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RESEARCH LETTER

Evaluation of insulin antibodies and placental transfer of insulin aspart in pregnant women with type 1 diabetes mellitus

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Abbreviations
HI human insulin
IAsp insulin aspart

To the Editor: The relevance of insulin antibodies to neonatal outcome in pregnant women with diabetes remains unclear [1–3]. In one study of 138 pregnant women with type 1 diabetes, insulin antibodies were detected in the cord blood of 95% of the offspring at birth [3]. However, there was no evidence that insulin antibodies caused the transfer of insulin across the placental barrier or influenced birthweight.

To date, no study has investigated insulin antibody levels in pregnant women with type 1 diabetes treated with an insulin analogue. In a recent randomised controlled trial we showed that prandial insulin aspart (IAsp), a rapid-acting insulin analogue, was as effective and well tolerated as soluble human insulin (HI) in pregnant women with type 1 diabetes [4, 5]. The aim of the present study was to assess maternal and cord blood insulin antibody levels in a subset of 97 women who participated in the trial [4, 5]. The evaluation was described in a post-initiation protocol amendment.

Methods

The study was performed in accordance with the Declaration of Helsinki and was approved by respective ethics committees and health authorities according to local regulations. Written informed consent was obtained from

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Table 1 Median levels of maternal blood serum insulin antibodies at baseline and gestational week 36

Treatment	Antibody	Maternal blood insulin antibody levels					
		Baseline		Gestational week 36		Change[a]	
		n	Median (%), range	*n*	Median (%), range	*n*	Median (%), range
IAsp + NPH	IAsp-specific	34	0.3 (−0.3–60.5)	46	0.2 (−0.3–45.0)	30	−0.1 (−15.5–0.3)
	HI-specific	34	−0.1 (−1.2–5.6)	46	−0.1 (−0.8–5.9)	30	0.0 (−0.8–0.4)
	Cross-reacting	34	6.9 (0.0–60.3)	46	3.7 (0.2–60.7)	30	−2.2 (−25.3–12.6)
HI + NPH	IAsp-specific	33	0.3 (−0.4–5.8)	37	0.3 (−0.3–33.3)	23	−0.1 (−2.1–2.9)
	HI-specific	33	−0.1 (−0.5–12.6)	37	0.0 (−0.5–13.5)	23	0.0 (−2.8–1.0)
	Cross-reacting	33	8.6 (−0.8–60.1)	37	4.1 (0.3–52.3)	23	−2.7 (−24.9–11.4)

[a] From baseline to gestational week 36
NPH, neutral protamine Hagedorn

participants before study entry. Sampling of maternal blood was performed at baseline and at gestational week 36 for the determination of maternal insulin antibodies (HI-specific, IAsp-specific and cross-reacting). Immediately after delivery, umbilical cord blood samples were obtained and analysed for specific and cross-reacting insulin antibodies (n=65) and serum insulin (IAsp and HI) (n=30).

Laboratory analyses Antibody analyses were performed by MDS Pharma Services (Fehraltorf, Switzerland) using a RIA method [6]. The results are expressed as the percentage of bound radioactivity relative to the total. Because of intra-assay variation, it is possible that samples with no or low levels of antibodies result in a negative value as a result of the subtraction of values from two assay series.

Insulin analysis was performed by Capio Diagnostik (Copenhagen, Denmark). Serum HI was measured by a commercially available two-site HI-specific ELISA (Dako, Glostrup, Denmark). Serum IAsp was measured by a two-site specific ELISA developed by Novo Nordisk. This assay is appropriate for measurements of IAsp at physiological concentrations (0–1,000 pmol/l) without cross-reactivity with HI. The total coefficient of variation for this assay is 14.7% [7] and the detection limit is 5.3 pmol/l; levels below this value were set to zero. Insulin assays measured total insulin. A number of samples had to be discarded. This was mainly due to haemolysis, particularly of the insulin samples (40%), and loss of sample stability as a result of late shipping (20% of the cord blood samples).

A total of 35 and 29 of the offspring whose mothers were treated with IAsp and HI, respectively, had valid cord blood insulin antibody measurements, whereas only 13 and 16 had valid cord blood insulin measurements. One participant treated with IAsp had cord blood measurements of 10,950 pmol/l for HI and 22.9 pmol/l for IAsp. As this unphysiologically high HI value suggested possible contamination, the result was considered invalid and all cord blood data for this participant were excluded from further analysis.

Statistics The development of maternal cross-reacting insulin antibodies, the relationship between cord blood and maternal cross-reacting insulin antibodies, and the relationship between birthweight and cord blood HI levels were analysed using linear regression. All values were logarithmically transformed. Treatment differences in cord blood HI levels were analysed using an ANOVA model with treatment as a fixed effect.

Table 2 Median levels of cord blood insulin antibody levels and cord blood insulin levels at delivery

Treatment	Antibody	Cord blood insulin antibody levels		Cord blood insulin levels at delivery			
				IAsp		HI	
		n	Median (%), range	*n*	Median (pmol/l), range	*n*	Median (pmol/l), range
IAsp + NPH	IAsp-specific	35	0.1 (−0.4–16.9)	13	0.0 (0.0–0.0)	12	67.5 (11.0–709)
	HI-specific	35	−0.02 (−0.3–1.7)				
	Cross-reacting	35	2.3 (0.2–34.0)				
HI + NPH	IAsp-specific	29	0.2 (−0.3–3.2)	16	0.0 (0.0–0.0)	16	286.5 (16.0–1,930)
	HI-specific	29	0.1 (−0.3–3.8)				
	Cross-reacting	29	4.4 (−0.0–44.2)				

NPH, neutral protamine Hagedorn

Results

Levels of IAsp-specific antibodies and HI-specific antibodies were low at baseline and remained low during pregnancy, with similar levels observed between treatment groups at gestational week 36 (Table 1). A small but significant decrease in cross-reacting insulin antibodies was observed during pregnancy: the estimated regression coefficient of cross-reacting insulin antibodies at baseline (for the two groups together) was 0.8266, which was significantly different from 1 (p=0.0120, R^2=0.7612). There was no treatment effect (p=0.9984). Duration of diabetes, pre-study use of IAsp or biphasic IAsp, and insulin dose were not correlated with level of cross-reacting antibodies at gestational week 36 in either treatment group (data not shown). There was a significant correlation between the level of cross-reacting insulin antibodies in cord blood and maternal cross-reacting insulin antibodies at gestational week 36 (regression coefficient=0.9271, $p<$ 0.0001, R^2=0.9067), but no treatment effect (p=0.9248). Two participants in the HI group had high levels of cord blood HI (1,150 and 1,930 pmol/l), but antibody levels were similar to the median levels observed in the cohort (Table 2). Sensitivity analyses excluding data from these two cord blood samples were performed for all analyses, with similar results. There was no correlation between birthweight and cord blood HI (p=0.1590). IAsp was undetectable in the cord blood of all participants, including four individuals who received an i.v. infusion of IAsp during delivery (Table 2). There was no detection of IAsp in the umbilical cord blood of participants with high levels of insulin antibodies.

Discussion

Our results, which suggest that insulin antibodies do not develop during pregnancy when using either IAsp or HI, are reassuring and comparable to previous data involving non-pregnant participants with type 1 and type 2 diabetes [6] and those with gestational diabetes mellitus treated with IAsp or insulin lispro [8, 9]. Overall, there was a small reduction in cross-reacting insulin antibodies in the IAsp group, perhaps similar to the decrease in levels seen with other types of antibody during pregnancy, such as serum antithyroglobulin [10]. The correlation between the level of maternal cross-reacting insulin antibodies and those in umbilical cord blood in the offspring is indicative of a transfer of maternal cross-reacting insulin antibodies across the placental barrier; however, there was no evidence to support the transfer of IAsp across the placenta.

This dataset on insulin antibodies in pregnancy is one of the largest reported to date and, we believe, provides new information on the use of insulin analogues in the management of type 1 diabetic pregnancy.

Trial registration: ClinicalTrials.gov NCT00365170

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Duality of interest D. R. McCance, M. Hod, R. Kaaja and F. Dunne, have no dual interests to declare. P. Damm has received honoraria from Novo Nordisk. E. R. Mathiesen has received honoraria, grants and lecture fees from Novo Nordisk, and lecture fees from Aventis. L. E. Jensen is an employee of and shareholder in Novo Nordisk. H. Mersebach is an employee of Novo Nordisk.

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