Available online at
ScienceDirect
www.sciencedirect.com
Elsevier Masson France
EM|consulte
www.em-consulte.com/en
Diabetes & Metabolism xxx (2016) xxx–xxx

Research letter

Wnt antagonist sclerostin and Dickkopf-1 in gestational diabetes

1. Introduction

Wnt secretory glycoproteins are a family of developmentally important signalling molecules that play important roles in embryonic induction, generation of cell polarity and specification of cell fate. Canonical and non-canonical Wnt pathways have been identified [1]. The importance of Wnt signalling in diabetes arose after the observation by Kanazawa et al. [2], who showed that a single polymorphism locus in the *WNT5B* gene may contribute to susceptibility to type 2 diabetes mellitus (T2DM) and may be involved in the pathogenesis of the disorder through regulation of adipocyte function. Emerging evidence also supports the effects of altered Wnt signalling on cardiovascular risk factors [1–3]. In animal models, the Wnt/β-catenin signalling pathway has been shown to contribute to modulation of insulin secretion, β-cell function and insulin signalling in skeletal muscle [4]. According to the canonical pathway, binding of the appropriate Wnt ligands to a co-receptor complex involving Frizzled and low-density lipoprotein receptor-related protein (LRP)-5 or -6 stabilizes cytoplasmic β-catenin protein, which translocates to the nucleus and activates the transcription of target genes. The Wnt/β-catenin canonical pathway is modulated by a number of factors, including secreted proteins such as Dickkopf-1 (Dkk-1) and sclerostin, which prevent formation of the Wnt–Frizzled–LRP5 complex by internalization of the LRP5/6 co-receptor and competitive binding to LRP5, respectively [1]. Also, Nuche-Berenguer et al. [5] demonstrated the upregulation of gene expression of Dkk-1 and sclerostin in T2DM rats, and revealed that sclerostin overexpression was associated with increased mRNA levels of activator LRP5 in insulin-resistant rats. Consistent with these findings, human studies have reported significantly higher serum sclerostin levels in T2DM patients than in controls; beyond T2DM, sclerostin is positively associated with the main features of the metabolic syndrome (MetS), including obesity and dyslipidaemia, as well as atherosclerotic disease [3,6].

Gestational diabetes (GDM) is defined as diabetes induced by pregnancy, but which resolves at the end of pregnancy. It usually develops in late pregnancy when insulin antagonistic hormones peak, leading to insulin resistance, glucose intolerance and hyperglycaemia. However, GDM may also lead to several serious maternal and foetal complications, and constitutes a significant risk factor for the subsequent development of T2DM and cardiovascular disease in later life [7].

To date, few data have been reported for the levels and associations of sclerostin in women diagnosed with GDM. As GDM can serve as a model of pre-T2DM and because sclerostin levels are increased in those with prediabetes, our present study investigated both sclerostin and Dkk-1 serum levels in women with GDM compared with healthy, non-diabetic pregnant controls, and also looked for possible associations between these Wnt antagonists and certain maternal/foetal outcomes.

2. Materials and methods

This was a case–control study involving pregnant women attending the Diabetes Outpatient Unit of the Department of Internal Medicine at the G. Martino University Hospital in Messina, Italy. Women were referred to the Unit for an oral glucose tolerance test (OGTT) for the detection of GDM. They were included in the study if they were aged ≥18 years and willing to give their informed consent. Exclusion criteria were the presence of renal or liver failure, severe heart failure or a psychiatric disorder. Pregnant women with established risk factors for GDM at gestational weeks 24–28 underwent a 75-g OGTT, with cut-off values of 5.1 mmol/L for fasting glucose, and 10.0 mmol/L and 8.5 mmol/L for 1-h and 2-h post-load glucose levels, respectively, and were considered eligible according to International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [6].

Over a period of 6 months (from October 2014 to April 2015), 35 consecutive women with GDM were recruited, while a group of pregnant women who were negative on the screening test were randomly selected, using a computer-generated randomization table, to serve as the control group.

Blood samples were drawn to check insulin, sclerostin and Dkk-1 levels at the same time as the OGTT. Insulin resistance was calculated by homoeostasis model assessment for insulin resistance (HOMA-IR). Enzyme immunoassays were used to measure levels of insulin (Beckman Coulter, Brea, CA, USA), sclerostin and Dkk-1 (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria), which had intra- and interassay coefficients of variation (CVs) < 7% for all analyses. The recruited women were followed until delivery. Information on the following parameters was collected: age; height; pregestational weight;

http://dx.doi.org/10.1016/j.diabet.2016.09.009
1262-3636/© 2016 Elsevier Masson SAS. All rights reserved.

Table 1
Main clinical characteristics of the studied population.

	Overall	Normal glucose tolerance	Gestational diabetes	*P* value
n	71	36	35	
Age (years)	33.8 ± 4.9	33.4 ± 5.3	34.1 ± 4.5	0.55
Height (cm)	162.7 ± 6.1	161.6 ± 6.0	163.9 ± 5.9	0.11
Pregestational weight (kg)	66.4 ± 13.5	62.5 ± 11.2	70.5 ± 14.7	0.01
Pregestational BMI (kg/m^2)	25.1 ± 5.0	24.1 ± 4.5	26.2 ± 5.4	0.08
Family history of diabetes (%)	59.5	33.3	66.7	0.12
Previous gestational diabetes (%)	14.3	0.0	20.0	0.53
Baseline BG on OGTT (mg/dL)	84.4 ± 10.1	76.5 ± 5.0	92.8 ± 6.8	<0.0001
1-h post-load BG on OGTT (mg/dL)	134.5 ± 36.2	113.7 ± 24.4	156.5 ± 33.7	<0.0001
2-h post-load BG on OGTT (mg/dL)	112.8 ± 28.8	96.1 ± 19.1	130.6 ± 26.8	<0.0001
Insulin on OGTT (uU/mL)	18.2 ± 11.0	13.1 ± 6.4	23.5 ± 12.1	<0.0001
HOMA-IR	3.5 ± 2.3	2.3 ± 1.2	4.8 ± 2.5	<0.0001
Sclerostin levels (pmol/L)	24.5 ± 15.2	25.0 ± 14.8	24.0 ± 15.8	0.79
Dkk-1 levels (pmol/L)	10.9 ± 3.9	11.4 ± 3.9	10.3 ± 4.0	0.26
Weight at delivery (kg)	77.1 ± 12.7	74.3 ± 10.9	80.1 ± 13.9	0.06
BMI at delivery (kg/m^2)	29.1 ± 4.7	28.5 ± 4.2	29.6 ± 5.2	0.32
Weight gain (kg)	10.5 ± 3.2	11.7 ± 2.8	9.1 ± 3.2	0.001
Gestational week at delivery (weeks)	38.6 ± 1.5	38.9 ± 1.6	38.3 ± 1.5	0.13
Caesarean section (%)	40.3	44.4	37.1	0.63
Female newborn (%)	52.8	50.0	48.6	0.99
Neonatal weight (g)	3112.4 ± 437.2	3144.4 ± 487.2	3078.5 ± 381.6	0.53
Apgar score at 1 min	9.2 ± 0.9	9.2 ± 0.8	9.2 ± 1.1	0.78
Apgar score at 5 min	9.8 ± 0.4	9.7 ± 0.4	9.8 ± 0.4	0.23

Data are presented as means ± SD or as percentages (%).
BMI, body mass index; BG, blood glucose; OGTT, oral glucose tolerance test; HOMA-IR, homoeostasis model assessment for insulin resistance.

family history of diabetes; history of previous GDM; weight at delivery; weight gain; gestational week at delivery; caesarean section rate; gender of the newborn; neonatal weight; and Apgar 1- and 5-min scores.

The study was conducted in accordance with the Declaration of Helsinki, and all participants gave their written informed consent.

2.1. Statistical analyses

Data were expressed as means ± SD for continuous variables and as percentages for categorical variables. The Kolmogorov–Smirnov test was used to test the normality of distribution of continuous variables. Clinical and demographic characteristics were compared using the chi-square test for categorical variables and the Kruskal–Wallis test for continuous variables. Pearson's correlation coefficient was employed to test correlations between sclerostin, Dkk-1, other well-known risk factors for GDM and pregnancy outcomes. Multivariate regression models were performed to analyze the association of sclerostin and Dkk-1 with GDM onset as well as the following covariates: age; pregestational body mass index (BMI); family history of diabetes; HOMA-IR; sclerostin; and Dkk-1. A *P* value <0.05 was considered statistically significant. Analyses were performed using IBM SPSS version 21.0 software (IBM Corp., Armonk, NY, USA).

3. Results

Overall, 71 women were included in our study; their main characteristics are presented in Table 1. On comparing women with normal glucose tolerance (NGT) with those with GDM, the latter had significantly higher pregestational weight, blood glucose levels at all OGTT points, insulin levels and HOMA-IR scores, and less weight gain (Table 1). No between-group differences were detected for age, height, family history of diabetes, history of previous GDM, or sclerostin or Dkk-1 levels. As for pregnancy outcomes, the two groups were not statistically different in terms of gestational week at delivery, caesarean section rate, newborn gender, neonatal weight and Apgar scores at 1 and 5 min (Table 1).

Correlation analyses showed that sclerostin correlated only with pregestational BMI; in contrast, Dkk-1 correlated with none of the tested variables. The multivariate regression model also showed no association between either sclerostin (OR: 0.98, 95% CI: 0.87–1.10) or Dkk-1 (OR: 1.73, 95% CI: 0.91–3.30) and GDM onset.

4. Discussion

Pregnancy is a challenging period for the mother's bones because of the development of the foetal skeleton; consequently, surrogate markers of bone resorption and bone formation may be observed to vary over time, with a predominance of bone resorption during the first and second trimesters. To date, however, maternal placental–foetal mineral homoeostasis has remained largely poorly understood, and there are few data regarding the role of Wnt antagonists, especially in GDM.

Our present study investigated serum levels of sclerostin and Dkk-1 in pregnant women with GDM and in NGT controls. No significant differences were found in maternal serum levels of both sclerostin and Dkk-1 in both groups of women; moreover,

Please cite this article in press as: Catalano A, et al. Wnt antagonist sclerostin and Dickkopf-1 in gestational diabetes. Diabetes Metab (2016), http://dx.doi.org/10.1016/j.diabet.2016.09.009

no associations could be found between sclerostin or Dkk-1 with the main metabolic maternal features or with foetal outcomes.

The close connection between bone tissue and glucose homoeostasis was recently highlighted, and osteocalcin (BGP), one of the very few osteoblast-specific proteins and, thus, a surrogate marker of bone formation, emerged as a hormone that can regulate β-cell proliferation, insulin secretion and insulin sensitivity. BGP has also been reportedly decreased in patients with T2DM and negatively correlated with fasting plasma glucose, HbA_{1c}, HOMA-IR and BMI, but increased with improved glycaemic control. Accordingly, the possible role of bone in controlling glucose homoeostasis has been hypothesized, and it has been even reported that BGP was significantly higher in women with GDM than in NGT women [6,8].

Both sclerostin and Dkk-1 interact with LRP5 and LRP6, and antagonize the canonical Wnt/β-catenin signalling pathway, the activation of which leads to an increased proliferation and differentiation of osteoblast precursor cells and reduced apoptosis of mature osteoblasts, while promoting the ability of differentiated osteoblasts to inhibit osteoclast differentiation [1]. Therefore, it may be speculated that Wnt antagonists could act as modulators of BGP production through regulation of osteoblast activity.

The Wnt/β-catenin signalling pathway is involved in the pathogenesis of obesity and T2DM [1,3]. It was also observed that the *TCF7L2* gene, which encodes a nuclear-binding factor for β-catenin, was associated with features of the MetS, including elevated systolic and diastolic blood pressures, and raised levels of glucose, cholesterol, triglyceride and uric acid [3].

However, in pregnant women, Platz et al. [9] recently reported that sclerostin does not correlate with any feature of the MetS, in contrast to what has been observed in other cohorts [6,10]. In fact, consistent with previous data obtained during pregnancy [9], our present study also observed no association between Wnt antagonist levels and our main maternal and foetal outcomes.

Although our study has a few limitations (small sample size, single time point of measurements), it is the first to investigate both sclerostin and Dkk-1 in a cohort of pregnant women whose delivery outcomes were recorded. Our present findings suggest that sclerostin and Dkk-1 do not play a significant role in the pathophysiology of GDM, although further research is needed to explore their associations with hormonal status and perhaps in different trimesters.

In conclusion, in our cohort of pregnant women, sclerostin and Dkk-1 were not associated with any adverse metabolic profile, and possibly do not play relevant roles in the pathophysiology of GDM.

Funding

This research received no specific grants from funding agencies in the public, commercial or not-for-profit sectors.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009;17:9–26.
[2] Kanazawa A, Tsukada S, Sekine A, Tsunoda T, Takahashi A, Kashiwagi A, et al. Association of the gene encoding wingless-type mammary tumor virus integration-site family member 5B (WNT5B) with type 2 diabetes. Am J Hum Genet 2004;75:832–43.
[3] Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320–3.
[4] Elghazi L, Gould AP, Weiss AJ, Barker DJ, Callaghan J, Opland D, et al. Importance of β-catenin in glucose and energy homeostasis. Sci Rep 2012;2:693.
[5] Nuche-Berenguer B, Moreno P, Portal-Nuñez S, Dapía S, Esbrit P, Villanueva-Peñacarrillo ML. Exendin-4 exerts osteogenic actions in insulin-resistant and type 2 diabetic states. Regul Pept 2010;159:61–6.
[6] Daniele G, Winnier D, Mari A, Bruder J, Fourcaudot M, Pengou Z, et al. Sclerostin and insulin resistance in pre-diabetes: evidence of a cross talk between bone and glucose metabolism. Diabetes Care 2015;38:1509–17.
[7] International Association of Diabetes Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676–82.
[8] Winhofer Y, Handisurya A, Tura A, Bittighofer C, Klein K, Schneider B, et al. Osteocalcin is related to enhanced insulin secretion in gestational diabetes mellitus. Diabetes Care 2010;33:139–43.
[9] Platz M, Stepan H, Schrey S, Kralisch S, Wurst U, Lossner U, et al. Serum levels of sclerostin in cardiometabolic disorders during pregnancy. Cytokine 2015;76:591–3.
[10] Catalano A, Pintaudi B, Morabito N, Di Vieste G, Giunta L, Bruno ML, et al. Gender differences in sclerostin and clinical characteristics in type 1 diabetes mellitus. Eur J Endocrinol 2014;171:293–300.

A. Catalano [a,*,1]
B. Pintaudi [b,1]
N. Morabito [a]
L. Giunta [a]
S. Loddo [a]
F. Corrado [c]
R. D'Anna [c]
A. Lasco [a,2]
A. Di Benedetto [a,2]

[a] *Department of Clinical and Experimental Medicine, University Hospital of Messina, Messina, Italy*
[b] *SSD Diabetology, Ca'Granda Niguarda Hospital, Milan, Italy*
[c] *Department of Pediatric, Gynecological, Microbiological and Biomedical Sciences, Messina, Italy*

* Corresponding author at: Department of Clinical and Experimental Medicine, University Hospital of Messina, Via C. Valeria, 98125 Messina, Italy. Tel.: +39 090 2213946; fax: +39 090 2935162.
E-mail address: catalanoa@unime.it (A. Catalano)
[1] These authors contributed equally.
[2] Senior authors.

Received 16 September 2016

Received in revised form 26 September 2016

Accepted 28 September 2016

Please cite this article in press as: Catalano A, et al. Wnt antagonist sclerostin and Dickkopf-1 in gestational diabetes. Diabetes Metab (2016), http://dx.doi.org/10.1016/j.diabet.2016.09.009