The Journal of Obstetrics and Gynaecology

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Journal of Obstetrics and Gynaecology

ISSN: 0144-3615 (Print) 1364-6893 (Online) Journal homepage: https://www.tandfonline.com/loi/ijog20

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To cite this article: Catherine McCormack, Shalem Leemaqz, Denise Furness, Gustaaf Dekker & Claire T. Roberts (2019): Do raised two-hour pre-pregnancy insulin levels confer the same risks of developing GDM, as raised fasting levels, in recurrent miscarriage patients?, Journal of Obstetrics and Gynaecology, DOI: 10.1080/01443615.2019.1672139

To link to this article: https://doi.org/10.1080/01443615.2019.1672139

Published online: 02 Dec 2019.

JOURNAL OF OBSTETRICS AND GYNAECOLOGY
https://doi.org/10.1080/01443615.2019.1672139

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ORIGINAL ARTICLE

Do raised two-hour pre-pregnancy insulin levels confer the same risks of developing GDM, as raised fasting levels, in recurrent miscarriage patients?

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ABSTRACT

This study questioned whether raised pre-pregnancy two-hour (2 h) insulin levels, measured in recurrent embryonic miscarriage (RM) patients via a 75 g Oral Glucose Tolerance Test (OGTT), are associated with an increased risk of gestational diabetes mellitus (GDM) in a subsequent pregnancy. Patients had a 75 g OGTT and insulin levels evaluated ($n = 170$). 54.1% had normal glucose and insulin levels, 45.9% had levels indicating hyperinsulinism (HI). In the 98 patients who achieved a pregnancy, the prevalence of GDM was 3.7% in those without HI, and 35.7% in the patients who only had raised 2 h insulin levels. While HI has been described as a risk factor for miscarriages only in relation to raised fasting (basal) insulin levels, this study demonstrated that raised 2 h insulin levels predict an increased risk of GDM in a subsequent pregnancy. Thus raised 2 h insulin levels likely confer a similar risk to raised fasting insulin levels in RM patients.

IMPACT STATEMENT

- **What is already known on this subject?** Fasting hyperinsulinism is known to be associated with an increased risk of gestational diabetes mellitus (GDM) in pregnancy. Hyperinsulinism, as reflected by the fasting (basal) insulin levels >20mU/L, has been recognized as a risk factor for recurrent miscarriages, particularly in patients with polycystic ovarian syndrome (PCOS), in the World literature. Raised two-hour insulin levels have not been considered as a risk factor in the literature before.
- **What do the results of the study add?** We have demonstrated a 10-fold increase in the development of GDM in patients with fasting insulin resistance, and/or raised 2h insulin levels, and an almost 10-fold increase in patients with only raised 2h levels. 58.8% of the patients who subsequently developed GDM only had raised 2h levels and would have been missed with routine testing.
- **What are the implications of these findings for clinical practice and/or further research?** Our study has demonstrated that GDM was three times more prevalent in the patients with only raised 2h levels, than in those only with raised fasting levels, reflecting insulin resistance/hyperinsulinism. Insulin studies including 2h insulin levels are therefore an important factor to consider when working up these patients. Insulin studies pre-pregnancy may be useful in identifying women at risk of suffering miscarriages or of developing GDM in a subsequent pregnancy.

KEYWORDS

Recurrent miscarriages; hyperinsulinism; insulin resistance; gestational diabetes mellitus

Introduction

Fasting hyperinsulinism is known to be associated with an increased risk of gestational diabetes mellitus (GDM) in pregnancy. Hyperinsulinism, as reflected by the fasting (basal) insulin levels >20mU/L, has been recognized as a risk factor for recurrent miscarriages, particularly in patients with polycystic ovarian syndrome (PCOS) (Boomsma et al. 2008; Glueck et al. 2008; Wang et al. 2011). Some investigators have shown an increased prevalence of insulin resistance in women who suffer recurrent pregnancy losses (Craig et al. 2002). Gestational diabetes mellitus (GDM) and maternal obesity have been shown to be independently associated with adverse neonatal and maternal outcomes, and both share metabolic characteristics such as hyperglycaemia, increased insulin resistance and hyperinsulinaemia (Catalano et al. 2012).

First we found that a significant proportion of our patients undergoing investigations for recurrent miscarriages had markedly raised two-hour (2 h) insulin levels, with normal fasting insulin and normal glucose levels. Given that fasting hyperinsulinism is known to be associated with an increased risk of gestational diabetes mellitus (GDM) in pregnancy, we followed these patients in a subsequent pregnancy to establish if raised 2 h levels did in fact confer as similar a risk of developing GDM as fasting hyperinsulinism. If so, it may be possible that raised two-hour levels are also a risk factor for miscarriages and should be routinely tested in a recurrent miscarriage work-up.

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Methods

Study population

We conducted a prospective cohort observational study as part of the PAPO study. (Prediction of Adverse Pregnancy Outcomes – ACTRN126090002542910).

Patients referred to the recurrent miscarriage clinic from July 2010 until January 2013 were evaluated for possible causes of recurrent miscarriages, including genetic, structural, autoimmune, endocrinologic, thrombophilic, metabolic and lifestyle factors. Patients were included if they had two or more documented (positive bloods tests/ultrasound examinations) losses with the same partner. Written informed consent was obtained from each patient. They were then followed up in a subsequent pregnancy. The study was approved by the Hospital Ethics Committee. (REC1481/6/09).

Data collection

All patients had their tests performed at the same laboratory and were included if all requested tests were performed. A 75 g oral glucose tolerance test (OGTT) was performed after a 10 h fast, and fasting and two-hour glucose and insulin levels were recorded. Demographic information was collected during the face-to-face visits with the physician, and BMI calculated. Patients' body mass index (BMI) was determined as weight divided by the height in metres squared. A BMI of 18–24.9 is regarded as normal, 25–29.9 as overweight and $\geq$30 as obese.

Insulin was measured using the Advia Centaur analytical system. This insulin assay detects recombinant insulin analogues in addition to endogenous insulin (Clinical and Laboratory Standards Institute (CLSI) 2006). The fasting insulin levels accepted as normal by our laboratory are 0–12mU/L. We accepted the glucose:insulin ratio $>$4.5 and insulin levels $<$20 mU/L as normal, and a glucose:insulin ratio $<$4.5 and insulin levels $\geq$20 mU/L as fasting hyperinsulinism. Normal two-hour levels in our laboratory are 10–40 mU/L. In our normal group the mean two-hour insulin levels were 25.88, and so we used three standard deviations from this mean as our cut-off for the diagnosis of two-hour hyperinsulinism 52.6 mU/L, as there are no guidelines in the literature.

GDM was defined as a fasting glucose greater than 5.5 mmol/L, and/or a 2 h level $>$8.0 mmol/L, following a 75 g GTT, at 28 weeks of gestation.

Statistical analysis

Univariate analyses were performed on maternal demographics including age, BMI, previous miscarriages and ethnicity, comparing between women with and without hyperinsulinism. The association between GDM and fasting and two-hour insulin levels was examined using Fishers exact test, with further analysis using Logistic regression adjusted for age, BMI and ethnicity to estimate the odds ratios and corresponding 95% confidence intervals. R version 3.3.2 was used to perform the analyses and results are considered statistically significant at 5% significance level ($p < .05$).

Given that the GDM prevalence in South Australia was 5.8% at the beginning of the study, we calculated that a sample size of 90 patients would be needed to achieve 90% power to detect a doubling of the GDM rate with alpha 0.05. We had 98 patients who went on to achieve pregnancy and live birth.

Results

Overall 182 patients were recruited. Twelve patients did not complete the study, had incomplete results, or were already pregnant at the initial test, and were not included. 170 undertook the required 75 g GTT and completed the study; 44 (25%) of patients did not achieve a pregnancy during the data collection period; 28 (22.2%) had miscarriages, and 98 achieved a live birth (77.8%).

Women were predominantly Caucasian Australians (Caucasian 81.8%, Indian 3.5%, Asian 4.7%, African 1.8% and Middle Eastern 8.2%). The ages ranged from 19 to 45 years, and the numbers of previous losses from 2 to 10 (Table 1).

92 (54.1%) patients were found to have normal glucose and insulin levels. 78 (45.9%) were found to have raised fasting levels and/or 2 h levels and 3 (3.8%) had impaired GTT's. In the group with increased insulin levels (hyperinsulinism/insulin resistance; HI/IR), 8.9% had raised fasting insulin, 23.1% had raised fasting and 2 h levels, and 67.9% had raised 2 h insulin levels only, with normal glucose levels. The normal fasting insulin levels ranged from 0.5 to 19 mU/L, and the raised (hyperinsulinism) levels ranged from 20 to 78 mU/L in the hyperinsulinism group (Figure 1). The 2 h insulin levels ranged from 2.8 to 49 mU/L in the normal group and from 60 to 370 mU/L in the group with hyperinsulinism (Figure 2).

Ninety-eight patients achieved an ongoing pregnancy following the testing. In these pregnancies, in the group of women with raised 2 h insulin levels who did not develop GDM, the insulin levels ranged from 60 to 120 mU/L, and in the group who did develop it, insulin levels ranged from 62 to 170 mU/L (Figure 3). The association between 2 h HI and GDM was significant ($p = .0013$).

There was a clear association between Ethnicity and HI (overall $p = .0026$). Although the numbers in some of the groups were small, nearly 79% of the women of Middle Eastern origin had HI.

Table 1. Demographics of patients attending Recurrent Pregnancy Loss clinic.

RM Clinic	No HI	HI	*p* value
N (%)	92 (54)	78 (46)	–
AGE (years)	22–43	21–45	ns
Mean	33.8	33.7	ns
Miscarriages			
Range	2–10	2–8	ns
Mean	3.20	3.22	ns
BMI (kg/m^2)			
20–24.9	48 (52)	29 (37)	ns
$\geq$25	44 (48)	49 (63)	ns
Ethnicity			0.0026
Caucasian	83 (90)	56 (72)	
Asian	5 (5)	3 (4)	
Middle Eastern	3 (3)	11 (14)	
Indian	1 (1)	5 (6)	
African	0 (0)	3 (4)	

Hi: Hyperinsulinism; ns: non-significant.

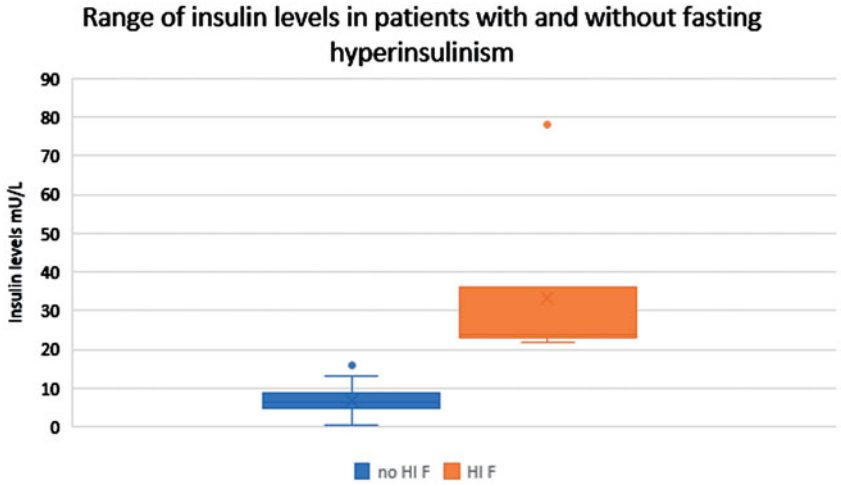

Figure 1. Pre-pregnancy fasting insulin levels in patients with and without hyperinsulinism. Hyperinsulinism was defined as insulin levels >20mU/L or a fasting glucose to insulin ratio <4.5.

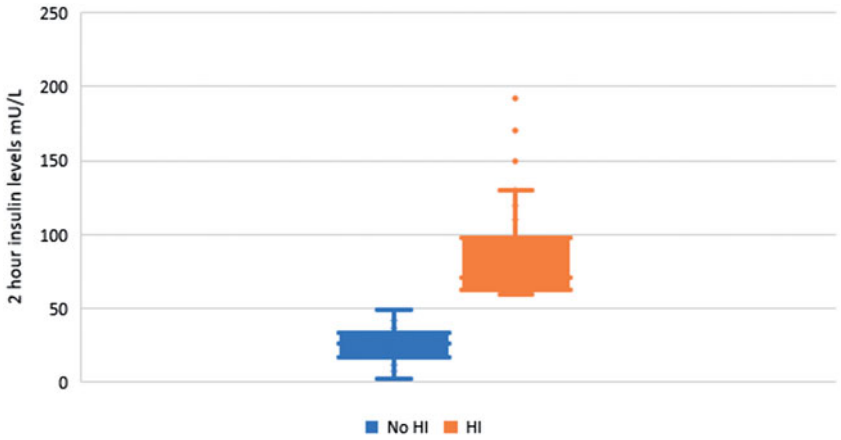

Figure 2. Pre-pregnancy 2-hour insulin ranges in patients with and without hyperinsulinism. Hyperinsulinism was defined in this study as a level >60mU/L.

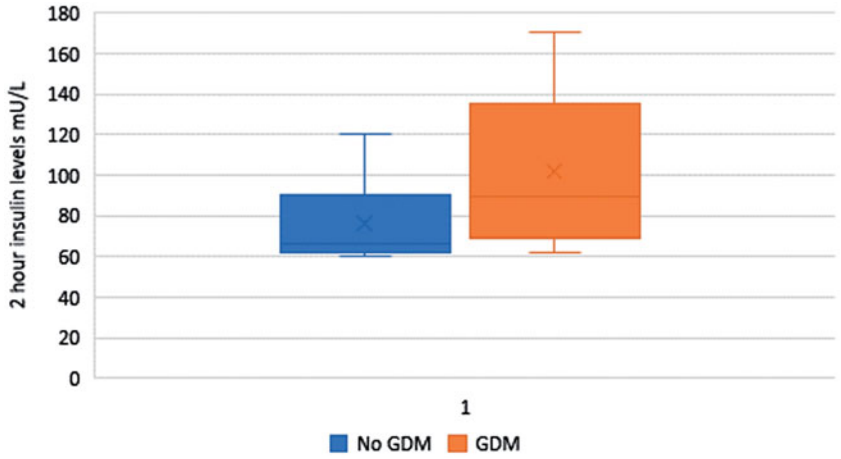

Figure 3. Pre-pregnancy raised 2-hour insulin levels (hyperinsulinism). Women who developed Gestational Diabetes Mellitus in a subsequent pregnancy, versus those with hyperinsulinism who did not.

Table 2 shows the prevalence of GDM in each group. Overall, 38.6% in those with raised fasting and/or two-hour levels developed GDM in a subsequent pregnancy. Of the patients who developed GDM in the subsequent pregnancy after the detection of abnormal insulin levels, 17.7% were the group who only had raised fasting insulin levels, and 58.8% were those who only had raised 2 h levels; 23.5% had both high fasting and 2 h insulin levels. After correcting for age, ethnicity and BMI, the risk of developing GDM with raised fasting or 2 h insulin levels was increased by between 10 and 28-fold (Table 2).

Although we used a conservative three standard deviations from the mean in our normal group to identify a cut-off for our high two-hour insulin group, this could be

Table 2. Associations between hyperinsulinism and Gestational Diabetes Mellitus, in the Recurrent Miscarriage clinic.

Insulin levels	No GDM	GDM	Total	% GDM	% Group	OR (95% CI)	*p*
Normal fasting – normal 2-hour	52	2	54	3.7%	2%	1	–
Normal fasting – high 2-hour	18	10	28	35.7%	10.2%	10.21 (1.81–5.64)	.0085
High fasting – normal 2-hour	2	3	5	60%	7.1%	28.22 (2.55–312.79)	.0065
High fasting – high 2-hour	7	4	11	36.4%	4.1%	11.32 (1.40–91.58)	.0229
TOTAL	79	19	98				

reduced to two standard deviations, which would probably increase the detection rate of HI in women with subsequent GDM.

Discussion

Women who suffer recurrent miscarriage form a heterogeneous group. However, previous reports link insulin resistance with recurrent miscarriage in a sub-set of patients (Wang et al. 2011). We have demonstrated a 10-fold increase in the development of GDM in patients with fasting insulin resistance, and/or raised 2 h insulin levels, and an almost 10-fold increase in patients with only raised 2 h levels. 58.8% of the patients who subsequently developed GDM only had raised 2 h levels and would have been missed with routine testing.

Most of the available literature considers hyperinsulinism to be reflected only by the fasting (basal) levels. Wang et al. (2011) considered the fasting, one, two and three-hour levels, however the patients were in the first trimester of pregnancy. They found a significant difference in both the insulin and glucose levels at one, two and three hours of testing, even when they excluded patients with polycystic ovarian syndrome (PCOS). They concluded that there is an increased incidence of insulin resistance in recurrent miscarriage patients, in the first trimester of pregnancy, and suggested that only testing for fasting insulin and glucose in recurrent miscarriage patients, would be inadequate to demonstrate insulin resistance (Wang et al. 2011).

Jakubowicz et al. (2004) found that hyperinsulinaemia led to reduced concentrations of insulin-like growth factor binding protein-1 (IGFBP-1) and glycodelin in early pregnancy, thereby increasing the chance of miscarriages. Glycodelin is an immunomodulatory protein involved in implantation, and Glycodelin -A is found in abundant levels in the decidua in early pregnancy. It plays an important role both in placental development and fetomaternal defence. Abnormal levels are associated with unexplained infertility and recurrent pregnancy loss (Glueck et al. 2008). Interestingly, insulin can negatively regulate the concentrations of glycodelin and IGFBP-1, and this may be the mechanism by which it increases the risk of miscarriages.

Hyperinsulinemia may also increase the level of plasminogen activator inhibitor-1 and induce villous thrombosis, thereby reducing the blood supply to the placenta and leading to trophoblastic hypoplasia, resulting in miscarriage (Gordon et al. 1995).

Lee et al. (2016) hypothesised that HI could cause an uncontrolled diabetic-like state in the fetal environment resulting in increased first trimester losses. High insulin levels have been shown *in vitro* to increase the transport of glucose by first trimester cytotrophoblasts independent of glucose level probably by upregulation of the GLUT1 glucose transporter system (Lee et al. 2016).

In conclusion, our study has demonstrated GDM was three times more prevalent in recurrent miscarriage patients with raised 2 h insulin levels than in those with raised fasting levels, reflecting insulin resistance/hyperinsulinism. Insulin studies including 2 h levels are an important factor to consider when working up these patients. This finding needs to be replicated in other populations. A full work-up should be performed on all patients, and all presumed 'causes/associations' of miscarriages addressed. Insulin studies pre-pregnancy may be useful in identifying women at risk of having miscarriages or developing GDM in a subsequent pregnancy and therefore identify women who may benefit from early intervention, such as diet and life style interventions.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

CTR was supported by a National Health and Medical Research Council of Senior Research Fellowship ([GNT1020749).]. (http://www.nhmrc.gov.au).

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