the increased maternal and fetal morbidity of diabetes during pregnancy. The benefits of early delivery to avoid fetal death or shoulder dystocia must be balanced against the morbidity linked to preterm birth, especially the respiratory morbidity.

The link between GDM and spontaneous preterm birth is still controversial. Hedderson *et al*[14] showed in a large cohort study that GDM was an independent risk factor for spontaneous preterm birth (RR = 1.42, 95%CI: 1.15-1.77). On the other hand, Yogev *et al*[15] found that the rate of spontaneous preterm delivery was not increased in GDM compared to non-GDM patients. Nevertheless, both studies found a relationship between higher glucose values in the oral glucose tolerance test (OGTT) or higher mean blood glucose levels and preterm birth.

Metabolic disorders

Hypoglycemia: The link between macrosomia, increased cord C-peptide levels that reflects fetal insulin secretion, and neonatal hypoglycemia has long been known. The data collected by the HAPO study confirmed this relationship: neonatal hypoglycemia was strongly associated with elevated cord serum C-peptide levels[16]. The infant of a diabetic mother is

at risk of transient hyperinsulinism, which prevents at birth the normal activation of metabolic pathways producing glucose and ketone bodies, and causes increased glucose consumption by tissues[17].

The exact incidence of hypoglycemia in case of maternal diabetes is difficult to assess due to the various definitions used for neonatal hypoglycemia in the literature. The rate of intravenously treated hypoglycemia was reported between 5% to 7% in two large studies[18,19]. Comparisons with the risk observed in healthy newborns are difficult also because monitoring of blood glucose at birth was different according to the mother was diabetic or not in most of the studies. At last, in many studies, blood glucose level in neonates is checked soon after birth, although the pathologic significance of low blood glucose levels immediately after birth, in the absence of specific symptoms, is still questioned. Indeed, an immediate fall in blood glucose concentration is observed after birth because of the interruption of placental supply, reaching a nadir between 1 and 2 h in healthy term infants[20]. Normal levels at this period cannot be distinguished from abnormal ones in asymptomatic infants and the incidence of hypoglycemia is likely to be overestimated[21]. From 3 h of age, blood glucose then rises spontaneously, even in the absence of any nutritional intake, due to the activation of metabolic regulatory pathways. Therefore, in the absence of abnormal clinical signs, the first blood glucose measurement is recommended after the second feed, which generally allows infants who cannot manage adequate early glucose homoeostasis to be identified[21].

There is currently no consensus on the indications for systematic glucose blood monitoring in asymptomatic infants born to diabetic mothers. It seems reasonable to consider that LGA or growth restricted infants (< 10th percentile) born to diabetic mother may benefit from blood glucose concentration check at 3 to 6 h intervals during the first day of life. On the other hand, normal-grown infants of mothers with diet-controlled GDM should not be monitored[22].

For newborns with no clinical signs, therapeutic intervention can be considered starting at a threshold value of 0.36 g/L (2.0 mmol/L). Early and frequent breastfeeding remains the key in preventing hypoglycemia, whatever the infant's BW, as far as he/she is able to feed autonomously. Therefore, infants of diabetic mothers should be kept aside their mother, in the absence of significant complications requiring a transfer to a special care neonatal unit. Even in mildly or moderately symptomatic infants with low blood glucose levels, sustained breastfeeding, or eventually formula supplements should be tried first, provided a satisfactory clinical response is obtained[22]. In case the infant is unable to feed, an IV glucose supplementation (3-6 mg/kg per hour) should be provided at constant rate of infusion, in order to avoid rebound hypoglycemia.

Hypocalcemia: Hypocalcemia can be defined by plasma calcium concentration below 2 mmol/L or ionized calcium concentration below 1.1 mmol/L, regardless of GA or BW. Transient neonatal hypocalcemia has been mainly reported in neonates of pregestational insulin dependent- diabetic mothers and may be partly related to maternal hypomagnesemia and subsequent fetal hypomagnesemia. The severity of hypocalcemia also appeared to be related to the severity of maternal diabetes, as calcium concentration in the neonates was negatively related to maternal HbA1c levels[23].

It seems that hypocalcemia is rarely of clinical significance, particularly in case of GDM, unless other complications are associated[24].

The mechanism is still unclear but seems to involve an abnormal calcium phosphorus metabolism during pregnancy with a decrease in calcium and vitamin D concentrations especially during the third trimester. Some studies have reported an association between GDM and low maternal vitamin D status, particularly with poor blood glucose control. Conversely, there are growing evidences that women who develop GDM are more likely to be vitamin D deficient[25]. Other factors like prematurity and perinatal asphyxia can contribute to low calcium levels[26].

Therefore, there is no indication to screen healthy baby for hypocalcemia and hypomagnesemia. When treatment is indicated, it consists to give oral vitamin D supplements and calcium gluconate orally or intravenously (40-60 mg/kg per day) and magnesium treatment according to plasma level.

Hyperbilirubinemia: Hyperbilirubinemia is more frequently observed in infants born to diabetic mothers. It is not a serious complication if non-toxic levels are diagnosed and treated, which is usually the case. The risk of nuclear icterus, the severe form of hyperbilirubinemia, is not reported in cases of diabetes as being more frequent. In the HAPO study, hyperbilirubinemia was weakly associated with maternal blood glucose levels[8]. Polycythemia could be one of the reasons, but additional mechanisms, such as preterm birth, poor liver conjugation are likely to be involved.

Hematologic disorders

It has been reported that infants of diabetic mothers may have polycythemia [hematocrit (Ht) higher than 65%]. Mechanisms evoked are reduced transplacental oxygen transport to the fetus and increased fetal oxygen consumption due to fetal hyperinsulinism. This may lead to fetal hypoxia and increased levels of fetal erythropoietin. However, no consistent correlation between plasma erythropoietin level and polycythemia has been reported in human. Increased insulin and IGFs levels can also increase red blood cells production. A strong positive correlation between maternal β-hydroxybutyrate levels and polycythemia was observed in a small observational study[27].

Normovolemic polycythemia seen in infants from diabetic mother can lead to hyperviscosity. Early symptoms are unspecific, feeding problems, plethoric aspect, acro-cyanosis, lethargy, hypotonia, respiratory distress, jitteriness and irritability, seizure (due to multiple cerebral infarcts), necrotizing enterocolitis, hyperbilirubinaemia and hypoglycemia have all been found associated. Polycythemia may also favor deep vessels thrombosis. Hypoglycemia may be aggravated in infants from diabetic mothers in case of polycythemia, due to increased glucose consumption by the increased red cell mass. Partial exchange transfusion with saline solution should be performed in symptomatic infants according to the formula (volume exchanged in mL): (Ht-55) x weight (kg) x 80/Ht.

Respiratory disorders

The rate and the risk of respiratory distress syndrome (RDS) in cases of GDM cannot be accurately established, due to insufficient precise data[28]. In a recent study from the French birth cohort in 2011, including 474 614 births, the risk for neonatal respiratory disorders was slightly but significantly increased in case of GDM [OR adjusted on mother's age and gestational age, 1.2 (1.1-1.3)] (personal data not yet published).

It is generally recognized that, besides RDS, infants born to diabetic mothers are exposed to increased risk of transient tachypnea of the newborn. This is more likely to happen after caesarian section due to delayed reduction of alveolar fluid at birth and when the infants have macrosomia. This was clearly showed in infants of T1D mothers[29].

Diabetes, but also maternal body mass index (BMI), is associated with a higher risk of persistent pulmonary hypertension (PPH). However, other independent risk factors like macrosomia and caesarean deliveries might be in the causal pathway between diabetes, overweight and PPH[30].

Cardiac disorders

Hypertrophic cardiomyopathy: Fetuses exposed to maternal hyperglycemia and hyperinsulinism, are prone to develop hypertrophic cardiomyopathy. It primarily affects the interventricular septum, but can extend to the myocardium in more severe cases[31].

Myocardial hypertrophy has been reported in both pregestational diabetes and GDM with a wide range of frequencies (between 25% to 75% of infants born to diabetic mothers)[32,33]. The incidence was lower in case of pure GDM comparing to pregestational diabetes[34]. The most recent studies showed that good maternal glycemic control does not entirely prevent interventricular septum hypertrophy and minor fetal cardiac function impairment, regardless of the type of diabetes[35,36]. Although myocardial hypertrophy is associated with an overall decrease in ventricular compliance and an increase in contractility of the left and right ventricles, it is most often asymptomatic. It can sometimes lead to severe morbidity and mortality, according to the severity and the extension of cardiac hypertrophy. Major septal hypertrophy can lead to subaortic stenosis and secondary mitral insufficiency. It is usually considered that heart hypertrophy resolves anatomically within few months. However, the long term effect of diabetic cardiomyopathy on heart function remains to be elucidated.

Cardiac malformations: Some data supports that GDM carries a small but significantly increased of congenital defects (ORs between 1.1 and 1.3), but it is much lower than in women with pregestational diabetes[37]. The malformations described are similar to those reported in pregestational diabetes, especially cardiovascular defects and anomalies involving the musculo-skeletal and central nervous systems[38,39].

The most commonly reported cardiac malformations include transposition of the great arteries, double outlet right ventricle, truncus arteriosus, hypoplastic left heart syndrome and ventricular septal defects[40].

Antenatal ultrasounds play an important role in monitoring fetal cardiac anatomy and function. Antenatal diagnosis of cardiac malformation is helpful to decide the place of birth when specific neonatal cardiologic care is needed. Babies of women with GDM should have an echocardiogram in the presence of clinical signs at birth associated with congenital heart malformations (cyanosis, murmur) or cardiomyopathy (heart failure).

Neurological impairments

Infants of diabetic mothers are prone to neurologic impairments, mainly due to perinatal asphyxia, birth traumas and metabolic disorders.

Perinatal asphyxia: Increased risk of perinatal asphyxia has been reported in diabetic pregnancies in a number of studies. The risks of perinatal asphyxia are increased in case of macrosomia, particularly when there is a shoulder dystocia[28,41]. Impaired fetal environment characterized by fetal hypoxia has also been evoked as a contributing factor.

However, in cases of GDM, the incidence of perinatal asphyxia, defined by a 5-min Apgar score < 7, was very low (1%-2%) in a study including more than a thousand neonates of GDM mothers[18]. In another study, umbilical arterial pH < 7.2 was about 15% in GDM group, comparable to the non-diabetic group[42]. In both studies, the incidence was not influenced by the treatment of maternal diabetes.

Metabolic disorders: Glucose is the main energy substrate for the brain. In newborns, hypoglycemia can lead to the situation where brain energy metabolism cannot be sustained. The consequences of low blood glucose levels depend on the availability of other substrates, such as lactate and ketone bodies also used by the brain to provide energy. These alternative substrates are not routinely measured, and the normal

threshold values are unknown. Their availability also depends on the clinical and nutritional status of the infant.

For infants presenting with clinical signs compatible with hypoglycemia, like apnoea, hypotonia, jitteriness, apathy, hypothermia, tremors and seizures, treatment must ensure that blood glucose levels remain above 0.45 g/L (2.5 mmol/L). An IV bolus dose of glucose (150-200 mg/kg) should be administered urgently, followed by a constant rate infusion. It is necessary to check that thereafter blood glucose concentrations stabilize within normal ranges (20). In case of clinical signs, Cornblath *et al*[43] have suggested that the Whipple triad should be fulfilled: a low blood glucose concentration; signs consistent with hypoglycemia; and resolution of signs and symptoms after restoring blood glucose concentrations to normal values. Therefore, if symptoms persist despite adequate treatment, other causes should be investigated, since these symptoms are not specific.

Symptoms from hypocalcemia are similar to those observed in hypoglycemia, but usually present later, between 24-72 h of life[31]. Then, blood calcium concentration should also be measured in the presence of symptoms suggestive of hypocalcemia.

Brachial plexus injuries: The spinal cord is vulnerable to birth trauma with symptoms related to palsies of the brachial plexus.

The most common type of brachial plexus injury, also called Erb's palsy, involved the cervical roots C5 to C7. The infant presents with internally rotated arm and flexed wrist. The second most common type called total plexus palsy involves cervical roots C5-C8 and sometimes thoracic root T1. The infant presents with a flaccid and insensitive arm and with clawed hand. Paralysis of the hemi-diaphragm is also observed when phrenic nerve is involved, leading to respiratory insufficiency and requirement of mechanical ventilation[44].

Incidence of brachial plexus palsy in newborns of diabetic mothers is low, between 0.2% and 3%. As a consequence, the risk could not be accurately measured[28].

Poor suckling: It has been shown in the early nineties that maternal GDM may impair neonatal behavior, leading to lethargy and hypotonia related to delayed neural maturation. More recently, poorer suckling patterns were found at day 3 only among infants of insulin-managed GDM mothers, but not in the diet-managed mothers[45]. This study confirmed some degree of neurologic immaturity during the early neonatal period.

Digestive impairment

Apart from the difficulties to feed because of poor suckling pattern, neonates of diabetic mothers may also exhibit neonatal small left colon, a cause of functional lower intestinal obstruction that can mimic Hirschsprung disease. The pathophysiology is unknown but it is significantly associated with maternal diabetes. The treatment is always conservative as long as intestinal perforation does not happen. Contrast enema is both diagnostic (abrupt transition zone at the splenic flexure) and curative, promoting the evacuation of meconium relieving the intestinal obstruction[46].

FACTORS THAT INFLUENCE THE SEVERITY OF NEONATAL ADVERSE OUTCOME IN GDM

The adverse neonatal outcomes described above are not constant in all cases but they are significantly influenced by the quality of maternal care and by maternal health. Furthermore, most of these complications are more likely to happen in macrosomic infants.

Maternal conditions and neonatal outcomes

Impact of maternal blood glucose levels and pregestational T2D on neonatal outcomes: Perinatal death, malformations and prematurity are mainly influenced by maternal glucose levels. As discussed above, there is also a linear relationship between glucose maternal level and the frequency of macrosomia[8]. Furthermore, the analysis of the risk of fetal malformation and perinatal death in case of GDM shows that undiagnosed pre-pregnancy T2D has a substantial impact on these serious perinatal complications.

There is a relationship between the malformation rate and maternal fasting blood glucose level[39,47]. This risk also increases with maternal BMI, and when GDM is diagnosed during early pregnancy[48,49]. Most major malformations occur very early in gestation during the embryonic stage. In diabetic pregnancies, they are attributable to unstable periconceptional glycemia. Maternal hyperglycemia results in excess glucose metabolism in the developing embryo that may alter various molecular chain reactions: (1) altered cell lipid metabolism, notably the production of prostaglandin E2 involved in the patency of the ductus arteriosus in utero[50]; (2) high glucose levels induce an excess production of reactive oxygen species which has been shown to cause oxidative stress and subsequently increase the risk for fetal malformations, notably neural tube defect[51]; and also (3) high glucose levels induce the activation of many proteins involved in apoptotic cell death, including members of the caspase families[52]. Although data on the molecular basis of diabetic embryopathy have improved during the last years, mechanisms are still incompletely understood[53].

These clinical and physiopathologic data suggest that the increased risk of congenital defects in GDM reported in some studies is likely to be related to the inclusion of women with undiagnosed T2D in the GDM

groups[54].

Unlike in pregestational diabetes, the increased rate of fetal deaths in the 2nd and 3rd trimesters of pregnancy is debatable in cases of GDM[55,56]. In a large cohort study, the rate of mortality was 16.2/1000 in the GDM group *vs* 12.5/1000 in the general population. Six weeks after delivery, women diagnosed with GDM were re-classified by a post-partum glucose tolerance test. Women having diabetes on post-partum test were considered as "newly presenting T2D". When those women were excluded from the GDM group, perinatal mortality was 8.9/1000 in the "true" GDM group, which was similar to the general population. Mortality was the highest in the groups with T2D diagnosed before and after pregnancy (respectively, 39.1/1000 and 56.2/1000)[57]. These data demonstrated that the increased risk of perinatal death reported in case of GDM in some studies, seems to be attributable to undiagnosed T2D.

Prematurity is one of the leading causes of neonatal death. As discuss above, higher maternal glucose levels were observed in case of prematurity in GDM pregnancies. Furthermore, one of the main causes of induced preterm delivery is maternal pre-eclampsia which is more commonly associated with T2D pregnancies[58].

Impact of maternal obesity in the complications of GDM: Maternal obesity is associated with worse perinatal outcome even in glucose-tolerant women. Macrosomia is the main complication reported in overweight or obese women, independently of diabetes[59-62]. It is well recognized that neonates born to obese women, even if normal glucose tolerant, have increased fat mass[63]. As in GDM, increased adiposity at birth is related to maternal excess of glucose and lipids availability, and placental transfer to the fetus[11].

The risk of fetal and infant deaths are two to three times greater for women with preconceptional obesity, after excluding pregnancies affected by congenital anomalies or pregestational diabetes[64]. It was recently showed that even modest increases in maternal BMI were associated with increased risk of fetal death, stillbirth, and neonatal, perinatal, and infant death. The relative risk per 5-unit increase in maternal BMI ranged from 1.15 to 1.24[65]. Maternal obesity is also associated with an increased risk of a range of structural anomalies, with the higher risk for neural tube defects[66,67]. It is interesting to note that the risk of particular malformations such as omphalocele and diaphragmatic hernia is increased in obese pregnant women, but not in case of diabetes[68,69].

There is a tight link between maternal obesity and diabetes in pregnancy. Indeed, the risk of GDM increases with maternal BMI[70]. The overall population-attributable fraction of GDM related to overweight was estimated at 46.2%[71].

The benefit effect of treatment of diabetes on neonatal outcomes is lower in obese women, even if targeted levels of glycemic control are achieved. Furthermore, when GDM is untreated or poorly controlled, overweight and obese women have a higher risk of poor neonatal outcome, compared to normal weight GDM women[42,72].

The combination of GDM and obesity shows a greater impact on pregnancy outcomes than either GDM or obesity alone. This cumulative risk was shown for macrosomia, newborn percent body fat and birth trauma[73]. It was also reported for a composite neonatal outcome (BW > 4000 g, birth trauma, shoulder dystocia, hypoglycemia, or jaundice)[74].

Effects of macrosomia on neonatal outcomes

It has long been reported that the delivery of macrosomic infants is associated with a higher risk for adverse neonatal morbidity such as birth injury, respiratory distress and hypoglycemia. Macrosomia (BW > 4500 g), regardless of the cause, is also in itself a risk factor for asphyxia and perinatal death[75].

Macrosomia increases the risk of shoulder dystocia, regardless of the cause. In the study by Zhang *et al*[75], the risk of birth injury was the highest for infants with a birth weight 4500-4999 g and ⩾ 5000 g, [ORs 2.4 (2.2-2.5) and 3.5 (3.0-4.2), respectively].

In the case of GDM, there is a particularly high risk of respiratory distress in newborns with a BW ⩾ 4000 g, compared to those with a BW of less than 4000 g [OR = 3.1 (1.11-8.65)][76]. In the other study, the risk of respiratory complications increased with increasing BW ⩾ 4000 g, irrespective of maternal diabetic status[22]. Furthermore, it seems that clinically significant hypertrophic cardiomyopathy without concomitant fetal macrosomia is rarely observed[40].

The analysis of the data collected by the HAPO study showed that neonatal hypoglycemia was strongly related to elevated cord C-peptide levels. High C-peptide levels are related to the importance of fetal hyperinsulinemia that favors fetal excess of growth. Therefore, infants with excessive size at birth are prone to develop hypoglycemia[16]. It was shown that when BW was ⩾ 4000 g the risk of hypoglycemia increased, but the risk was higher when BW ⩾ 4000 g was associated with maternal GDM[76]. In another study, the risk of hypoglycemia increased with increasing BW, irrespective of maternal diabetic status[77].

SIZE OF THE BURDEN IN LOW INCOME COUNTRIES

The prevalence of risk factors for diabetes during pregnancy are increasing all around the world because of increasing incidence of T2D and obesity and the shift of age at onset of diabetes to younger age groups. T2D is an occult disease that can remain undiagnosed, especially in young women of reproductive age. A recent study reported an estimated global prevalence of hyperglycemia in pregnancy worldwide of 170/1000

live births in 2013. A majority of cases occurred in low and middle income countries (91.6%). The prevalence varies widely around the world. The South-East Asia region had the highest prevalence with 23% of live births, followed by the Middle East and North Africa region with 22%[78].

A community-based prospective program in India, with universal screening for GDM, showed that the prevalence of GDM was 13.9%. The frequency varied widely across urban, semi-urban and rural areas, respectively 17.8%, 13.8% and 9.9%. The prevalence also varied according to maternal BMI. For BMI $\geqslant$ 25 mg/m^2, the incidence was up to 28.4%, 23.8% and 16.1% in urban, semi-urban and rural areas, respectively[79].

A recent analysis of data from World Health Organization (WHO)'s Global Survey on maternal and perinatal outcomes in 23 developing countries described the prevalence of macrosomia, one of the main complications of maternal diabetes and obesity[80]. There was a large variation in the prevalence of babies with BW $\geqslant$ 4 kg, ranging from 0.5% in India, to 15% in Algeria. Maternal diabetes and increased gestational BMI were significantly associated with macrosomia in all regions. For example, in Algeria, where 15% of the babies had a BW $\geqslant$ 4 kg, 25% of the mothers were obese (BMI $\geqslant$ 30 kg/m^2). In in Latin America countries, frequency of maternal obesity was more than 30% (Argentina, Mexico, and Paraguay).

It can then be estimated that the burden of neonatal complications is higher in developing countries than in high-income countries, because of the high incidence of maternal hyperglycemia and the absence of screening and treatment of maternal diabetes, and finally because of substandard neonatal care. This is probably even worst within the rural areas, because of limited financial and human resources.

CONCLUSION

It is indisputable that diabetes during pregnancy exposes the fetus and the neonates to increased adverse outcomes. These risks mainly depend on maternal health condition. Thus, awareness of maternal health prior and during pregnancy is essential to pediatricians to anticipate the severity of neonatal adverse outcome. Health systems in low income countries are often insufficiently structured to provide adequate screening and care to diabetic mothers. Such situations seriously increase the burden of adverse fetal and neonatal outcomes, probably still underestimated.

The current definition of GDM does not allow identifying pregestational diabetes from true GDM. The WHO recently proposed new criteria for the diagnosis and definition of hyperglycemia first detected in pregnancy which distinguishes the more serious diabetes in pregnancy from GDM[81]. This is a considerable advance as we say that risks of serious complications for fetuses and the neonates are much higher in true diabetes than in GDM. This will help to better understand the burden of hyperglycemia in pregnancy and its relationship with the growing prevalence of T2D. This will also probably allow in the future to determine precisely the risks linked to GDM compared to those linked to T2D. Such distinction will subsequently help better identifying risks in the neonatal period, but also later in life. Indeed, offspring of diabetic and obese women, or macrosomic infants, are more likely to be obese and to have diabetes and cardiovascular diseases in adulthood[3,4]. These long-term consequences of diabetes in pregnancy are going to be the burden of further generations.

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P- Reviewer: Tskitishvili E **S- Editor**: Tian YL **L- Editor**: A
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DOI: 10.4239/wjd.v6.i5.744

World J Diabetes 2015 June 10; 6(5): 744-751
ISSN 1948-9358 (online)

MINIREVIEWS

Emerging links between type 2 diabetes and Alzheimer's disease

Gumpeny R Sridhar, Gumpeny Lakshmi, Gumpeny Nagamani

Gumpeny R Sridhar, Gumpeny Lakshmi, Gumpeny Nagamani, Endocrine and Diabetes Centre, Department of Obstetrics and Gynecology, Andhra Medical College, Visakhapatnam 530002, India

Author contributions: Sridhar GR, Lakshmi G and Nagamani G contributed equally to this paper.

Conflict-of-interest: None.

Correspondence to: Gumpeny R Sridhar, MD, DM, FACE, FRCP, Endocrine and Diabetes Centre, Department of Obstetrics and Gynecology, Andhra Medical College, 15-12-15 Krishnanagar, Visakhapatnam 530002, India. sridharvizag@gmail.com
Telephone: +91-891-2566301
Fax: +91-891-2509427

Received: August 29, 2014
Peer-review started: August 30, 2014
First decision: November 27, 2014
Revised: December 4, 2014
Accepted: March 16, 2015
Article in press: March 18, 2015
Published online: June 10, 2015

Abstract

Type 2 diabetes mellitus and Alzheimer's disease are both associated with increasing age, and each increases the risk of development of the other. Epidemiological, clinical, biochemical and imaging studies have shown that elevated glucose levels and diabetes are associated with cognitive dysfunction, the most prevalent cause of which is Alzheimer's disease. Cross sectional studies have clearly shown such an association, whereas longitudinal studies are equivocal, reflecting the many complex ways in which the two interact. Despite the dichotomy, common risk and etiological factors (obesity, dyslipidemia, insulin resistance, and sedentary habits) are recognized; correction of these by lifestyle changes and pharmacological agents can be expected to prevent or retard the progression of both diseases. Common pathogenic factors in both conditions span a broad sweep including chronic hyperglycemia *per se*, hyperinsulinemia, insulin resistance, acute hypoglycemic episodes, especially in the elderly, microvascular disease, fibrillar deposits (in brain in Alzheimer's disease and in pancreas in type 2 diabetes), altered insulin processing, inflammation, obesity, dyslipidemia, altered levels of insulin like growth factor and occurrence of variant forms of the protein butyrylcholinesterase. Of interest not only do lifestyle measures have a protective effect against the development of cognitive impairment due to Alzheimer's disease, but so do some of the pharmacological agents used in the treatment of diabetes such as insulin (especially when delivered intranasally), metformin, peroxisome proliferator-activated receptors γ agonists, glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Diabetes must be recognized as a risk for development of Alzheimer's disease; clinicians must ensure preventive care be given to control and postpone both conditions, and to identify cognitive impairment early to manage it appropriately.

Key words: Cognition; Insulin resistance; Insulin; Butyryl-cholinesterase; Dementia

Core tip: Type 2 diabetes mellitus is a risk factor for future development of Alzheimer's disease, the most prominent cause of cognitive failure in the elderly. Common pathogenic mechanisms underpin both

conditions. Therapeutic strategies in prevention (lifestyle changes) and pharmacological agents (biguanides, intranasal insulin, thiazolidinediones, glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors could also be useful against Alzheimer's disease.

Sridhar GR, Lakshmi G, Nagamani G. Emerging links between type 2 diabetes and Alzheimer's disease. *World J Diabetes* 2015; 6(5): 744-751 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/744.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.744

INTRODUCTION

The prevalence of diabetes mellitus is inexorably rising as evidenced by data from local, national and international studies[1-3]. Improved care of diabetes as well as general increase of longevity are predictive of greater proportion of elderly in the general population. It is well known that the critical problems of the aged relate to impaired activities of daily living and of cognitive decline. It is also recognized that compared to persons without diabetes, those with diabetes have dementia two to three times more commonly[4]. The economic and social burden on the health-care system as well as on care-providers would be enormous. It is essential if methods are available, to prevent or postpone the onset of both conditions; lifestyle changes appear to be effective in preventing both conditions[5].

CAUSES OF DEMENTIA

Dementia may be broadly classed into Alzheimer's disease, vascular dementia, dementia with Lewy bodies and frontotemporal dementia[4]. Of these, Alzheimer's disease is the most common type[6].

Dementia in diabetes: Epidemiological studies

A number of epidemiological studies have shown an association of dementia with diabetes. In the Hisayama study (1995), the relative risk (RR) of Alzheimer's disease was 2.18 (95%CI: 0.97-4.9) and of vascular dementia 2.77 (95%CI: 2.59-2.97)[7]. A later publication from the same study group (2011) showed an RR of 2.05 (95%CI: 1.18-3.57) for Alzheimer's disease and 1.82 for vascular dementia (95%CI: 0.89-3.71)[8]. The Rochester Study (1997) reported differences in the risk of Alzheimer's disease based on gender: RR was 2.27 for men (95%CI: 1.55-3.31) and 1.37 for women (95%CI: 0.94-2.01)[9]. The well cited Rotterdam Study (199) showed a relative risk of 1.9 for Alzheimer's disease (95%CI: 1.2-3.1)[10] (Table 1).

Table 1 Dementia and diabetes

Relative risk of AD	Relative risk of vascular dementia	Ref.
2.18	2.77	[7]
2.05	1.82	[8]
1.9	-	[10]

AD: Alzheimer's disease.

Conflicting results: Cross sectional vs longitudinal studies

Although there is a consensus from cross-sectional studies that hyperglycemia causes cognitive impairment, results from longitudinal studies are conflicting[4]. Two recent longitudinal studies show up differences in the occurrence of Alzheimer's disease and glucose tolerance.

The Baltimore Longitudinal Study of aging prospectively assessed a cohort of community-dwelling individuals. An investigation was made to relate serial glucose intolerance, insulin resistance with brain β amyloid burden measured *in vivo* using carbon 11-labelled Pittsburgh Compound B. The latter is utilized to image β-amyloid (Aβ) *in vivo* with PET scan; ^{11}C-Pittsburgh compound-B (11C-PiB), a PET Aβ ligand, is widely employed for early diagnosis of Alzheimer disease. It allows quantitative analysis of Aβ burden. Derived as a carbon-11 labelled thioflavin-T amyloid dys, it binds to Aβ plaques with high specificity and affinity. ^{11}C-Pittsburgh compound-B (11C-PiB), correlates with the rate of cerebral atrophy. Pathological process for Alzheimer's disease was established at autopsy[11]. Analysis was carried out using grouped and continuous mixed-models analyses. The key result of the study was, there was *no* significant correlation of brain markers of Alzheimer with insulin resistance or glucose intolerance during the follow up period of 22.1 years (SD:8.0)[11].

In contrast, a group from the University of Washington, which evaluated whether higher glucose levels increase the risk of dementia in those without diabetes, found that they did[12]. Participants, without dementia were drawn from the Adult Changes in Thought study (839 men, 1228 women, mean baseline age 76 years). In all 35264 glucose levels and 10208 glycosylated hemoglobin levels were analyzed. They were followed up for a median of 6.8 years. 524 subjects developed dementia (74 of 243 with diabetes and 450 of 1228 without diabetes). Higher levels of average glucose levels were related to development of dementia in both groups, ie those with and without known diabetes. The authors conclude that "higher glucose levels may be a risk factor for dementia, even among persons without diabetes"[12].

Can the conflicting results of these two rigorous, well-designed studies be resolved? The Baltimore study used both neuroimaging as well as autopsy to identify Alzheimer's pathological processes. The Adult Changes in Thought study performed a 6 year follow up in a large group of well-defined elderly. In the former, insulin resistance and glucose intolerance were not a risk factor for Alzheimer's changes; in the

latter, higher glucose levels even among those without diabetes may be a risk factor for dementia.

The apparent differences can be attributed to the variety of pathological changes in diabetes leading to dementia including Alzheimer's disease: chronic hyperglycemia, hypoglycemia (acute and recurrent), glycosylated of proteins, vascular disease, endothelial dysfunction, inflammation, altered blood brain barrier, dyslipidemia, insulin resistance, genetic predisposition, amyloid deposition and depression, among others[13]. More sensitive methods of measuring brain volume as a surrogate of cognitive function may throw light[4]. In addition, there is a flaw in using brain markers such as plaques in diagnosis of Alzheimer's disease: subjects may have amyloid plaques, yet display no symptoms of Alzheimer's disease throughout their life. This discrepancy between the presence of plaques and Alzheimers could play a role for a lack of finding a link between diabetes and Alzheimer's disease.

Hyperglycemia

Hyperglycemia is a recognized risk factor for cognitive impairment as shown by the ACCORD-MIND study and others[14,15]. Biological reasons for such changes were ascribed to neural damage following advanced glycosylated end products and oxidative stress, osmotic stress damaging the blood brain barrier and resultant leak of toxic substances leading to further damage of nervous structures[4]. In addition to chronic hyperglycemia as assessed by glycated hemoglobin, glycemic variability was also proposed to contribute to cognitive dysfunction. Measurements by continuous glucose monitoring revealed cognitive function was better correlated with diurnal variation in blood glucose[16]. Post prandial glucose levels could also be a contributing factor, acting *via* oxidative stress[4].

Hypoglycemia

Although severe hypoglycemia was shown to be associated with dementia in the elderly[17], when hypoglycemia is avoided by careful treatment as in the DCCT/EDIC study, there was no association between hypoglycemia and cognitive dysfunction[18]. In the elderly however, hypoglycemia, when coupled with atherosclerosis leads to organic brain damage which is often irreversible[4].

Role of insulin in brain

Cognition may be affected not only by alterations in the level of glucose, but also *via* the action of insulin. Upon transport through the blood brain barrier, insulin binds to its receptors, and is involved in modulating cognitive function. A large number of insulin receptors occur in brain areas related to memory such as the hippocampus and cerebral cortex. In addition it also aids the release of β-amyloid peptide extracellularly, and increases the expression of the enzyme which degrades insulin, insulin degrading enzyme (IDE)[4]. As the latter also degrades β-amyloid peptide, insulin deficiency results in accumulation of β-amyloid peptide. Both hyperinsulinemia and hyperglycemia were shown to increase neuritic plaque formation[19].

Information is being available about the origin of insulin in the brain and its role in cognition. Originally, brain was considered to be insulin insensitive because insulin did not influence the glucose uptake by the bulk brain. However insulin has been shown to be a neuroregulatory peptide playing a role in food intake and in monitoring the energy stores of the body[20]. Interestingly a role for insulin in modulation of memory and cognition is also emerging. Its action has been observed in regions associated with reward recognition such as hippocampus, and in global cognition and memory. Rather than passing across the blood brain barrier from the periphery, insulin appears to be produced locally for action as a neurotransmitter, regulated by glucose levels. In addition a para-arteriolar pathway for transport at the level of micro-vasculature has been proposed[20].

The role of insulin in the pathogenesis of Alzheimer disease has been described. Insulin can modulate Aβ peptide *in vitro*. The peptide is well known as a neuropathological hallmark of AD. Low levels of insulin in the brain can decrease the Aβ release into extracellular compartments. In addition hypoinsulinemia in the central nervous sytem can lower the levels of insulin-degrading enzyme, thereby impairing Aβ clearance. In all, chronic hyperinsulinemia in the peripheral circulation, along with decreased uptake of insulin into the brain can lead to dysregulation of Aβ and inflammation[21,22].

Role of microvascular disease in cognitive decline

Studies have shown that retinopathy and nephropathy are associated with impairment of cognition[22,23]. Small vessels in both organs arise from a similar embryonic antecedent and share similar structures; it is conceivable therefore that insults (*e.g.*, increased polyol pathway activity, myo-inositol dysmetabolism) result in similar adverse reactions in frontal lobe of the cerebral cortex leading to cognitive decline[4]. However, evidence is not unequivocal. Retinopathy is related to microvascular changes in diabetes. Because the retina shares many features with the brain, both developmental, anatomical (*e.g.,* microvascular bed) and physiological (*e.g.,* blood-tissue barrier), changes in retina were suggested to presage brain pathological processes. Alzheimer's disease is known to involve the retina, such as the macula and the optic disc. It has been suggested that pathological changes in the retina such as macular deposits, reduced thickness of retinal nerve, cupping of optic disc and retinal microvascular changes may be related to cognitive dysfunction and Alzheimer disease[24].

Insulin resistance and Alzheimer's disease

Downstream insulin signaling acts through a complex interplay involving phosphatidylinositol 3-kinase (P13K0 and mitogen activation protein kinase (MAPK).

The latter is associated with most metabolic effects of insulin. Unlike its peripheral effects, the action of insulin in the central nervous system is dependent on its crossing the blood brain barrier *via* direct transfer[25] through an insulin receptor protein, which is selectively distributed. In addition there is also local synthesis of insulin in the CNS. Unlike peripheral insulin receptors, those in the CNS differ in terms of structure, function and size. They are highly populated in the olfactory bulb, hypothalamus, cortex, cerebellum, hippocampus and are expressed in both neurons and glia[26].

The physiological effects of insulin in the brain are unlike those in the periphery: in an animal model it suppressed food intake and increased the level of glucose[27], acting in a way as its own counterregulation[25]. Compared to its weight, the brain depends on a larger amount of glucose for its metabolic needs compared to other tissues. Glucose reaches it *via* facilitated diffusion transported by glucose transport proteins. In the brain, insulin does not have a major effect on either the transport of glucose to the brain or its basal metabolism[28,29]. While it is not a major regulator of glucose metabolism in the brain, insulin indirectly affects neurons by modulating neurotransmitter release, neural growth, tubulin activity, nerve survival and synaptic plasticity[26]. In humans, insulin improves cognition independent of its effects on peripheral glucose[30].

Whereas acute increases of insulin improve cognition, chronic hyperinsulinemia can adversely affect neuronal function *in vitro* by increasing susceptibility to toxin and stress-induced effects[31]. Glycated proteins and inflammatory mediators could also have a pathogenic role[25].

Protein aggregation, diabetes and Alzheimer's disease

Protein aggregation has been suggested to be an underlying pathogenic factor between type 2 diabetes and Alzheimer's disease[32]. A number of hypotheses were proposed to explain why a biological protein can be transformed into a pathological entity with the ability to self-assemble: aging, high concentrations of the protein, mutation of amino acids or abnormal post-translational modification, modulated by environmental factors[32].

Alzheimer's disease is associated with accumulation of neurofibrillary tangles and amyloid fibers leading to neuronal cell loss. Amyloid β peptides form from cleavage of amyloid β-protein precursor seen in plaques. It is organized as amyloid fibrils, which are linear aggregates[32]. Diabetes is also characterized by localized and progressive amyloid deposition in the pancreatic β cell islets[33]. Common features of amyloid deposited in both Alzheimer's disease and diabetes include: linear appearance, with a beta-sheet structure, which begin to form from spherical oligomers that can self-assemble[34]. The islet amyloid peptide is secreted by β cells of the pancreas and consists of 37 amino acids.

ApoE-ε4

Expression of ApoE-ε4, which is related to diabetes as well, increases the risk of early onset Alzheimer's disease. It has increased ability to deposit Aβ, which is neurotoxic, and also impair its clearance[35]. ApoE-ε4 is less protective against oxidative stress and leads to cholinergic dysfunction seen in Alzheimer's disease, besides modifying the cholesterol transporter protein ABCA1.

Other potential associations

In addition other associations are also being recognized as risk factors for both diabetes and Alzheimer's disease: weight gain, acting perhaps through defective leptin signaling and increased formation of advanced glycation end products, which could have a pathogenic role in the amyloid plaques deposited in the brain[35]. Other emerging associations include disturbances in sleep and the circadian rhythm[36,37], and iron overload in brain among persons with obesity[38].

Insulin like growth factor

Animal studies have provided intriguing evidence that loss of insulin-like growth factors, along with insulin could lead to age-dependent brain atrophy with cognitive decline[39]. Insulin and its growth factors maintain brain protein content; their replacement can prevent brain protein loss, cell degeneration and demyelination. Lack of insulin and growth factors, which are common to type 2 diabetes and to Alzheimer's disease could therefore play a role in their pathogenesis and provide therapeutic targets in their treatment[39].

Butyrylcholinesterase

Butyrylcholinesterase, belonging to the esterase family of enzymes that also contain acetylcholinesterase[40] has been evaluated in relation to insulin resistance, cardiovascular disease, obesity and dyslipidemia[36]. Variant forms of the enzyme with little or no activity exist in isolated geographic populations[41] with apparently no adverse health effects[42]. Butrylcholinesterase was studied in relation to both type 2 diabetes mellitus and to Alzheimer's disease[43-45]. with studies suggesting a possible protective effect against Alzheimer's disease and risk for fronto-temporal dementia[46].

A number of hypothesis were put forward for the association of butyrylcholinesterase with type 2 diabetes mellitus and Alzheimer's disease[33,36]. Plaques in the brains of individuals with dementia had higher levels of butyrylcholinesterase, which is also localized in neurofibrillary tangles. It was also shown to attenuate amyloid formation[47].

Similarly in type 2 diabetes, butyrylcholinesterase may modify the expression of insulin resistance or by way of amyloid fibril deposition in β cells of the pancreas[33]. It was shown to interact with amylin

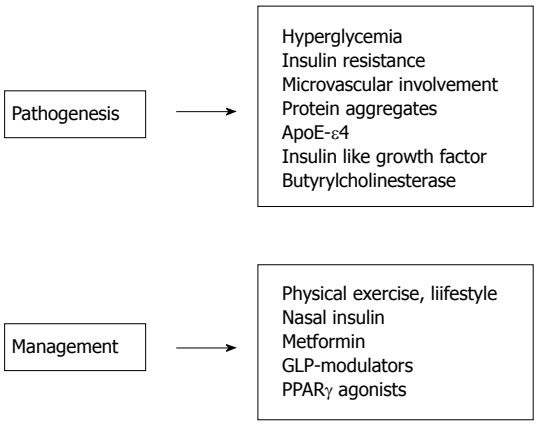

Figure 1 Links between Alzheimer's disease and type 2 diabetes mellitus.

and attenuate the formation of amylin fibril as well as its oligomer. When applied to cultured β cells, it was protective against amylin cytotoxicity[47]. Butyrylcholinesterase was shown to participate in the progression of metabolic syndrome to type 2 diabetes mellitus. A majority of subjects with type 2 diabetes show extracellular deposits formed by islet amyloid polypeptide (IAP), adjacent to the β cells. Elevated levels of IAP are found in conditions of insulin resistance. Disturbed balance of sympathetic and parasympathetic nervous system could participate in metabolic syndrome. Lower vagal activity could in part be caused by increased hydrolysis of acetylcholine mediated by increased butyrylcholinesterase[48]. Lowering of acetylcholinesterase results in reduced parasympathetic signals and increased ratio of sympathetic signals[48]. In addition BChE was reported to attenuate the formation of Aβ amyloid fibrils[47]. Essentially subjects with metabolic syndrome had elevated levels of BChE compared to those with type 2 diabetes and with controls. *In vitro* interaction of BChE was observed with amylin. It interacted with amylin, and attenuated the formation of both amylin fibril and oligomer formation, showing that it can protect cultured β cells from cytotoxicity due to amylin[47]. Thus increased BChE seen in metabolic syndrome could protect pancreatic β cells by reducing toxic amylin oligomer formation[47].

A bioinformatics study suggested the following sequences ($E < e^{-5}$) were associated with both type 2 diabetes and Alzheimer's disease: butyrylcholinesterase precursor K allele (NP_000046.1), acetylcholinesterase isoform E4-6 precorsor (NP_000656.1) and apoptosis-related acetylcholinesterase (1B41|A). In an animal study, streptozotocin-induced diabetes was associated with elevated butyrylcholinesterase activity, lowered superoxide dismutase and impaired cognitive function assessed by Morris water maze method[49]. Being low-grade inflammatory conditons, both type 2 diabetes mellitus and Alzheimer's may be target conditions for utilization of butyrylcholinesterae as a biomarker[50], as well as a treatment target[33].

The principal drugs currently available to manage Alzheimer's disease act *via* modifying butyrylcholinesterase levels. Renewed interest in the possible role of "missing genes" in people who are apparently healthy may aid in uncovering new treatment modalities[51]. Individuals with variant butyrylcholinesterae activity genes may be a potential group for such long-term follow up studies vis a vis their propensity or protection against diabetes and Alzheimer's disease[40].

THERAPEUTIC IMPLICATIONS

The interest of linking type 2 diabetes mellitus and Alzheimer's disease lies not in science, but more in translational science: how understanding the common pathogenesis can help in prevention and treatment of both conditions. Other than the scope for future therapies, evidence is now available for the currently available drugs used in diabetes[52] to have a modulatory effect on the cognitive decline due to Alzheimer's disease.Along with its action on AMPK, metformin has been recently shown to influence the incretin system by increasing the secretion of glucagon-like peptide-1 (GLP-1)[53]. GLP-1 and GIP receptors are known to be expressed in the brain, and direct activation of these receptors may be a potential strategy to treat Allzheimer's disease[54]. In addition, metformin also influences gut microbiome along with its other putative actions in the management of diabetes mellitus[55]. The use of drugs used in diabetes mellitus such as metformin, GLP-1 mimetics (exenatide and liraglutide) and peroxisome proliferator-activated receptors γ agonists may all be of potential benefit in the prevention and management of Alzheimer disease[56].

When a theoretical basis for insulin to affect brain responses was studied in clinical practice, a recent review of 8 published studies on effect of intranasal insulin on cognition, comprising 328 participants showed first of all, no significant adverse effects. Generally the authors concluded that "the limited clinical experience suggests potential beneficial cognitive effects of intranasal insulin"[57]. In a single study, use of 20 IU intranasal insulin showed improved immediate recall in Apoε4(-) subjects but not in Apoε4(+) subjects.

Other than correcting hyperglycemia, some of the conventional antidiabetic agents were shown to affect cognition. Metformin protected brain against oxidative stress in rats, and by preventing apoptosis[54]. However caution must be exercised because of metformin leading to increased biogenesis of Alzheimer's amyloid peptides[58] and increased risk of cognitive impairment[59]. Thiazolidinediones, which act at the nuclear receptors of insulin-sensitive tissues, affect transcription of genes affecting lipid and glucose metabolism. Early studies suggested that this group of drugs may favourably affect cognition, before the clinical use tapered due to their adverse drug effect profile.

Pharmacological agents modulating the incretin

system have now become mainstream in many markets world-wide. Pathological studies showed that sitagliptin lowered APP and Aβ deposition in the hippocampus of transgenic Alzheimer's disease mice[53]. However whether it does so by lowering glucose levels or independently remains to be clarified. Interest arises because liraglutide, another drug in the same group had similar protective effects (Figure 1).

CONCLUSION

In conclusion, diabetes mellitus and Alzheimer's disease are both common and increasing in incidence in the aging population. Recent evidence has shown common pathogenic factors operating in both conditions. Thereby common preventive and therapeutic agents may be used in their prevention and treatment. Physicians caring for the elderly must be aware of the increased risk of the other when one condition is present. Common pathogenesis and therapeutic agents make it possible to manage both using similar lifestyle changes and pharmacological agents.

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P- Reviewer: Hssan M, Hwu CM, Laher I **S- Editor**: Tian YL
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World Journal of Diabetes

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DOI: 10.4239/wjd.v6.i5.752

World J Diabetes 2015 June 10; 6(5): 752-758
ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Patient attitudes about financial incentives for diabetes self-management: A survey

Katherine S Blondon

Katherine S Blondon, Division of General Internal Medicine, University Hospitals of Geneva, 1205 Geneva, Switzerland

Author contributions: Blondon KS solely contributed to this paper.

Ethics approval: By the Institutional Review Board of the University of Washington.
Informed consent: Statement available online, participants gave their online consent by accepting to participate.
Conflict-of-interest: No funding to disclose.
Data sharing: No additional data are available.

Correspondence to: Katherine S Blondon, MD, PhD, Division of General Internal Medicine, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1205 Geneva, Switzerland. kblondon@uw.edu
Telephone: +41-79-5534323
Fax: +41-22-3729235

Received: November 27, 2014
Peer-review started: November 28, 2014
First decision: January 20, 2015
Revised: March 3, 2015
Accepted: April 1, 2015
Article in press: April 7, 2015
Published online: June 10, 2015

Abstract

AIM: To study the acceptability of incentives for behavior changes in individuals with diabetes, comparing financial incentives to self-rewards and non-financial incentives.

METHODS: A national online survey of United States adults with diabetes was conducted in March 2013 (n = 153). This survey was designed for this study, with iterative testing and modifications in a pilot population. We measured the demographics of individuals, their interest in incentives, as well as the perceived challenge of diabetes self-management tasks, and expectations of incentives to improve diabetes self-management (financial, non-financial and self-rewards). Using an ordered logistic regression model, we assessed the association between a 32-point score of the perceived challenge of the self-management tasks and the three types of rewards.

RESULTS: Ninety-six percent of individuals were interested in financial incentives, 60% in non-financial incentives and 72% in self-rewards. Patients were less likely to use financial incentives when they perceived the behavior to be more challenging (odds ratio of using financial incentives of 0.82 (95%CI: 0.72-0.93) for each point of the behavior score). While the effectiveness of incentives may vary according to the perceived level of challenge of each behavior, participants did not expect to need large amounts to motivate them to modify their behavior. The expected average amounts needed to motivate a 5 lb weight loss in our population and to maintain this weight change for a year was $258 (interquartile range of $10-100) and $713 (interquartile range of $25-250) for a 15 lb weight loss. The difference in mean amount estimates for 5 lb and 15 lb weight loss was significant (P < 0.001).

CONCLUSION: Individuals with diabetes are willing to consider financial incentives to improve diabetes self-management. Future studies are needed to explore incentive programs and their effectiveness for diabetes.

Key words: Patient incentives; Diabetes self-management; Motivation; Weight loss; Patient engagement

Core tip: Patient incentives have shown potential in modifying behaviors such as smoking cessation or weight loss. This online survey for individuals with diabetes explores their attitude towards incentives (financial, non-financial and self-rewards) for diabetes self-management. Although nearly all participants showed positive expectations about financial incentives, they favored financial incentives for less challenging behaviors, and non-financial incentives for more challenging behaviors. This survey also enquired about expected amount of incentives, in particular for a 5 lb weight loss, maintained over a year.

Blondon KS. Patient attitudes about financial incentives for diabetes self-management: A survey. *World J Diabetes* 2015; 6(5): 752-758 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/752.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.752

INTRODUCTION

Behavioral changes are key part of diabetes self-management[1], but they are difficult to implement and maintain. Setting goals is a key step to behavior change. Findings from a group of Swedish investigators about patients' willingness to pay to improve their diabetes self-management emphasize the importance of certain goals for patients, such as weight loss, less frequent or severe hypoglycemic events and lower HbA1c levels[2].

Financial incentives have shown potential in supporting patients to modify their behaviors[3], such as for smoking cessation[4] or weight loss[5,6]. Acceptability and feasibility of incentives may vary with different health behaviors, as the complexity of the behaviors vary widely. The effect and type of incentives for one-time vaccinations[7] may differ from incentives for repeated, constant efforts for weight loss[8]. Financial incentives for health and wellness are being used by a rapidly growing number of employers, with nearly 90% companies using such measures in the 2013 survey from Fidelity Investments and the National Business Group on Health (57% increase since 2009)[9]. While some authors have suggested using financial incentives for diabetes self-management behaviors, evidence for incentivized diabetes self-care is still scarce[10]. There is to our knowledge only one study on patient incentives for diabetes self-management, comparing incentives to peer mentors or usual care[11]. Although this study did not find a significant benefit, the sample was small and the reward design was solely based on the outcome (change in HbA1c value).

Incentives programs can reward different types of goals. Some programs provide outcome-based incentives, where rewards are given for achieved final results (*e.g.*, HbA1c or BMI, body mass index). These outcome-based rewards can favor individuals who are healthier at baseline. Other programs reward the total amount of behavior change-related results, thus favoring individuals who are more obese, for example, who then have more weight to lose. Finally, some incentive programs reward the process, such as attendance at group sessions, or tracking and reporting results. These are more equitable, because it is more attainable for all participants. Furthermore, prior research suggests that process-based rewards may be more effective than outcome-based rewards, and that associating them could have an additional effect.

Incentives programs also differ in the rewards that are given. Some programs have financial incentives, others offer vouchers and discounts[12], and yet others propose badges and stars without financial stakes. Self-rewards are another type of reward, which one gives oneself for reaching self-defined goals. Rewards are typically used to address present bias, the tendency to value small immediate rewards over large rewards in the distant future. Controversies about using financial incentives for long-term behavior change have been raised due to the "undermining effect[13,14]". Long-term behavior changes are driven by intrinsic motivation, or inherent motivation, rather than by extrinsic motivation, which are rewards that are external to the behavior[15]. Prior studies on rewards have shown that the removal of extrinsic rewards can results in a decrease in intrinsic motivation, the so-called undermining effect[13,14].

The goal of this study was to explore patients' expected responses to financial and non-financial incentives to improve their diabetes self-management. We hypothesized that expected responses to the type of incentive (financial, non-financial or self-reward) would differ with the perceived level of challenge of the incentivized behavior. We also wanted to explore the amount of money participants considered necessary to motivate behavior change. These results can help guide the creation and implementation of new patient-centered approaches for diabetes self-management.

MATERIALS AND METHODS

Setting

The survey was developed by the authors through an iterative process of editing and was tested on two groups of altogether 15 students and faculty at the University of Washington. The feedback led to modifications to simplify the survey, and resulted in a final set of 10 questions in addition to demographic information. Two individuals of the test groups had diabetes. The survey was available in English.

This online survey was launched to a panel of

Table 1 Overview of survey questions

Survey question themes
Three most difficult and three easiest diabetes self-management tasks:
Tracking health parameters
Choosing foods
Adapting medications or insulin
Affording healthcare
Adjusting meds and diet around unexpected events
Perceived challenges for living with diabetes (detailed in Table 2)
Perceived helpfulness of incentives for motivating healthier behaviors:
Cash rewards (weekly, monthly or yearly)
Tangible rewards, *e.g.*, vouchers (weekly, monthly or yearly)
Decrease in future insurance premium
Non-financial rewards (badges, stars)
Social support (sharing with friends)
Self-reward (setting money aside for a self-set goal)
Potential sources of funding for the rewards (employer, insurance, self-paid)
Estimated amount needed to lose weight and keep it off for a year:
To lose 5 lb
To lose 15 lb

three hundred members of a commercial survey website in March 2013. This company has a large panel of international volunteer members who can be filtered according to personal information such as age or country of residence. Eligible members receive recruitment emails, and choose freely whether to respond or not. All participants gave their informed consent prior to their inclusion in the study. Participants who complete the proposed surveys receive website points, which can be exchanged for tangible gifts.

Recruitment

Our survey was administered in English, and was restricted to United States residents. It was open until 300 participants responded. We also filtered out individuals younger than 18 years old. The survey was open to individuals with type 1 or type 2 diabetes, but excluded those with gestational diabetes. We did not offer any supplementary compensation for completing the survey. (We had no involvement with the reward points that are part of the website). The study was approved by the Institutional Review Board of the University of Washington.

Measures

Participants completed the 10-question survey on diabetes self-management, goals and barriers (Table 1). We asked them to identify the three most difficult and the three easiest barriers among the following diabetes self-management tasks: tracking health, choosing foods, cooking appropriate meals, adapting medications or insulin, affording healthcare, adjusting meds and diet around unexpected events. We then asked them which diabetes-related behaviors were challenging: structuring a daily routine around diabetes management, impact on social relationships, thinking about diabetes all the time, social support from family, friends and workplace, changing foods, seeing how others think of them, and understanding the relationships between glucose, diet and exercise.

We asked the participants to anticipate their responses to different rewards to help improve diabetes self-management: financial rewards, non-financial rewards and self-rewards. For the financial rewards, we asked participants to estimate the helpfulness of rewards, according to their frequency (weekly, monthly or annual) and type (cash, vouchers or reduced annual insurance premium) to improve their health behaviors. We used a 4-point scale with our predicted small sample to avoid having a neutral option. For the non-financial rewards, we enquired about the anticipated effect of receiving stars or badges, or sharing results with friends, in helping improve their behavior. Finally, we asked participants about the helpfulness of self-paid rewards (*e.g.*, setting money aside for a self-set goal) in improving health behaviors.

We explored who the participants thought should pay for the rewards to improve their health behaviors (health insurance companies, employers or self-paid rewards). We adapted two questions from Long *et al*[16]'s survey instrument, which were: "If you were overweight, how much money would you need to receive to persuade you to lose 5 pounds and keep it off for 1 year?" and likewise for a 15-pound weight loss. Finally, we explored their use of mobile technology (in general and for diabetes).

Statistical analysis

We conducted descriptive analyses describing means and distributions. We defined financial incentive as any cash incentive (weekly, monthly or yearly), any voucher or reduction in insurance premium. We considered recognition by badges or stars and sharing results with friends as a non-financial incentive. Helpfulness of incentives was a binary variable, defined as not helpful or helpful (somewhat helpful to very helpful). To explore associations between diabetes tasks and type of reward, we used a logistic regression model to compare expectations of self-management tasks perceived as difficult or easy. We proceeded similarly for the behaviors that are related to diabetes self-management, separating not helpful or somewhat challenging behaviors from those that are challenging. We then created a score ranging from 0 (easy) to 24 (hard) based on how difficult these behaviors were perceived to be (Table 2). Using a logistic regression model, we studied the association between the score and the three types of reward. In this model, we also analyzed the effect of age and of weight loss motivation, using the estimated amount needed to lose 5 lb and keep it off during a year. We used a cutoff of $30000 to avoid bias from outliers in these analyses.

The percentage of complete cases was 76%. Missing covariate data were infrequent (≤ 3%) other than for income (7%). Missing data were multiple-

Table 2 Challenges of diabetes self-management behaviors

Behavior	*n* (%)
Having to structure my daily routine around diabetes management	77 (50.3)
Coping with the impact of diabetes on my social relationships	64 (41.8)
Thinking about diabetes all the time	74 (48.4)
Having insufficient support from family and/or friends	52 (34.0)
Having insufficient support from my workplace	51 (33.3)
Seeing how other people think of me	88 (57.5)
Having to change what I eat	49 (32.0)
Understanding the relationships between glucose, diet and exercise	72 (47.1)
Overall mean	65.9 (43.1)

For the score, each behavior was rated 0 to 3 points (total of 0 to 24 points).

Table 3 Participant characteristics by type of diabetes

Diabetes	Type 1	Type 2[1]	*P*-value
Value	17 (11.1)	136 (88.9)	
Female	6 (35.3)	71 (52.2)	0.20
Age (x)	39.6 ± 3.5	44.8 ± 1.3	0.18
United States. region			0.06
West	1 (5.9)	26 (19.1)	
Midwest	4 (23.5)	29 (21.3)	
Northeast	9 (52.9)	30 (22.1)	
South	3 (17.6)	51 (37.5)	
Hispanic	1 (7.6)	13 (9.3)	0.80
White race	14 (84.7)	120 (88.9)	0.14
Education (highest attained level)			0.98
High school	8 (47.1)	61 (44.9)	
College	5 (29.4)	40 (29.4)	
Graduate school	4 (23.5)	35 (25.7)	
Income > $50000/yr	7 (42.9)	72 (52.9)	0.45
Current smoker	4 (21.8)	32 (23.5)	0.87
Smartphone user	14 (80.6)	53 (38.8)	0.005

[1]Limited to white and black race. Data are expressed as mean ± SD or *n* (%).

imputed with 10 imputed datasets using imputation by chained-equations[17]. The imputation model included the covariates used in all our analysis (with dependent variables), as well as the region of residence. Categorical variables were compared using χ^2 tests. *P* values from regression models were derived from Wald tests with robust standard errors. A *P*-value < 0.05 determined statistical significance. No interaction was tested. All analyses were conducted on Stata 11 (Stata Corporation, Texas).

RESULTS

Out of the 300 responders, 153 participants were eligible and consented to participate. Excluded participants differed from the inclusion group only by the higher proportion of female individuals (69.4% *vs* 50.4%). Age, region of residence, race, ethnicity, type of education and income were not statistically different among inclusion and exclusion groups.

The included participants had a mean age of 44.2 ± 1.2 years, with 50.4% women. Graduate school education was achieved by 25.5%, and 34.1% had an annual income of > $75.000/year. Nearly a quarter of the participants were smokers (23.0%) at the time of the survey. Smartphone ownership was 43.7%. We present the detailed participant characteristics by type of diabetes in Table 3. There were 11.1% individuals with type 1 diabetes and 88.9% individuals with type 2 diabetes. Although the mean age was not statistically different, participants with type 1 diabetes were significantly more likely to have a smartphone. Individuals with type 1 diabetes were located more in the northeast area of the United States. and less in the West and South areas.

Almost all participants (96.7%) had positive expectations from the use of incentives (financial or non-financial) to improve their diabetes self-management. Only six individuals were not interested in incentives. There was no significant difference in demographic characteristics (age, gender, race/ethnicity, education, income) among those interested in incentives or not. While all individuals with type 1 diabetes were interested in incentives, six individuals who were not interested in incentives had type 2 diabetes. Forty-five percent of participants with an interest in incentives were smartphone users, although there was no significant difference in smartphone use between those with or without interest in incentives.

Overall, the participants expected financial incentives to motivate themselves more than non-financial rewards (96.0% *vs* 60.0%), and 70.2% of individuals expected self-rewards to be helpful in improving diabetes self-management. The self-management tasks rated as the three easiest were: keeping track of health parameters, making food choices and cooking appropriate meals. The tasks considered most difficult were: affording diabetes costs, and adjusting diet and medications around unexpected events. Participants expected financial incentives to help improve food choices and healthcare costs the most, whereas self-rewards were expected to help improve adjustments to unexpected events the most. Non-financial incentives were expected to help improve adapting insulin doses the most.

We studied the participants' responses to the three types of incentives according to whether these factors were considered a challenge for diabetes self-management or not. We found that overall, 94% expected a positive outcome with financial incentives, compared with 60% with non-financial incentives, and 69% with self-rewards. Participants expected financial incentives to help improve the food habit changes the most, whereas non-financial incentives and self-rewards were expected to improve support from the workplace and impact on social relationships the most.

We assess the association of perceived level of challenge (0 is easy, 24 is challenging) and type of incentive (financial, non-financial and self-rewards) in Table 4. We found in the unadjusted analysis that for

Table 4 Effect of behavior score on the 3 types of rewards

Score	OR financial incentive (95%CI)	*P*-value	OR non-financial incentive (95%CI)	*P*-value	OR self-reward (95%CI)	*P*-value
Score	0.82 (0.72-0.93)	0.002	1.06 (1.01-1.10)	0.01	1.00 (0.96-1.04)	0.98
Score adjusted for age	0.82 (0.72-0.93)	0.002	1.06 (1.01-1.11)	0.01	1.00 (0.96-1.05)	0.94
Score adjusted for weight loss motivation[1]	0.83 (0.73-0.95)	0.005	1.07 (1.02-1.11)	0.006	1.01 (0.96-1.05)	0.75

[1]Estimated amount needed to lose 5 lb and maintain it for a year.

an increase in the behavior score by one point, the odds ratio comparing expected response to financial incentives with no response to financial incentives would be 0.82 (P = 0.002). When comparing expected responses to non-financial incentives to no response to these incentives, we found an OR of 1.05 (P = 0.01). The level of perceived difficulty with these behaviors was not associated with expecting to respond to self-rewards in our dataset (OR = 1.00, P = 0.98). We obtained similar results when adjusting for age or for the weight loss motivation (assessed by the amount needed to lose 5 lb and keep it off for a year). This means that when these behaviors are perceived to be more difficult, the participant less expected to respond to financial incentives. Yet for non-financial incentives, when more behaviors are perceived to be difficult, participants expected to respond more to non-financial incentives.

We asked participants to provide an estimated amount needed to motivate losing 5 and maintaining it for a year, and the amount for a 15 lb weight loss. We excluded 2 outliers for each analysis, using a $30000 cutoff. To motivate a 5 lb weight loss, participants gave estimates that ranged from $0 (12 participants) to $2.15 billion. On average, the estimated amount to motivate people to lose 5 lb was $258, with a median of $50 and interquartile range of $10 to 100. When asked how much money participants needed to lose 15 lb and keep it off for a year, the range of responses was identical. They expected themselves to be motivated with an average of $713 (median of $100, IQR $25-250). Only 8 participants responded with $0 for this question. The difference between these two estimates was statistically significant (P < 0.001).

DISCUSSION

In this national web-based survey of individuals with diabetes, we explored perceptions of financial and non-financial incentives to improve diabetes self-management. We found that nearly all surveyed individuals were interested in incentives, with no difference in socioeconomic status, or demographic features (age, race, gender and SES). In fact, the only significant difference was in the type of diabetes, as all individuals with type 1 diabetes were interested in incentives.

Smartphones offer a unique opportunity to monitor behaviors[18] and provide rewards. Smartphone use was reported by nearly half of surveyed individuals interested in incentives and smartphone adoption is increasing rapidly[19]. The easy availability of smartphones creates opportunities for low-effort tracking and immediate gratification through smartphone applications with reward systems. This short delay favors the efficacy of feedback for behavior changes[20], and is a unique possibility offered by these devices. Many applications already exist for diabetes management[21,22], including applications with non-financial reward systems[23]. Applications with financial rewards are also available to motivate users to exercise, and recent developments like near field communication technologies facilitate money management on mobile devices. Using a smartphone app could also allow for better individualization of the reward program, by adapting to each user's stage of disease and self-management, and by identifying areas that are more challenging for each person.

Individuals overall were optimistic about the effectiveness of incentives, and expected financial incentives to be a stronger motivation than non-financial incentives for behavior change. Furthermore, when considering how difficult behavior changes were perceived to be, using a 32-point score, we found that participants expected to be significantly less likely to use financial incentives for more challenging behavior changes in the unadjusted analysis. In fact, participants were more likely to use non-financial incentives when facing the difficult behaviors. Interestingly, the perceived level of difficulty for behavior change was not associated with the use of self-rewards. These findings persisted after adjusting for age and weight loss motivation.

Our findings suggest that financial incentives could have a potential role to play in motivating select behavior changes for diabetes self-management. The different response by perceived level of challenge suggest that perhaps a combination of incentives is needed to improve the various self-management skills. In Polonsky *et al*[24]'s recent study about perceived obstacles for glucose self-monitoring, avoidance behaviors (including forgetting, lack of time or reminders of diabetes) were predictors of a low frequency of glucose testing. Whether avoidance also predicts low success of other self-care behaviors is uncertain. Based on our findings, avoidance behaviors that are perceived to be less challenging could have a positive response to financial incentives, while more

challenging behaviors would require the use of non-financial incentives.

The amounts of financial incentives are important to consider. An interesting finding from our survey is the relatively low amounts of money that participants expected as incentive for weight loss ($258 for the 5 lb weight loss and $713 for the 15 lb weight loss), particularly if we consider that current out-of-pocket costs for diabetes are estimated at $350-500/mo[25,26]. This concurs with findings from another study which explored the use of financial incentives in diabetes, where participants suggested $25 per month for tracking and reporting glucose results[27]. Employers typically employ similar amounts for action-based incentives, or rewards for taking action (joining a weight loss program, for example) after going through a risk assessment[28]. Prior research has found that very large amounts can lead to lower performances, because the individual feels pressure to perform well. Likewise, amounts that are too small lead to lower performances, even lower than those who do not have any incentive[29]. These considerations suggest that our participants' intuitions about the reward amounts are in the right ballpark, although this needs to be evaluated empirically.

The strengths of our study include its focus on the use of incentives, both financial and non-financial, in a diabetic population. Although the relatively moderate number of participants may limit its generalizability, its web-based modality allowed us to recruit nationwide. This modality however also has its limitations, as the participants are self-selected, and received tokens in exchange for taking part in the survey (no compensation was given by the investigators, this was solely a feature of the survey company). Our population may therefore be disproportionately biased in their interest in incentives. A final limitation to our study lies in its design as a survey, as we assess the participants' expected response to incentives, which may differ from them their actual response to incentives. Future studies are needed to confirm our findings, and to further explore the acceptability and feasibility of incentives for diabetes self-management in a larger population.

According to our nationwide survey, patients with diabetes are willing to consider using incentives, both financial and non-financial, to improve diabetes self-management. While the financial incentives may be more effective for behavior changes that are perceived as less challenging, non-financial incentives may be useful for the more challenging behaviors. Participants did not expect to need large amounts to motivate them to modify their behavior. Our findings suggest that the effectiveness of incentives could vary, and may depend on the perceived difficulty of the incentivized task. Future studies are needed to confirm these results in interventional studies on larger populations.

ACKNOWLEDGMENTS

I acknowledge Klasnja P, Pratt W and the rest of the iMed team for their help in preparing the survey.

COMMENTS

Background

Financial incentives have shown potential for modifying behaviors such as smoking cessation. Diabetes self-management requires many behavior changes, which may benefit from such incentives. Little is known about patient attitudes in response to incentives for diabetes self-management.

Research frontiers

Although incentives have been studied for certain behaviors, the current research hotspot is patient acceptance of incentives for diabetes self-management, in particular for the goals, type (financial, non-financial or self-reward) and amount of incentives.

Innovations and breakthroughs

Beyond patient attitudes and expectations about their response to incentives, this survey provides suggestions about goal-setting in reward programs, in relation to the perceived level of challenge of certain behaviors. Individualization of goals and rewards could be feasible with the exponential adoption of mobile devices, which could both track and reward behaviors ubiquitously.

Applications

The results of this study can help guide future interventional studies with incentives, both in goal-setting in terms of types of behaviors, types of rewards, and amounts for financial incentives.

Terminology

Financial incentives include all rewards relating to monetary rewards or equivalents such as vouchers (cash or discount for a future purchase, for example). Non-financial incentives are rewards such as badges or stars in social networks that provide recognition from others for an achieved feat. Self-rewards are rewards that a person gives themselves when they reach a predefined goal, often planned as small rewards that accumulate to reach a large, final reward.

Peer-review

In this well-written paper, the author investigates the patient willingness to change diabetes self-management behaviors in response to rewards. The reviewers found this approach to improve patient engagement interesting. The results can help guide the creation and implementation of new patient-centered approaches for diabetes self-management.

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P- Reviewer: Tomkin GH, Vestergaard ET **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Zhang DN

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i5.759

World J Diabetes 2015 June 10; 6(5): 759-773
ISSN 1948-9358 (online)

SYSTEMATIC REVIEWS

Diabetic nephropathy in Africa: A systematic review

Jean Jacques N Noubiap, Jashira Naidoo, Andre P Kengne

Jean Jacques N Noubiap, Internal Medicine Unit, Edéa Regional Hospital, PO BOX 100 Edéa, Cameroon
Jashira Naidoo, Department of Medicine, Groote Schuur Hospital, University of Cape Town, 7925 Observatory, Cape Town, South Africa
Jashira Naidoo, Andre P Kengne, Non-Communicable Diseases Research Unit, South African Medical Research Council, 7505 Cape Town, South Africa

Author contributions: All authors contributed to this work.

Conflict-of-interest: None for all co-authors.

Correspondence to: Andre P Kengne, Professor, Medical Research Council of South Africa, PO Box 19070 Tygerberg, 7505 Cape Town, South Africa. andre.kengne@mrc.ac.za
Telephone: +27-21-9380529
Fax: +27-21-9380460

Received: December 8, 2014
Peer-review started: December 9, 2014
First decision: January 20, 2015
Revised: February 18, 2015
Accepted: March 16, 2015
Article in press: March 18, 2015
Published online: June 10, 2015

Abstract

AIM: To determine the prevalence and incidence of diabetic nephropathy in Africa.

METHODS: We performed a systematic narrative review of published literature following the MOOSE Guidelines for Meta-Analysis and Systematic Reviews of Observational Studies. We searched PubMed-MEDLINE for all articles published in English and French languages between January 1994 and July 2014 using a predefined strategy based on the combination of relevant terms and the names of each of the 54 African countries and African sub-regions to capture the largest number of studies, and hand-searched the reference lists of retrieved articles. Included studies reported on the prevalence, incidence or determinants of chronic kidney disease (CKD) in people with diabetes within African countries.

RESULTS: Overall, we included 32 studies from 16 countries; two being population-based studies and the remaining being clinic-based surveys. Most of the studies (90.6%) were conducted in urban settings. Methods for assessing and classifying CKD varied widely. Measurement of urine protein was the most common method of assessing kidney damage (62.5% of studies). The overall prevalence of CKD varied from 11% to 83.7%. Incident event rates were 94.9% for proteinuria at 10 years of follow-up, 34.7% for end-stage renal disease at 5 years of follow-up and 18.4% for mortality from nephropathy at 20 years of follow-up. Duration of diabetes, blood pressure, advancing age, obesity and glucose control were the common determinants of kidney disease.

CONCLUSION: The burden of CKD is important among people with diabetes in Africa. High quality data from large population-based studies with validated measures of kidney function are still needed to better capture the magnitude and characteristics of diabetic nephropathy in Africa.

Key words: Diabetes; Diabetes nephropathy; Chronic kidney disease; Epidemiology; Prevalence; Incidence; Mortality; Africa; Systematic review

Core tip: Chronic kidney disease is a serious health threat for people with diabetes in Africa, with prevalence

figures ranging from 11% to 83.7%. The incidence estimates suggest that 95% of people with diabetes may have proteinuria after 10 years from diabetes diagnosis; about 35% may develop end-stage renal disease after 5 years and 18% die from nephropathy after 20 years of disease duration. Hypertension, obesity, poor glycemic control and diabetes duration are the main risk factors of chronic kidney disease among diabetic patients in Africa. High quality data are needed to refine the epidemiology of diabetic nephropathy on the continent.

Noubiap JJN, Naidoo J, Kengne AP. Diabetic nephropathy in Africa: A systematic review. *World J Diabetes* 2015; 6(5): 759-773 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i5/759.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i5.759

INTRODUCTION

Africa, like the rest of the world, is experiencing an increasing prevalence of diabetes alongside other non-communicable diseases, mainly as a result of urbanization, sedentary lifestyles, obesity and population growth and ageing[1]. Estimates for 2013 by the International Diabetes Federation (IDF) indicate that the number of adults with diabetes in the world will expand by 55%, from 381.8 million in 2013 to 591.9 million in 2035[2]. The largest increase of the population with diabetes will occur in sub-Saharan Africa, with a projected growth of 109.6%, from 19.8 million in 2013 to 41.5 million in 2035[2].

Diabetes causes significant morbidity, disability and early mortality. Diabetes has been identified as a major contributor in several other important diseases, both non-communicable diseases such as cardiovascular disease and renal disease[3,4], and communicable diseases such as invasive bacterial infections[5,6]. Mortality attributable to diabetes in sub-Saharan Africa was estimated to account for 8.6% of the total death in 2013[7]. Diabetic nephropathy (DN) is one of the most common complications of diabetes. The prevalence of DN is increasing steeply along with the diabetes epidemic[8]. Approximately one third to half of patients with diabetes develops renal manifestations[8-11]. DN is associated with increased premature mortality, end-stage renal disease and need to renal replacement therapy, cardiovascular diseases, and escalating health-care costs[8].

DN has been suggested to be more frequent among patients with diabetes in Africa as compared to those in the developed world due to delayed diagnosis, limited screening and diagnostic resources, poor control of blood sugar and other risk factors, and inadequate treatment at an early stage[7,12,13]. However, evidence to support the burden of kidney diseases in people with diabetes in Africa remains very patchy, and we are not aware of any effort to synthesize existing data on the occurrence of kidney disease in African populations with diabetes. Accordingly, the aim of this review is to provide a comprehensive overview of the published evidence on the occurrence of nephropathy in African people with diabetes.

MATERIALS AND METHODS

Data sources and search strategy

A systematic narrative review of published literature was performed following the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies[14]. We searched MEDLINE *via* PubMed for articles published in English and French on DN in Africa between January 1994 and July 2014, using a predefined strategy based on the combination of relevant terms and the names of each of the 54 African countries and African sub-regions to capture the largest number of studies. The data search was limited to human studies. The last search date was October 22, 2014. Search histories are provided in Table 1. Once duplicate references were removed the titles and abstracts of the references were screened. The references of included articles were scanned to identify additional articles of interest.

Study selection and data extraction

We included cross-sectional, case-control or cohort studies of subjects with diabetes mellitus resident in African countries reporting the prevalence or incidence or progression of DN. We excluded studies of populations of African origin residing outside Africa; case series (sample size less than 50 subjects), letters, comments and editorials; studies not published in English or French. Two investigators (JJNN, APK) independently identified articles and sequentially screened them for inclusion (Figure 1). Disagreements were solved by a third investigator (JN). Full text articles were reviewed by two investigators (JJNN and APK) who independently extracted data regarding study setting and design, study population characteristics and prevalence or incidence of DN.

RESULTS

We identified 730 articles, of which 73 were reviewed in full-text; 32 met the inclusion criteria (Figure 1)[15-46].

Characteristics of included studies

Characteristics of the included studies are summarized in Table 2. The 32 studies were performed in 16 countries, with a geographical distribution covering all the African regions. However, more than half the studies [18 (56.3%)] were from South Africa (five), Nigeria (four), DR Congo (three) and Ethiopia (three).

Only two population-based studies were identified. In Democratic Republic of Congo, between March and April 2007, Makulo *et al*[35] studied pathologic

Table 1 Search history PubMed

Search	Search terms	Hits
1	Diabetes[tw] OR Diabetes mellitus[tw] OR Type 1 diabetes[tw] OR Type 1 diabetes mellitus[tw] OR T1DM[tw] OR Type 2 diabetes[tw] OR Type 2 diabetes mellitus[tw] OR T2DM[tw] OR Hyperglycemia[tw] OR Glucose intolerance[tw]	445204
2	Renal insufficiency[tw] OR Renal failure[tw] OR Renal injury[tw] OR Renal disease[tw] Kidney insufficiency[tw] OR Kidney failure[tw] OR Kidney injury[tw] OR Kidney disease[tw] OR End-stage renal disease[tw] OR End-stage renal failure[tw] OR End-stage kidney disease[tw] OR End-stage kidney failure [tw] OR End stage renal disease[tw] OR End stage renal failure[tw] OR End stage kidney disease[tw] OR End stage kidney failure [tw] OR Microalbuminuria [tw] OR Micro-albuminuria OR Macroalbuminuria [tw] or Macro-albuminuria [tw]	154354
3	# 1 AND # 2	20388
4	Diabetic nephropathy [MeSH Terms]	19406
5	# 3 OR # 4	34221
6	(((((("Africa"[MeSH] OR Africa*[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR "Burkina Faso"[tw] OR Burundi[tw] OR Cameroon[tw] OR "Canary Islands"[tw] OR "Cape Verde"[tw] OR "Central African Republic"[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR "Democratic Republic of Congo"[tw] OR Djibouti[tw] OR Egypt[tw] OR "Equatorial Guinea"[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR "Guinea Bissau"[tw] OR "Ivory Coast"[tw] OR "Cote d'Ivoire"[tw] OR Jamahiriya[tw] OR Jamahiryia[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Libya[tw] OR Libia[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mayote[tw] OR Morocco[tw] OR Mozambique[tw] OR Mocambique[tw] OR Namibia[tw] OR Niger[tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR "Sao Tome"[tw] OR Senegal[tw] OR Seychelles[tw] OR "Sierra Leone"[tw] OR Somalia[tw] OR "South Africa"[tw] OR "St Helena"[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR "Western Sahara"[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw] OR "Central Africa"[tw] OR "Central African"[tw] OR "West Africa"[tw] OR "West African"[tw] OR "Western Africa"[tw] OR "Western African"[tw] OR "East Africa"[tw] OR "East African"[tw] OR "Eastern Africa"[tw] OR "Eastern African"[tw] OR "North Africa"[tw] OR "North African"[tw] OR "Northern Africa"[tw] OR "Northern African"[tw] OR "South African"[tw] OR "Southern Africa"[tw] OR "Southern African"[tw] OR "sub Saharan Africa"[tw] OR "sub Saharan African"[tw] OR "subSaharan Africa"[tw] OR "subSaharan African"[tw]) NOT ("guinea pig"[tw] OR "guinea pigs"[tw] OR "aspergillus niger"[tw]))))))	354928
7	# 5 AND # 6	1065
8	#4 Limits: 1994/01/01 to 2014/10/22 and studies done in Humans	918

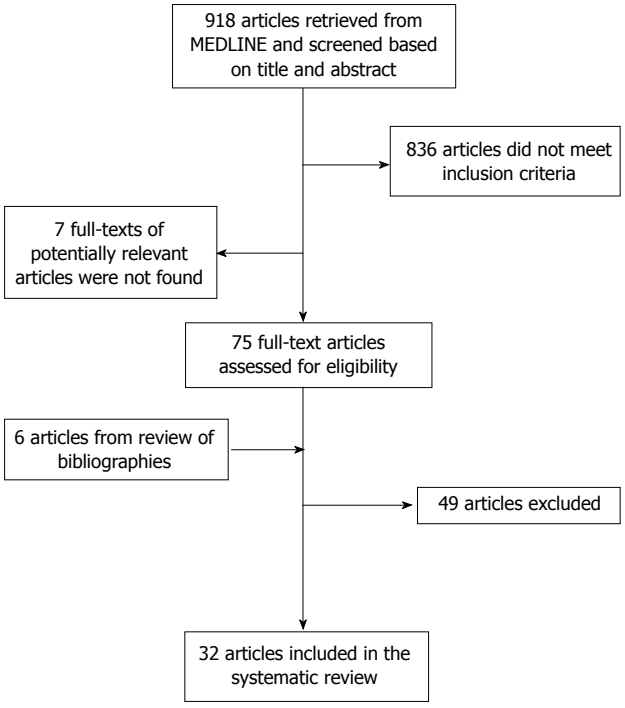

Figure 1 Flow diagram of study selection.

albuminuria among 81 diabetic patients identified through a population-based survey on the prevalence of diabetes involving 1898 participants[35]. Pruijm *et al*[39] in Seychelles in 2004, conducted a large-scale population-based estimate of the prevalence of microalbuminuria among 1218 adults. All other studies were clinic-based surveys conducted mostly in diabetic clinics. There were three cohort studies (two prospective and one retrospective), one case-control study and the other 28 studies were cross-sectional with non-random sampling. Only three (9.4%) studies were conducted in rural settings.

Methods of assessment and classification of chronic kidney disease (CKD) varied widely. The studies assessed kidney function by urine protein [20 (62.5%) studies], urine albumin-to-creatinine ration (ACR) [9 (28.1%) studies], and estimation of glomerular filtration rate (GFR) by Cockcroft-Gault formula [3 (9.4%) studies] or by MDRD formula [4 (12.4%) studies]. Six studies (18.8%) measured kidney function by two methods, and renal biopsy was not performed in any study.

Prevalence of CKD

As depicted in Table 3, the overall prevalence of CKD varied from 11% in Tunisia to 83.7% in Tanzania[20,29]. In studies where proteinuria was used to assess CKD, the prevalence varied from 5.3% in South Africa to 53.1% in Cameroon (study with a small sample size)[32,44]. When considering the estimation of the GFR, the prevalence ranged from 4.6% in Tanzania to 43.1% in Nigeria (study with a small sample size)[15,33].

Incidence of CKD

A study in South Africa investigated the long-term incidence of proteinuria among T2DM patients. After 12 years of follow-up or death, 94.9% (56/59) had a proteinuria with a mean duration from diabetes onset to proteinuria of 9.7 (5.9) years[31]. In another study in South Africa, found that 18.4% of T1DM patients had

Table 2 General characteristics of studies of chronic kidney disease in people with diabetes in Africa

Ref.	Country	Period	Design	Setting	Sample size	Mean or median age (yr)	Male (%)	Type and duration of diabetes (yr)	Duration FUP	Method for CKD assessment			
										Proteinuria	MDRD	Urine ACR	Cockroft-Gault
Motala *et al*[37], 2001	South Africa	Not precised	Retrospective cohort study	Clinic, urban	219	39.5 T1DM; 58.4 T2DM	19.6	16.10 T1DM; 18.6 T2DM	At least 10 yr	persistent proteinuria (Dipstick)			
Elbagir *et al*[26], 1995	Sudan	Jan-July 1992	Cross-sectional, self-selected sampling	Clinic, urban	128	31.5 (15-75)	48.4	Insulin-treated; 9 (1-40)	NA	Proteinuria (Dipstick)			
Sobngwi *et al*[44], 1999	Cameroon	Not precised	Cross-sectional, self-selected sampling	Clinic, urban	64	37.4 normotensive T1DM; 51.7 normotensive T2DM; 57.9 hypertensive T1DM	57.8	6.7 normotensive T1DM; 4.7 normotensive T2DM; 4.8 hypertensive T1DM	NA	Proteinuria			
Katchunga *et al*[30], 2010	DR congo	2005-2007	Cross-sectional, self-selected sampling	Clinic, urban	98	58 (10.4)	35.7	7.3 T2DM	NA		MDRD (corrected for Blacks)		
Choukem *et al*[22], 2012	Cameroon	Jan 2008-Oct 2010	Cross-sectional, self-selected sampling	Clinic, urban	420	56.7	49	4 (1-9) T2DM	NA	Proteinuria (Dipstick)			
Keeton *et al*[31], 2004	South Africa	Not precised	Prospective cohort, self-selected sampling	Clinic, urban	59	62	35.6	17.8 T2DM	12 yr			Urine ACR	
Pruijm *et al*[39], 2008	Seychelles	2004	Cross-sectional; random sex and age-stratified sample	Population	1218 (whole sample, including diabetic patients)	Not precised	45.9	Newly diagnosed patients	NA			Urine ACR	
Alebiosu[16], 2003	Nigeria	Jan 2000 June 2001	Cross-sectional, self-selected sampling	Clinic, urban	342	6.5 T1DM; 9.4 T2DM	53.8	26 T1DM; 53.4 T2DM	NA	Persistent proteinuria			
Bouaziz *et al*[20], 2012	Tunisia	Jan 2008 Dec 2010	Cross-sectional, self-selected sampling	Clinic, urban	73	59.3	23.3	T2DM 10.6	NA	Proteinuria			
Ajayi *et al*[15], 2014	Nigeria	Not precised	Retrospective cross-sectional	Clinic, urban	65	Not available	Not available	T2DM	NA		MDRD		
Levitt *et al*[32], 1997	South Africa	July-December 1992	Cross-sectional, stratified random sampling	Clinic, urban	243	56.4	38.3	8 T2DM and T1DM	NA	Persistent proteinuria		Urine ACR	
Majaliwa *et al*[34], 2007	Tanzania	June 2005-Feb 2006	Cross-sectional, self-selected sampling	Clinic, urban	99	12.6	42.4	4.76 T1DM	NA	Proteinuria			
Marshall *et al*[36], 2013	Rwanda	June 2009-Nov 2010	Cross-sectional, self-selected sampling	Clinic, urban	286	18.6	46.5	3.4 T1DM	NA	Proteinuria		Urine ACR	
Alebiosu *et al*[18], 2003	Nigeria	Sept 1999-August 2002	Cross-sectional, self-selected sampling	Clinic, urban	465	Not precised	Not precised	T2DM	NA	Proteinuria			
Gill *et al*[28], 2005	South Africa	From 1982 to 2002	Prospective cohort, self-selected sampling	Clinic, urban	88	22 at onset	52	T1DM	20 yr				
Djrolo *et al*[24], 2001	Benin	Not indicated	Cross-sectional	Clinic, urban	152	53.3	65.8	T1DM and T2DM	NA	Proteinuria			

Rotchford *et al*[43], 2002	South Africa	1999	Cross-sectional, self-selected sampling	Clinic, rural	253	56.5	26.9	42.2; T1DM and T2DM	NA			Urine ACR	
Rissassi *et al*[42], 2009	DR congo	11 june 2008 to 30 july 2008	Cross-sectional, self-selected sampling	Clinic, urban	181	19.1	38.7	57.6 T1DM	NA			Urine ACR	
Rahlenbeck *et al*[40], 1997	Ethiopia	January - April 1995	Cross-sectional, self-selected sampling	Clinic, urban	170	31.4 T1DM; 56.7 T2DM	60	5.9 T1DM; 6.0 T2DM	NA	Proteinuria			
Wanjohi *et al*[45], 2002	Kenya	June 2000 - January 2001	cross-sectional, self-selected sampling	Clinic, urban	100	53.7	37	10.3 T2DM	NA	Albuminuria			
Nambuya *et al*[38], 1996	Uganda	1 January 1993 - 10 August 1994	Cross-sectional, self-selected sampling	Clinic, urban/urban (origin of participants)	252	Not precised	46.4	45 (range 30-69) T2DM and T1DM	NA	Proteinuria			
Rasmussen *et al*[41], 2013	Zambia	February - April 2011	Cross-sectional, self-selected sampling	Clinic, rural	101	50 (range 50-68)	37.3	T2DM and T1DM	NA			Urine ACR	
Bentata *et al*[19], 2013	Morocco	From September 2006	Prospective cohort study	Clinic, urban	72	29.5	69.4	17 (11-20) T1DM	5 yr	Proteinuria	MDRD		
Gill *et al*[27], 2008	Ethiopia	Not precised	Cross-sectional, self-selected sampling	Clinic, rural	105	41	70.5	7 T1DM and T2DM	NA			Urine ACR	
Bouzid *et al*[21], 2011	Tunisia	June 2006 - July 2008	Cross-sectional, self-selected sampling	Clinic, urban	689	60	39.3	11 T2DM	NA	Proteinuria			Cockroft-Gault
Janmohamed *et al*[29], 2013	Tanzania	October 2011 - March 2012	Cross-sectional, self-selected sampling	Clinic, urban	369	54 (IQR 45-62)	46.6	6 (3-11) T1DM (6.2%) and T2DM (93.8%)	NA				Cockroft-Gault
Danquah *et al*[23], 2012	Ghana	August 2007 - June 2008	Cross-sectional, self-selected sampling	Clinic, urban	675	54.7	25	T2DM	NA	Proteinuria			
Lutale *et al*[33], 2007	Tanzania	July 2003 - March 2004	Cross-sectional, self-selected sampling	Clinic, urban	244	T1DM 21(range 4-44.8) T2DM 53 (range 23.5-85)	46.3	T1DM 3 (0-17) T2DM 4 (range 0-25)	NA	Proteinuria			Cockroft-Gault
Worku *et al*[46], 2010	Ethiopia	October 2008	Cross-sectional, self-selected sampling	Clinic, urban	305	44.4	62.9	T1DM and T2DM; 53.4% less than 5 yr and 33.8% 5-9 yr	NA	Proteinuria			
Makulo *et al*[35], 2010	DR Congo	30 March - 24 April 2007	Cross-sectional, self-selected sampling	Population-based, Urban	81	Not precised	Not precised	No precision	NA		MDRD	Urine ACR	
Eghan *et al*[25], 2007	Ghana	January - July 2005	Cross-sectional, self-selected sampling	Clinic, urban	109	54.1	28	T1DM and T2DM 10.7	NA	Proteinuria			
Alebiosu *et al*[17], 2004	Nigeria	January 2000 - June 2001	Case (T2DM with persistent proteinuria-control (T2DM patients nephropathy)	Clinic, urban	162	53.4	50	T2DM 9.4 cases, 5.5 controls	NA				

ACR: Albumin-to-Creatinine Ratio; FUP: Follow-up; MDRD: Modification of diet renal disease; NA: Not applicable.

Table 3 Prevalence and incidence of chronic kidney disease in people with diabetes across studies in Africa

Ref.	Country	Sample size	Type of diabetes	Duration of follow-up	Diagnostic criteria for CKD	Prevalence	Incidence	Comments
Motala *et al*[37], 2001	South Africa	219	T1DM and T2DM	16.10 (4.9) T1DM; 18.6 (5.7) T2DM; at least 10 yr	Persistent proteinuria (dipstix proteinuria on three or more consecutive occasions over 18 mo in the at absence of infection or cardiac failure)	Not applicable	24.6%	
Elbagir *et al*[26], 1995	Sudan	128	Insulin-treated	Not applicable	Proteinuria (≥ 30 mg/dL)	22%	Not applicable	
Sobngwi *et al*[44], 1999	Cameroon	64	T1DM and T2DM	Not applicable	Proteinuria	53.1%	Not applicable	
Katchunga *et al*[30], 2010	DR Congo	98	T2DM	Not applicable	MDRD: CKD stage ≥ 2 according to the National Kidney foundation	18.1%	Not applicable	
Choukem *et al*[22], 2012	Cameroon	420	T2DM	Not applicable	Proteinuria (30 mg/24 h)	31%	Not applicable	
Keeton *et al*[31], 2004	South Africa	59	T2DM	12 yr	Urine Albumin-to-Creatinine Ratio (no detail)		After 12 yr of follow-up or death, 94.9% (56/59) had a proteinuria with a mean duration from diabetes onset to proteinuria of 9.7 (5.9) yr	83% (49/59) had an elevated SCr at the end of the study and in 66.1% (39/59) the SCr level had doubled during the study
Pruijm *et al*[39], 2008	Seychelles	1218	All types	Not applicable	Microalbuminuria: Urine Albumin-to-Creatinine Ratio 3.4-33.9 mg albumin/mmol creatinine	36.1%	Not applicable	
Alebiosu[16], 2003	Nigeria	342	T1DM and T2DM	Not applicable	Persistent proteinuria	28.4%	Not applicable	
Bouaziz *et al*[20], 2012	Tunisia	73	T2DM	Not applicable	Microalbuminuria: < 2.8 g/mol for women and < 2.3 g/mol for men	11%	Not applicable	
Ajayi *et al*[15], 2014	Nigeria	65	T2DM	Not applicable	MDRD: eGFR ≤ 60 mL/min per 1.73 m^2	43.1%	Not applicable	
Levitt *et al*[32], 1997	South Africa	243	T2DM and T1DM	Not applicable	Urine Albumin-to-Creatinine Ratio > 3.4 mm/mmol Persistent proteinuria (for at least 3 consecutive visits)	36.7% 5.3%	Not applicable	
Majaliwa *et al*[34], 2007	Tanzania	99	T1DM	Not applicable	Proteinuria (no detail)	29.3%	Not applicable	
Marshall *et al*[36], 2013	Rwanda	286	T1DM	Not applicable	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g Macroalbuminuria or overt nephropathy: Urine Albumin-to-Creatinine Ratio ≥ 300 mg/g	Microalbuminuria: 21%; Macroalbuminuria: 5%	Not applicable	
Alebiosu *et al*[18], 2003	Nigeria	465	T2DM	Not applicable	Proteinuria and eGFR	41.1%	Not applicable	The method for the estimation of the GFR is not indicated
Gill *et al*[28], 2005	South Africa	88	T1DM	20 yr	Persistent dipstick proteinuria		Death of renal cause after 20 yr = 18.4% (9/49)	Death due to chronic renal failure after 20 yr of follow-up was 9/49 (after exclusion of lost to follow)
Djrolo *et al*[24], 2001	Benin	152	T1DM and T2DM	Not applicable	Proteinuria (no detail)	20%	Not applicable	
Rotchford *et al*[43], 2002	South Africa	253	T1DM and T2DM	Not applicable	Microalbuminuria > 2.5 mg/mmol in men or 3.5 mg/mmol in women	46.4%	Not applicable	

Rissassi *et al*[42], 2009	DR congo	181	T1DM	Not applicable	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g Macroalbuminuria: Urine Albumin-to-Creatinine Ratio ⩾ 300 mg/g	21.9% (microalbuminuria) and 7.3% (macroalbuminuria)	Not applicable	
Rahlenbeck *et al*[40], 1997	Ethiopia	170	T1DM and T2DM	Not applicable	Microalbuminuria: > 30 mg/L Macroalbuminuria: > 300 mg/L	T1DM: 32% (microalbuminuria) and 15% (macroalbuminuria) T2DM: 37% (microalbuminuria) and 20% (macroalbuminuria)	Not applicable	
Wanjohi *et al*[45], 2002	Kenya	100	T2DM	Not applicable	Proteinuria ⩾ 20 mg	26%	Not applicable	
Nambuya *et al*[38], 1996	Uganda	252	T1DM and T2DM	Not applicable	Proteinuria (no detail)	17.1%	Not applicable	Newly diagnosed patients
Rasmussen *et al*[41], 2013	Zambia	101	T1DM and T2DM	Not applicable	Microalbuminuria: ACR = 3.5-35.0 for women and 2.5-25.0 mg/mmol for men Macroalbuminuria were ACR > 35.0 for women and > 25.0 for men	Microalbuminuria: 23.8% Macroalbuminuria: 8.9%	Not applicable	There were 33 patients with diabetes alone, and 68 patients with diabetes and hypertension
Bentata *et al*[19], 2013	Morocco	72	T1DM	5 yr	Microalbuminuria: albumin excretion rate 30-300 mg/24 h Macroalbuminuria: albumin excretion rate > 300 mg/24 h Nephrotic proteinuria: albumin excretion rate ⩾ 3000 mg/24 h Renal failure: eGFR < 60 mL/min (MDRD)	At the time of enrollement Microalbuminuria: 48.6% Macroalbuminuria: 36.1% Nephrotic proteinuria: 15.3%	The incidence of end stage renal disease after 5 yr: 34.7%	Urinary assays done on admission were repeated on three specimens at three-monthly intervals
Gill *et al*[27], 2008	Ethiopia	105	T1DM and T2DM	Not applicable	Nephropathy: ACR > 25.0 mg/mmol and retinopathy present Microalbuminuria: ACR > 2.5 and < 25.0 mg/mmol in men and > 3.5 and < 25.0 mg/mmol in women	Nephropathy: 2% Microalbuminuria: 51%		Urinary ACR levels (to assess microalbuminuria and nephropathy) were done on 59 patients, as those with haematuria and/or urinary infection were excluded
Bouzid *et al*[21], 2011	Tunisia	689	T2DM	Not applicable	CKD: eGFR < 60 mL/min per 1.73 m^2 (Cockroft-Gault) Microalbuminuria: albumin excretion rate 30-300 mg/24 h Macroalbuminuria: albumin excretion rate > 300 mg/24 h	CKD: 19.8% Microalbuminuria: 13% Macroalbuminuria: 10.1%	Not applicable	Macroalbuminuria was significantly associated with CKD ($P < 0.00001$)
Janmohamed *et al*[29], 2013	Tanzania	369	T1DM and T2DM	Not applicable	CKD: eGFR < 60 mL/min per 1.73 m^2 (Cockroft-Gault) or microalbuminuria (> 20 mg/L) or overt protienuria	CKD: 83.7% eGFR < 60 mL/min per 1.73 m^2: 24.7% Microalbuminuria: 45.8% Overt proteinuria: 34.1%	Not applicable	
Danquah *et al*[23], 2012	Ghana	671	T2DM	Not applicable	Proteinuria ⩾ 20 mg/L	43%	Not applicable	
Lutale *et al*[33], 2007	Tanzania	244	T1DM and T2DM	Not applicable	Microalbuminuria: AER 20-200 μg/min Macroalbuminuria: AER > 200 μg/min Renal failure: eGFR < 60 mL/min per 1.73 m^2:	Microalbuminuria: 12.1% (T1DM); 9.8% (T2DM) Macroalbuminuria: 1.1% (T1DM); 7.2% (T2DM) Renal failure: 4.6% (T1DM); 22% (T2DM)	Not applicable	

Worku *et al*[46], 2010	Ethiopia	305	T1DM (38%) and T2DM (62%)	Not applicable	Proteinuria (no detail)	15.7%	Not applicable	
Makulo *et al*[35], 2010	DR Congo	81	No precision	Not applicable	Microalbuminuria: ACR 30-299 mg/g Macroalbuminuria: ACR ⩾ 300 mg/g Renal failure: eGFR < 60 mL/min per 1.73 m^2	Microalbuminuria: 43.5% Macroalbuminuria: 12% Renal failure: 21.4%	Not applicable	
Eghan *et al*[25], 2007	Ghana	109	T1DM and T2DM	Not applicable	Microalbuminuria: ACR 30-300 mg/g	43.1%	Not applicable	
Alebiosu *et al*[17], 2004	Nigeria	162	T2DM	Not applicable	Not applicable	Not applicable	Not applicable	The study did not assess the prevalence or incidence of diabetic nephropathy, but its predictors

ACR: Albumin-to-Creatinine Ratio; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; CKD: Chronic kidney disease; eGFR: Epidermal growth factor receptor.

died from renal nephropathy after 20 years of follow-up[28]. In a recent study in Morocco, the incidence of end-stage renal disease after 5 years was 34.7%[19].

Risk factors of CKD

Twenty studies (62.5%) reported factors associated with CKD in diabetic patients (Table 4). However, in most studies the method to assess this association was imprecise. In cross-sectional studies, correlates of CKD included systolic and diastolic high blood pressure, long duration of diabetes, older age, dyslipidemia, obesity[16-22,25,26,29-31,33,36,40,42-44,46]. In a study in Cameroon, T2DM patients with systolic hypertension and diastolic hypertension were respectively 1.45 (95%CI: 1.15-1.84; P = 0.006) and 1.33 (95%CI: 1.06-1.66; P = 0.026) times more likely to have nephropathy[22]. Two studies in Rwanda and South Africa respectively showed that a one year increase in the duration of T1DM increased by 0.86 (95%CI: 0.77-0.96; P = 0.008) the odds of microalbuminuria[36], and that T1DM and T2DM patients with a duration of diabetes greater than 10 years were 4.19 times (95%CI: 1.93-9.10; P < 0.001) more likely to have microalbuminuria[43]. Poor glycemic control as measured by HbA1c was also a strong predictor of nephropathy. For instance, HbA1c level greater than 10% and 14% were respectively associated with a 2.6 fold (95%CI: 1.1-6.4) and a 4.69 (95%CI: 1.65-13.3; P = 0.004)[42,43]. A 1 g/dL decrease in hemoglobin level has been found to be associated with end-stage renal disease (OR 3.18, 95%CI: 1.47-6.87; P = 0.003)[19]. Studies in Nigeria showed that left ventricular hypertrophy, stroke, myocardial infarction and peripheral arterial disease were more frequent in T2DM patients with nephropathy, especially those with advanced stages[17,18].

DISCUSSION

Diabetic nephropathy is a common and morbid complication of diabetes and the leading cause of CKD in the developed world. The lack of renal registries means that there are no reliable statistics about the burden of CKD in people with diabetes in the majority of African countries. The current systematic review identified 32 relevant studies published over the last 20 years on kidney diseases in people with diabetes residing in Africa. Prevalence rates ranged from 11% to 83.7% for the overall CKD, 5.3% to 53.1% for CKD based on proteinuria, and 4.6% to 43.1% for CKD based on eGFR. Incident event rates were 94.9% for proteinuria at 10 years for follow-up, 34.7% for ERSD at 5 years of follow-up and 18.4% for mortality from nephropathy at 20 years of follow-up. Diagnosed duration of diabetes, blood pressure variables, advancing age, obesity and to some extend glucose control were the common determinants of kidney disease in people with diabetes. Studies were overwhelmingly hospital-based studies; half of them originated from four countries while variable definitions and methods for assessing nephropathy had been used across studies.

The most recent overview of CKD in populations within Africa was completed in 2012, and was restricted to sub-Saharan African Countries[47]. This review identified 90 articles representing data from 21 countries, with over half of the studies originating from South Africa, Nigeria and Ethiopia alones. Across 21 studies deemed to be of medium to high quality by the investigators, the pooled prevalence of CKD was 13.9% (95%CI: 12.2-15.7), with substantial heterogeneity across studies. The prevalence in people with diabetes ranged from 4% to 24% based essentially on proteinuria defined CKD[47]. In our review without applying quality criteria, we found much higher prevalence of CKD, regardless of the definition. In four studies published in 2013 for instance, the prevalence of microalbuminuria ranged between 21% and 45%. Although issues with the quality of the studies preclude direct comparisons, it is likely that nephropathy is

Table 4 Risk factors for chronic kidney disease in people with diabetes

Ref.	Country	Sample size	Type of diabetes	Diagnostic criteria for CKD	Risk factor	Measure of association		Factors adjusted for	Comments
						Effect size	*P*-value		
Motala *et al*[37], 2001	South Africa	219	T1DM and T2DM	Persistent proteinuria	Not assessed				
Elbagir *et al*[26], 1995	Sudan	128	Insulin-treated	Proteinuria	Age		$P = 0.006$		
					Duration of diabetes		$P = 0.003$		
					Systolic BP		$P = 0.0001$		
					Diastolic BP		$P = 0.001$		
					Serum cholesterol		$P < 0.05$		
Sobngwi *et al*[44], 1999	Cameroon	64	T1DM and T2DM	Proteinuria	Duration of diabetes		$P = 0.04$		
					Diastolic BP		$P = 0.01$		
Katchunga *et al*[30], 2010	DR Congo	98	T2DM	MDRD (corrected for Blacks); CKD stage ≥ 1 according to the National Kidney foundation	Hypertension	aOR: 2.49 (0.98-6.34)	$P = 0.04$	Age, duration of diabetes, BMI	
Choukem *et al*[22], 2012	Cameroon	420	T2DM	Proteinuria (30 mg/24 h)	Systolic BP	aOR: 1.45 (1.15-1.84)	$P = 0.006$		
					Diastolic BP	aOR: 1.33 (1.06-1.66)	$P = 0.026$		
					Pulse pressure	aOR: 1.35 (1.06-1.71)	$P = 0.0007$		
					Mean arterial pressure	aOR: 1.42 (1.13-1.78)	$P = 0.006$		
Keeton *et al*[31], 2004	South Africa	59	T2DM	Urine Albumin-to-Creatinine Ratio (no detail)	High entry serum creatinine		$P < 0.006$		These are risk factors for death from chronic renal failure (compared with the patients who were still alive at follow-up)
					BMI < 28		$P < 0.003$		
					Severe retinopathy		$P < 0.002$		
					Mean glucose level of > 14 mmol/L		$P < 0.035$		
									By the end of study 47 of the 59 patients had died; the cause of death not established in 2 patients. Death was due to chronic renal failure in 17 cases
Pruijm *et al*[39], 2008	Seychelles	1218	All types	Microalbuminuria: Urine Albumin-to-Creatinine Ratio 3.4-33.9 mg albumin/mmol creatinine	Not assessed				Risk factors were investigated in the whole study population in both diabetics and non-diabetics
Alebiosu[16], 2003	Nigeria	342	T1DM and T2DM	Persistent proteinuria	Not assessed				
Bouaziz *et al*[20], 2012	Tunisia	73	T2DM	Microalbuminuria: < 2.8 g/mol for women and < 2.3 g/mol for men	Family history of nephropathy		$P = 0.0289$		Comparison of T2DM patients with nephropathy with those without nephropathy
					Smoking		$P = 0.0056$		
					Insulin therapy		$P = 0.0310$		
					Glitazones therapy		$P = 0.0115$		
					Anti-hypertensives (not ACE inhibitor)		$P < 0.0001$		
					Lipid-lowering agents		$P < 0.0001$		
Ajayi *et al*[15], 2014	Nigeria	65	T2DM	MDRD: eGFR ⩽ 60 mL/min per 1.73 m^2	Not assessed				
Levitt *et al*[32], 1997	South Africa	243	T2DM and T1DM	Urine Albumin-to-Creatinine Ratio > 3.4 mm/mmol and Persistent proteinuria (for at least 3 consecutive visits)	Not assessed				

Majaliwa *et al*[34], 2007	Tanzania	99	T1DM	Proteinuria (no detail)	Missing insulin doses		*P* = 0.045	Not available	
Marshall *et al*[36], 2013	Rwanda	286	T1DM	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g	Age (increase)	aOR: 0.86, 95%CI: 0.77-0.96	*P* = 0.009	Each variable is adjusted for the others	These are risk factors of microalbuminuria. There was no factor associated to macroalbuminuria
					Duration of diabetes (one year increase)	aOR: 0.86, 95%CI: 0.77-0.96	*P* = 0.008		
					Diastolic BP (increase)	aOR: 0.86, 95%CI: 0.77-0.96	*P* = 0.004		
					HBA1c (increase)	aOR: 0.86, 95%CI: 0.77-0.96	*P* = 0.047		
Alebiosu *et al*[18], 2003	Nigeria	465	T2DM	Proteinuria and eGFR (no detail)	Hypertension, left ventricular hypertrophy, stroke and myocardial infarction were more frequent in advanced stages of nephropathy	Not available	*P* < 0.05	Not available	Patients with advanced stages of nephropathy (IV and V) were compared with those with stages ⩽ III
Gill *et al*[28], 2005	South Africa	88	T1DM	Persistent dipstick proteinuria	Not assessed				
Djrolo *et al*[24], 2001	Benin	152	T1DM and T2DM	Proteinuria (no detail)		Not available	Not available	Not available	Proteinuria was more frequent in insulin-treated patients compared those on oral antidiabetic treatment. The prevalence of proteinuria also increased with the duration of diabetes
Rotchford *et al*[43], 2002	South Africa	253	T1DM and T2DM	Microalbuminuria > 2.5 mg/mmol in men or 3.5 mg/mmol in women	Duration of diabetes > 10 yr	4.19 (1.93-9.10)	< 0.001	Model contains duration of diabetes, BMI, HbA1c, age and hypertension	
					BMI > 33	0.27 (0.08-0.48)	0.002		
					HbA1c > 14%	4.69 (1.65-13.3)	0.004		
					Hypertension	2.11 (1.07-4.17)	0.031		
Rissassi *et al*[42], 2009	DR congo	181	T1DM	Microalbuminuria: Urine Albumin-to-Creatinine Ratio = 30-299 mg/g	Duration of diabetes > 5 yr	4.1 (1.9-8.4)		No precision	
				Macroalbuminuria: Urine Albumin-to-Creatinine Ratio ≥ 300 mg/g	Age > 18 yr	2.9 (1.3-6.2)			
					HbA1c > 10%	2.6 (1.1-6.4)			
Rahlenbeck *et al*[40], 1997	Ethiopia	170	T1DM and T2DM	albuminuria: > 30 mg/L	Duration of diabetes	Beta = 0.061, SE = 0.018 for T1DM	< 0.001	Hypertensive patients excluded	
					Systolic blood pressure	Beta = 0.027, SE = 0.005 for T1DM	< 0.001		
Wanjohi *et al*[45], 2002	Kenya	100	T2DM	Proteinuria ≥ 20mg	None identified				
Nambuya *et al*[38], 1996	Uganda	252	T1DM and T2DM	Proteinuria (no detail)	None assessed				
Rasmussen *et al*[41], 2013	Zambia	101	T1DM and T2DM	Microalbuminuria: ACR = 3.5-35.0 for women and 2.5-25.0 mg/mmol for men Macroalbuminuria were ACR > 35.0 for women and > 25.0 for men	None assessed				
Bentata *et al*[19], 2013	Maroc	72	T1DM	End-stage renal disease: eGFR < 15 mL/min	Hemoglobin blood (per 1 g/dL decrease)	3.18 (1.47-6.87)	0.003	No precision	These are independent risk factors for ESRD in type-1 diabetes patients with diabetic nephropathy
					Diastolic blood pressure (per 1 mmHg increase)	1.15 (1.04-1.27)	0.006		

Gill *et al*[27], 2008	Ethiopia	105	T1DM and T2DM	Nephropathy: ACR > 25.0 mg/mmol and retinopathy present Microalbuminuria: ACR > 2.5 and < 25.0 mg/mmol in men and > 3.5 and < 25.0 mg/mmol in women	None assessed			
Bouzid *et al*[21], 2011	Tunisia	689	T2DM	Renal failure: creatinine clearance < 60 mL/min (Cockroft-Gault)	Older age Hypertension Long duration of diabetes Higher BMI Dyslipidemia	Not provided	< 0.00001 < 0.00001 < 0.001 0.02 0.01	
Janmohamed *et al*[29], 2013	Tanzania	369	T1DM and T2DM	CKD: eGFR < 60 mL/min per 1.73 m^2 (Cockroft-Gault) or microalbuminuria (> 20 mg/L) or overt proteinuria	Older age	1.03 (1.00-1.05)	0.03	Adjustment made, but no precision
Danquah *et al*[23], 2012	Ghana	671	T2DM	Proteinuria ≥ 20mg/l	Not assessed			
Lutale *et al*[33], 2007	Tanzania	244	T1DM and T2DM	Abnormal proteinuria: AER > 20 μg/min	Duration of diabetes Elevated systolic blood pressure Elevated serum creatinine	0.090 (0.049- 0.131) 0.012 (0.003-0.021) 0.011 (0.002- 0.020)	< 0.0001 0.010 0.016	Predictors in the model: diabetes duration, Systolic BP, age, serum creatinine
Worku *et al*[46], 2010	Ethiopia	305	T1DM and T2DM	Proteinuria (no detail)	Duration of diabetes T2DM on insulin	Not provided	0.001 0.018	
Makulo *et al*[35], 2010	DR Congo	81	No precision	Microalbuminuria: ACR 30-299 mg/g Macroalbuminuria: ACR ≥ 300 mg/g Renal failure: eGFR < 60 mL/min per 1.73 m^2	Not assessed			
Eghan *et al*[25], 2007	Ghana	109	T1DM and T2DM	Microalbuminuria: ACR 30-300 mg/g	Duration of diabetes Serum creatinine Blood urea nitrogen Urine potassium		0.04 0.05 0.01 0.0061	The associations were assessed by comparing patients with and without microalbuminuria
Alebiosu *et al*[17], 2004	Nigeria	162	T2DM	No precision	Duration of diabetes Serum total cholesterol Alcohol > 30 mg/d Peripheral vascular disease Stroke		< 0.05 < 0.05 < 0.05 < 0.05 < 0.05	The study assessed the predictors of diabetic nephropathy comparing T2DM patients with and without nephropathy

CKD: Chronic kidney disease; BMI: Body mass index; ACR: Albumin-to-Creatinine Ratio; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; eGFR: Epidermal growth factor receptor.

more frequent in population with diabetes within Africa than in developed countries. The review by Stanifer *et al*[47] also identified many challenges and limitations, which largely apply to the current study.

The most important aspect in assessing incidence and prevalence of diabetic nephropathy in Africa is currently different diagnostic criteria for CKD. There are no clear definitions on DN. The 2012 KDIGO CKD classification assesses diabetes related kidney changes according to urinary albumin-to-creatinine ratio based on early morning spot urine samples[48]. Quantification of proteinuria in assessing CKD is controversial as no optimal test exists. The National Institute for Health and Clinical Excellence (NICE) guidance has recommended that an early morning urinary ACR should be preferred to other tests of proteinuria, because ACR offers greater sensitivity for the detecting lower, but clinically significant, levels of proteinuria[49]. Almost all the studies included in our review utilized urine tests to diagnose CKD, but only nine studies used ACR. Inconsistencies in the way and manner of reaching a diagnosis of DN in Africans are explained at least in part by issues relating to availability and accessibility of screening or diagnostic tools. Swanepoel *et al*[50] have reviewed in detail some of the problems associated with nephrology in Africa and discussed the role of lack of amenities in diagnosing renal diseases. Another challenge to making the diagnosis of diabetic nephropathy in Africa is the degree to which other causes of chronic kidney disease have been excluded. A standard armamentarium of tests would include tests looking for HIV, hepatitis B and C, brief collagen screen, syphilis exclusion and other tests would have to be based on history and physical exam.

The classification of CKD is important in the definition of DN and has a few limitations that are universally acknowledged: eGFR underestimates kidney function and there is discordance in the estimates across different estimators[51]; isolated microalbuminuria is a normal feature of aging, inflammation, vascular pathologies, smoking, diet and obesity which are all frequent in diabetes; decline in kidney function is an expected phenomenon with advanced age, just like diabetes risk increases with age. Further considerations to CKD classifications and DN definition limitations is that current guidelines take no notice of the single most important risk factor associated with CKD namely hypertension, which is present in over 50% of people with type 2 diabetes.

Risk factor association was not assessed in 12 of the 32 studies, however common risk factors included were hypertension, raised BMI, HbA1c and duration of diabetes. Despite advances in management over the last three decades, many people with diabetes still develop CKD. This may be partly explained by the poor achievement of blood pressure and blood glucose targets. Recently the JNC 8 guidelines have added to the controversy of various blood pressure targets needed for diabetic patients that would assist in preventing progression to CKD. Optimal targets when reached, however have shown to aid in progression to progression. Another risk factor pertinent to the developing world is the socioeconomic status of individuals in the causative role of diabetic nephropathy. Weil *et al*[52], in 2010 reviewed factors associated with disadvantage that may increase the risk of diabetic kidney disease, and the barriers to care that hinder attempts to provide an adequate therapeutic response[52].

Several mechanisms underlying the pathogenesis of diabetic nephropathy have been suggested and include glomerular hyperfiltration; hyperglycemia and the increased production of advanced glycation end products; hypoxia-inflammation and the activation of cytokines. Hyperfiltration commonly occur in early in the course of diabetes and involves glucose-dependent dilation of the afferent arteriolar dilation, and the enhanced filtration area secondary to the increase in the number of mesangial cells and capillary loops. Molecular level action involves vasoactive mediators like insulin-like growth factor 1, transforming growth factor beta, nitric oxide, prostaglandin, glucagon and vascular endothelial growth factor[53]. Other hallmarks of diabetic nephropathy include nodular diabetic glomerulosclerosis and diffuse glomerulosclerosis, mediated at least in part by inflammatory processes and immune cells activity[53]. Interstitial fibrosis and tubular atrophy are also seen early in DN, with the underlying pathogenetic mechanism being similar to those in progressive non diabetic renal disease[54].

Diabetic nephropathy ultimately occurs only in susceptible individuals with diabetes; which susceptibility is determined by the combined effect of genetic predisposition and non-genetic factors. Genetic susceptibility to diabetic nephropathy is by nature polygenetic. Whole-genome scanning studies have identified several chromosomal regions linked with diabetic nephropathy; however, the pathophysiologic function of such genetic regions has yet to be fully elucidated. Genetic polymorphisms may explain the familial clustering of diabetic nephropathy[55]. Some studies have suggested some detrimental effect of the double-deletion (DD) polymorphism of the angiotensin-converting enzyme (ACE) genotype on disease progression[56]. Non-genetic determinants of diabetic nephropathy include among others socioeconomic factors, dietary factors, poor hyperglycemic control, hypertension, obesity and early life factors[57,58]. Hypertension appears to be a strong correlate of disease progression in Black people[59,60].

The current review has some limitations. Included studies were mostly based on small samples, with different study designs and most of the studies were cross sectional with only two being retrospective cohorts and one case-control. A large proportion were based in urban clinics with and most of the populations studied were that attending a general diabetic clinic and the results may not be generalizable

to primary care populations. Ideally chronic kidney disease should not be diagnosed on the basis of single measurements of serum creatinine and albuminuria, and standard baseline investigations are needed to exclude other causative kidney disease, although there is precedence for this in other studies in the West as well. Finally, detection of microalbuminuria was one the most frequent method to assess the presence of diabetic nephropathy. As microalbuminuria is more a quantitative estimate of endothelial/vascular dysfunction than of diabetic nephropathy, the incidence and prevalence rate of diabetic nephropathy have probably been overestimated when assessing kidney function by urine protein.

In conclusion, the current review gives a small glimpse of the larger numbers of CKD in diabetics in Africa compared to Western society. CKD is a substantial health burden among diabetic patients on the African continent, with prevalence varying from 11% to 83.7% depending on the method of assessment. Estimates suggest that 95% of diabetics may have proteinuria after a 10 years duration of diabetes, about 35% may have an end-stage renal disease after 5 years and 18% die from nephropathy after 20 years of disease duration. Risk factors of CKD include mainly hypertension, obesity, poor glycemic control and disease duration. Better surveillance of diabetes is a necessary first step toward its prevention and control, which is now recognized as an urgent priority. An electronic database in African regions would be ideal to assist in this entity although it is presumed that we are light years away from that. At a primary care level it is very plausible that with early detection, proper screening, and management, the impact of diabetic nephropathy may be better mitigated to lessen its impact on society and healthcare.

COMMENTS

Background

African countries are experiencing an epidemics of diabetes mellitus. Diabetic nephropathy is one the most frequent complications of diabetes mellitus. Several studies on the epidemiology of diabetic nephropathy have been conducted in Africa, but there is no previous published work which synthesizes evidences from this study to provide an overview of the disease on the continent.

Research frontiers

Epidemiological data on diabetic nephropathy in Africa are sparse. These data are important to quantify the magnitude of the disease and assist the formulation of strategies to reduce the impact of nephropathy on people with diabetes in Africa.

Innovations and breakthroughs

This review is the first to synthesize relevant data on diabetic nephropathy in Africa. The authors performed extensive electronic and manual bibliographic searches to determine the prevalence and incidence of diabetic nephropathy on the continent. Although the quality of data was not optimal, estimates suggest that the prevalence of diabetic nephropathy vary between 11%-83.7%. About one third of diabetic patients have end-stage renal disease after 5 years and about one fifth die from nephropathy after 20 years of disease duration. Hypertension, obesity, poor glycemic control and disease duration are the main risk factors of chronic kidney disease among diabetic patients in Africa.

Applications

This review shows that the burden of chronic kidney disease is important among people with diabetes in Africa. The findings will have implications for policy, practice and future research on diabetic nephropathy on the continent.

Terminology

Diabetic nephropathy is an alteration of the function of the kidneys due to diabetes mellitus. It is associated with substantial morbidity and mortality.

Peer-review

The authors of the present manuscript performed extensive electronic and manual bibliographic research to determine the prevalence and incidence of kidney disease in people with diabetes mellitus within countries in Africa. Overall the review is well written.

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P- Reviewer: de Oliveira JMF, Laghmani K **S- Editor**: Ji FF
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