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Research letter

Gradual increase in advanced glycation end-products from no diabetes to early and regular gestational diabetes: A case-control study

Abbreviations

1h-PG	plasma glucose value 1 h after 75-g oral glucose tolerance test
2h-PG	plasma glucose value 2 h after 75-g oral glucose tolerance test
AGEs	advanced glycation end-products
BMI	body mass index
DIP	diabetes in pregnancy
eGDM	early gestational diabetes mellitus
FINDRISC	Finnish Diabetes Risk Score
FPG	fasting plasma glucose
GDM	gestational diabetes mellitus
SD	standard deviation
WG	weeks of gestation

Introduction

Given the increasing incidence of unknown type 2 diabetes (T2D), a new subset of dysglycaemia during pregnancy was introduced in the recommendations of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) [1] and endorsed in France [2]. Pregnant women are currently diagnosed with diabetes in pregnancy (DIP) when fasting plasma glucose (FPG) values are $\geq$ 7 mmol/L. As early diagnosis of DIP could prevent delay in treatment, screening was suggested for dysglycaemia not only after 24 weeks of gestation (WG), but also at the first antenatal visit. Thus, intermediate early hyperglycaemia, usually called 'early gestational diabetes mellitus' (eGDM), is defined as FPG levels in the 5.1–6.9 mmol/L range [1,2]. This condition has been observed in 7.2–11.9% of pregnant women and currently accounts for up to 48.8% of all GDM cases in high-risk populations (see Cosson et al. for a review [3]).

High FPG in early pregnancy when insulin resistance has not yet increased could indicate either unrecognized prediabetes (in cases of eGDM) or unrecognized diabetes (in cases of DIP) prior to pregnancy, as glycaemic status before pregnancy is rarely known. Therefore, the present study explored markers that might reflect metabolic status before pregnancy. Our hypothesis was that levels of cutaneous advanced glycation end-products (AGEs) and the Finnish Diabetes Risk Score (FINDRISC) would differ according to glycaemic status, with a gradual increase from no GDM through to regular GDM, eGDM and DIP.

Material and methods

Study design

This was a single-centre observational study that analyzed the electronic medical records of female subjects. The study was conducted according to the principles expressed in the Declaration of Helsinki. Due to the observational nature of the study, there was no need for informed consent from participants. Data were analyzed anonymously, and our database was declared to the French Committee of Data Protection and Civil Liberties [Commission Nationale de l'Informatique et des Libertés (CNIL), number 1704392v0].

Definitions of glycaemic status during pregnancy

The standard policy is to universally screen women by measuring FPG at the beginning of pregnancy and, if previous screening is normal or not done, then after 24 WG, using a 75-g oral glucose tolerance test (OGTT), and measuring FPG and plasma glucose at 1 h and 2 h after glucose intake (1h-PG and 2h-PG, respectively) [1,2]. DIP was defined as FPG $\geq$ 7.0 mmol/L and/or 2h-PG $\geq$ 11.1 mmol/L; eGDM was defined as FPG $\geq$ 5.1 mmol/L in early pregnancy, and regular GDM as FPG 5.1–6.9 mmol/L and/or 1h-PG $\geq$ 10.0 mmol/L and/or 2h-PG 8.5–11.0 mmol/L during OGTT performed after 22 WG. Only those women who had normal early FPG levels were included in the group with 'regular GDM'. Finally, 'no GDM' was defined as FPG $<$ 5.1 mmol/L and 1h-PG $<$ 10.0 mmol/L and 2h-PG $<$ 8.5 mmol/L during late OGTT, with an early FPG $<$ 5.1 mmol/L.

Subjects with eGDM and GDM

Pregnant women newly diagnosed with dysglycaemia were referred to a 1-day diabetes programme. During this programme, the subject's family and personal history was recorded, and a clinical examination was performed. Prepregnancy body mass index (BMI) was calculated using the declared weight before pregnancy (in kg) divided by the current squared height (in m^2). Women with a FINDRISC $\geq$ 12 were considered at high-risk of prevalent dysglycaemia [4]. In addition, women whose skin autofluorescence was measured between August 2015 and July 2016 were prospectively included in the study.

Pregnant women with no GDM

The no GDM group included outpatients who visited obstetricians/gynaecologists at our hospital and agreed to have their skin autofluorescence measured. Only age and GDM screening were known for these subjects.

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Skin autofluorescence

The level of cutaneous AGEs was non-invasively measured through their fluorescent properties using an AGE Reader® device (DiagnOptics BV, Groningen, Netherlands) [5–7]. The method and its validation are described in Appendix A (see supplementary material associated with this article online).

Statistical analyses

Continuous variables were expressed as means ± SD and compared by one-way analysis of covariance (ANCOVA) or Mann–Whitney's *U* test for adequacy. The significance of differences in proportions was tested by Chi^2 test. Statistical analyses were carried out using SPSS software (SPSS Inc., Chicago, IL, USA). The level of significance for all tests was $P < 0.05$.

Results

Characteristics of the study population

A total of 188 women had their skin autofluorescence measured. From the 34 women with normal OGTT results at 22 GW, 12 who had normal FPG levels at their first antenatal visit (4.44 ± 0.39 mmol/L at 11.4 ± 3.5 WG) were selected. Later in pregnancy, their FPG values during OGTT at 25.7 ± 2.9 WG were 4.33 ± 0.39 mmol/L (1h-PG: 6.67 ± 1.11 mmol/L and 2h-PG: 6.11 ± 0.94 mmol/L). Also selected, from among the 84 women with GDM diagnosed at 22 WG, were 48 who had had normal FPG values during early pregnancy. In addition, 62 women had eGDM and eight had DIP.

The characteristics of these 118 women with dysglycaemia are presented in Table 1, and show that their FINDRISC progressively increased from regular GDM to eGDM and DIP statuses.

Skin autofluorescence according to GDM categories

Skin autofluorescence (AGEs, in arbitrary units) gradually increased from no GDM (1.79 ± 0.32) to regular GDM (1.99 ± 0.47), eGDM (2.11 ± 0.48) and DIP (2.42 ± 0.34) significantly ($P = 0.015$; $P = 0.021$ after adjusting for age; $P = 0.021$ after adjusting for age and ethnicity), with greater proportions of women having AGE levels > mean + 1 standard deviation (SD) for their age (41.7%, 45.8%, 54.8% and 100%, respectively; $P = 0.032$ before and after adjusting for ethnicity) or > mean + 2 SD for age (8.3%, 10.4%, 25.8% and 50%, respectively; $P = 0.021$, and $P = 0.045$ before and after adjusting for ethnicity, respectively; Fig. 1).

Discussion

The present study shows that pregnant women with dysglycaemia but no known diabetes have higher levels of skin autofluorescence than women without dysglycaemia. Furthermore, independently of age and ethnicity, there is a gradual increase in skin autofluorescence from no GDM to regular GDM, eGDM and DIP, suggesting that previous lifetime glucose exposures differed across these categories. Such results are in line with a progressive increase in FINDRISC from regular GDM to eGDM and DIP. Overall, our results suggest that eGDM should be considered an intermediate entity between normal glucose metabolism and preexistent prediabetes/diabetes prior to pregnancy.

Previously, de Ranitz-Greven et al. [8] reported similar levels of skin autofluorescence in pregnant women with GDM at the time of diagnosis and pregnant women without diabetes. In another study, they found that skin autofluorescence levels were higher in women with preexisting diabetes than in women with and without GDM [6]. These results suggest that the higher levels of skin autofluorescence were further elevated in women with preexisting diabetes and long-standing, pronounced hyperglycaemia. This is

Table 1
Characteristics of the studied pregnant women according to glycaemic status.

	Regular GDM ($n = 48$)	eGDM ($n = 62$)	DIP ($n = 8$)	*P*
Characteristics				
Age (years)	32.3 ± 4.3	33.1 ± 5.4	32.0 ± 4.3	0.689
Pregravid BMI (kg/m^2)	27.0 ± 5.0	28.7 ± 5.8	27.5 ± 4.9	0.255
BMI categories				0.739
Normal (%)	18 (38.3)	18 (29.5)	2 (25.0)	
Overweight (%)	16 (34.0)	19 (31.1)	3 (37.5)	
Obese (%)	13 (27.7)	24 (39.3)	3 (37.3)	
Caucasian origin (%)	14 (29.2)	13 (21.0)	0 (0)	0.167
Personal history of GDM (%)	11 (22.9)	20 (32.3)	6 (75.0)	0.013
Personal history of macrosomic infant (%)	6 (12.5)	5 (8.1)	2 (25.0)	0.324
Pregravid hypertension (%)	0 (0)	2 (3.2)	0 (0)	0.399
At least one risk factor for GDM (%)	40 (83.3)	55 (88.7)	8 (100)	0.376
Twin pregnancy (%)	2 (4.2)	1 (1.6)	1 (12.5)	0.257
Family history of type 2 diabetes (%)	14 (29.2)	29 (49.2)	5 (62.5)	0.075
FINDRISC (units)	10.3 ± 3.9	11.6 ± 3.6	13.6 ± 3.8	0.037
FINDRISC ≥ 12 units (%)	17 (36.2)	32 (52.5)	6 (75.0)	0.066
GDM				
Early screening (%)				
Time of early screening (WG)	12.2 ± 3.6	12.3 ± 4.6	11.0 ± 3.7	0.762
Early fasting plasma glucose (mmol/L)	4.67 ± 0.28	5.44 ± 0.39	7.06 ± 2.5	< 0.001
Late screening (%)				
Time of late screening (WG)	25.9 ± 2.9	NA	28.0 ± 6.1	0.539
Late fasting plasma glucose (mmol/L)	4.89 ± 0.61	NA	5.44 ± 1.11	0.336
1-h plasma glucose (mmol/L)	9.56 ± 1.61	NA	11.83 ± 1.33	0.072
2-h plasma glucose (mmol/L)	8.22 ± 1.56	NA	12.0 ± 0.28	0.001

Data are *n* (%) or means ± SD.
GDM: gestational diabetes mellitus; eGDM: early gestational diabetes mellitus; DIP: diabetes in pregnancy; BMI: body mass index; FINDRISC: Finnish Diabetes Risk Score; WG: weeks of gestation; NA: non-applicable.

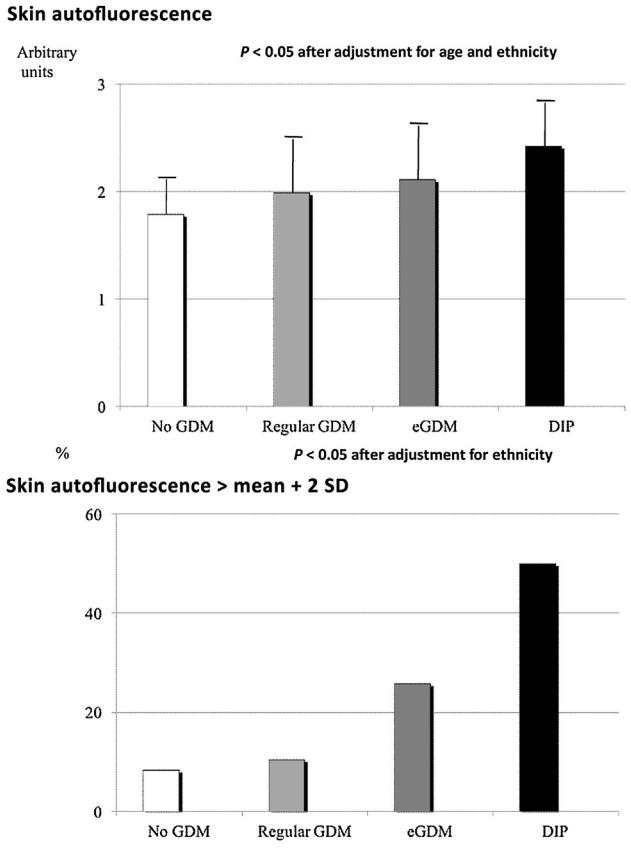

Fig. 1. Skin autofluorescence levels, used to measure advanced glycation end-products, are graphically presented according to glycaemic status. GDM: gestational diabetes mellitus; eGDM: early gestational diabetes mellitus; DIP: diabetes in pregnancy; SD: standard deviation.

consistent with our observation of higher skin autofluorescence in women with DIP.

Physiologically during normal pregnancy, FPG concentrations progressively decrease, with a plateau at around 10–20 WG. Thereafter, insulin resistance increases and 'regular' (or late) GDM may arise [9]. Therefore, our proposed hypothesis was that high FPG levels during early pregnancy might indicate unidentified dysglycaemia before pregnancy and, indeed, our present results are consistent with this hypothesis: skin autofluorescence gradually increased in control subjects who developed GDM at 22 WG but who had normal FPG in early pregnancy, and in women with eGDM and, finally, in women with DIP. More important, this trend persisted even after adjusting for two major confounding factors: age [5]; and ethnicity [8].

These findings are in agreement with previous characterizations of eGDM (see Cosson et al. for a review [3]). First, early high FPG levels during pregnancy are associated with the classic risk factors for T2D. Second, women with eGDM have lower insulin sensitivity compared with women with regular (late) GDM and those with normal glucose tolerance. Third, women with eGDM rather than regular GDM are more likely to have dysglycaemia in the early postpartum period and to develop T2D after delivery in the long term.

Overall, the gradually increasing FINDRISC from regular GDM to eGDM and DIP are also consistent with our present results. In fact, the FINDRISC depends on the classic risk factors for T2D [10], and is predictive of prevalent dysglycaemia in obese women [4]; it is also an accurate tool for identifying individuals at high-risk of developing T2D [10].

Maury et al. [7] suggested that skin autofluorescence levels were markers of metabolic memory in pregnant women. Forearm skin autofluorescence at 24–30 WG was reported to gradually decrease in women with previous diabetes, and also in those with GDM and previous hyperglycaemia, those with GDM but without previous hyperglycaemia and, finally, those with neither diabetes nor GDM. In their study, previous hyperglycaemia was defined as previous GDM, or having given birth to a macrosomic infant or having GDM diagnosed before 24 WG [7].

One strength of our study was that it was possible to differentiate between women with eGDM and those with definite regular GDM (abnormal OGTT at 22 WG, but normal FPG earlier in pregnancy). However, our study also has some limitations. Intraindividual changes in skin autofluorescence during pregnancy might account for the different levels measured early and late in pregnancy and, in fact, de Ranitz-Greven et al. [6] found an overall increase in skin autofluorescence from 26 to 38 WG. However, such a trend if confirmed in early pregnancy would then have resulted in lower skin autofluorescence levels when women with eGDM were tested. Furthermore, their findings also revealed that the decrease in skin autofluorescence during pregnancy was significant only in women without GDM [6]. Another limitation is that our results are based on a European multiethnic population attending a public university hospital, although the data were adjusted for ethnicity. Finally, the numbers of women with no GDM and regular GDM were limited in our series because of our deliberately high level of selection for these groups, a decision that appears to be crucial. In fact, at least 50% of those with eGDM no longer had GDM after 24 WG despite the lack of any specific care [3].

Conclusion

Our results based on skin autofluorescence levels suggest that eGDM could be the consequence of an intermediate state between normal glycaemic status and prediabetes before pregnancy. This might partly explain why women with eGDM have high FPG during early pregnancy despite the absence of physiological insulin resistance. Women with DIP have even higher glycaemic exposures prior to pregnancy.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data (Appendix A) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.diabet.2018.01.007.

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Circulating soluble klotho is not associated with an elevated ankle-brachial index as a surrogate marker of early arterial calcification in patients with type 1 diabetes mellitus and no evidence of renal dysfunction

Introduction

The conventional view of peripheral artery disease has evolved to now consider it a continuous pathway from arterial calcification to endoluminal atherosclerosis. In agreement with this idea, arterial stiffness secondary to vascular calcification is a strong independent predictor of cardiovascular (CV) events and all-cause mortality.

The *KL* gene encodes klotho, a 130-kDa single-pass transmembrane protein that is also an obligatory co-receptor of the phosphaturic hormone fibroblastic growth factor (FGF)-23 [1], expressed in distal renal tubules and parathyroid glands. Its extracellular domain can be cleaved, resulting in its presence in blood as a soluble protein (s-klotho) and suggested to protect against endothelial dysfunction by increasing nitric oxide availability. Klotho deficiency is associated with vascular disorders, including medial arterial calcification (MAC) [2]. Cross-sectional studies of chronic kidney disease (CKD), considered a state of accelerated ageing, have shown reduced s-klotho levels in all stages of disease [3]. In addition, s-klotho reduction may serve as a biomarker of premature diabetic nephropathy.

Increased arterial stiffness has been documented in young patients with type 1 diabetes mellitus (T1DM) [4]. Low s-klotho concentrations associated with early predictors of atherosclerosis and endothelial dysfunction have been also reported in these patients.

To provide new insights into the pathogenesis of MAC in T1DM, our present study aimed to address whether an association between circulating s-klotho and asymptomatic peripheral MAC is present in patients with T1DM and normal renal function.

Material and methods

Study population

This cross-sectional study involved 164 adult patients with T1DM, all of whom were aged < 30 years at the time of diagnosis, recruited consecutively at our outpatient clinic. Exclusion criteria included:

- ankle–brachial index (ABI) < 0.9;
- symptomatic claudication (by Edinburgh Claudication Questionnaire);
- previous diagnosis of peripheral artery disease, diabetic foot or leg amputation and;
- previous diagnosis of CKD, defined by the four-variable Modification of Diet in Renal Disease (MDRD-4) equation as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2, renal transplantation or renal replacement therapy. The presence of albuminuria < 300 mg/g was allowed.

Of our 164 study patients, 54 had MAC, defined as an ABI > 1.2, and were compared with 110 patients of similar gender distribution, age and eGFR, but presenting with normal ABI values (range: 0.9–1.2).

The study was approved by the local ethics committee, and all patients gave their written informed consent.

Clinical and anthropometric variables

The patients' medical records were reviewed for current medications and parameters related to their T1DM, CV risk factors, and coronary heart and cerebrovascular disease. Smoking status was also recorded. Anthropometric evaluation, which included weight, height, waist and hip circumferences, and body fat percentage in relation to total body weight, was performed by bioelectrical impedance (TBF-300A Body Composition Analyzer, Tanita Corporation, Tokyo, Japan).

Sampling

Venous blood samples were obtained after an overnight fast and a 24-h period of abstinence from alcohol and vigorous physical exercise. Analytical assessment of renal function (plasma creatinine, eGFR, urinary albumin-to-creatinine ratio in a random spot urine collection) was performed. Blood parameters also included fasting glucose, lipid profile, and urea, total protein, albumin, parathormone, alkaline phosphatase, HbA_{1c} and vitamin D concentrations.