Journal of Diabetes and Its Complications 33 (2019) 530–534

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Retinal vessel diameters confound the relationship of pregnancy to retinopathy and infant outcomes in T1D

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ARTICLE INFO

Article history:
Received 28 February 2019
Received in revised form 2 May 2019
Accepted 15 May 2019
Available online 23 May 2019

Keywords:
Diabetic retinopathy
Type 1 diabetes
Pregnancy
Retinal vessel diameters
Blood pressure
Severe infant abnormalities

ABSTRACT

Objectives: To determine whether: 1) retinal vessel diameters in pregnant and non-pregnant women with type 1 diabetes (T1D) alter the relationship of pregnancy to severity of diabetic retinopathy (DR); and 2) retinal vessel diameters in early pregnancy alter the relationship of severity of DR in the mother to severe adverse outcome in the infant.

Methods: Two cohorts of women with T1D, one composed of pregnant women and the other of non-pregnant women of child-bearing age, were recruited in Wisconsin. Baseline examinations (including retinal photography and collection of diabetes-related characteristics) were conducted, with follow-up approximately one year later. Retinal images were graded according to the modified Airlie House classification protocol, and retinal vessel diameters were measured from digitized images. The latter were included in analyses as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE).

Results: In multivariate models: 1) Pregnancy was significantly associated with both incidence (OR = 4.43, CI = 1.42–13.79) and progression (OR = 2.62, CI = 1.52–4.51) of DR. Neither CRAE nor CRVE were significant, but their addition modestly altered the associations of pregnancy to worsening DR; 2) Baseline retinopathy (OR = 1.28, CI = 1.05–1.57) was associated with severe adverse outcome in the infant. This association was unchanged by adjustment for retinal vessel diameters.

Conclusions: Pregnancy is associated with worsening DR in women with T1D. DR severity in early pregnancy is associated with severe adverse outcome in the infant. The retinal vessel diameters CRAE and CRVE were not associated with these outcomes but were modest confounders of the association of pregnancy to worsening DR.

1. Introduction

Diabetic retinopathy (DR) is a complication of diabetes usually first observable after about 5 years of diabetes. Severity tends to increase thereafter related to duration of diabetes, glycemic control, and blood pressure.[1–3] In addition, retinal vessel diameters have been associated with DR such that wider retinal venules are related to increased severity of DR and macular edema.[4] Pregnancy during the course of type 1 diabetes (T1D) is also associated with increased severity of DR.[5]

It is known that pregnancy is associated with vasodilation that may be related to endothelial function.[6–8] Further, placentas from diabetic pregnancies appear to have pathologic vascular changes.[9] Specifically, microvascular placental changes including chorangiosis (increased villous capillaries and thickening of blood vessel walls), edema of the villi, and thickened basement membranes have been noted in the placentas of patients with T1D.[10] It is possible that microvascular caliber changes observed in the retina in pregnancy may be biomarkers of pathology in placental circulation that are themselves associated with severe adverse outcomes in the infant.

The purpose of this investigation is to examine two different research questions: 1) Do retinal vessel diameters alter the association of pregnancy to the incidence and progression of DR at follow-up in women with T1D; 2) Do retinal vessel diameters, aside from baseline retinopathy level, further inform the odds of a severe adverse outcome in the infant of a mother with T1D?

2. Methods

2.1. Participants

Between 1982 and 1986, 190 pregnant women were recruited from high-risk pregnancy clinics associated with hospitals in Madison,

Conflicts of interest: None of the authors has any conflict of interest to disclose.

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https://doi.org/10.1016/j.jdiacomp.2019.05.012
1056-8727/

Neenah, and LaCrosse, Wisconsin. Of these, 158 were confirmed to have T1D and completed an initial evaluation within the first 20 weeks of gestation. These women were considered for analysis of severe adverse infant outcomes.

A comparison group of 279 women was recruited from participants identified for the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) who had T1D and were women of child-bearing age but were not pregnant at the time of recruitment. These two groups form the basis for analyses of incidence and progression of DR.

The groups were compared with regard to characteristics considered to be potential risk factors or confounders of the association between our predictors and outcomes. There were no significant differences in the presence of proteinuria, presence of DR, or retinal vessel diameters. There were significant differences in duration of diabetes, age, diastolic blood pressure, and total glycated hemoglobin (Table 1).

2.2. Protocols

All participants had a baseline evaluation as soon as possible following recruitment, at which time consent conforming to the tenets of the Declaration of Helsinki was obtained from participants following Institutional Review Board approval from the Human Subjects Committee of the University of Wisconsin-Madison. The study operations were performed in adherence to the Health Insurance Portability and Accountability Act. Examiners explained the study rationale and nature of the examinations before collecting pertinent information including current age, age at diagnosis, smoking history, and other health information via a standardized questionnaire. Blood pressure was measured according to the Hypertension Detection and Follow-Up Program protocol.[11] A blood sample was obtained from which total glycated hemoglobin was determined. Stereoscopic film fundus photographs of seven standard fields were taken for both eyes after dilation of the pupils. These photographs were graded for the presence and severity of DR according to a six-level modification of the Airlie House classification of DR,[12] in which levels are defined as follows:

- Level 10: no retinopathy
- Level 20: microaneurysms or blot hemorrhages only
- Level 30: microaneurysms and one or more of the following: retinal hemorrhages, but total of hemorrhages and microaneurysms less than those in standard photograph 2A of the Diabetic Retinopathy Study; hard exudates less than standard photograph 3; soft exudates questionably present; intraretinal microvascular abnormalities questionably present; venous beading questionably present; venous loops definitely present
- Level 40: microaneurysms and one or more of the following from definition of level 50 not met: hemorrhages and microaneurysms greater than or equal to standard photograph 2A; hard exudates greater than or equal to standard photograph 3; soft exudates definitely present; intraretinal microvascular abnormalities definitely present; venous beading definitely present; larger venous loops or reduplication definitely present
- Level 50: DRS Group 3, which we describe roughly as any 3 of the following in fields 4–7: hemorrhages and microaneurysms equaling or exceeding those in standard photograph 2A in at least two fields; soft exudates definitely present in >2 fields; intraretinal microvascular abnormalities definitely present in >2 fields; venous beading definitely present in >2 fields; intraretinal microvascular abnormalities present in 4 fields and greater than or equal to standard photograph 8A in >2 fields
- Level 60: proliferative diabetic retinopathy

Fundus photographs were digitized into a lossless file format. Retinal vessel diameters were measured from the digitized images by trained graders who were masked to subject characteristics. Retinal arterioles and venules located 0.5 to 1.0 optic disc diameters from the disc margin were measured using a semi-automated computer program (IVAN, University of Wisconsin-Madison; Ferrier, NJ). Following a standard protocol, the grader determined each vessel to be an arteriole or venule using the original color photographs for reference. On average, nine arterioles and nine venules were measured per eye. Retinal vessel measurements were summarized using measurements from the six largest arterioles and six largest venules to produce the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE), respectively.[13]

Information about health of the infants was abstracted from hospital delivery charts and the subsequent hospital stay. Information varied among the hospitals and practitioners but in most cases included delivery type, gestational age, birth weight, birth length, birth heart rate, resuscitation status, and Apgar scores at 1 and 5 min. Congenital anomalies were recorded in the delivery suites or at some time prior to discharge. Diagnoses were coded according to the ICD 9.[14] Autopsy reports were reviewed when available for stillborn infants and for those infants who were born alive but did not survive the initial hospitalization. Maternal report was accepted as evidence of abortion.

Participants were invited to complete a follow-up examination approximately a year later (postpartum, for those who had been pregnant), where the same examination and photography protocols were followed.

2.3. Definitions

Severe adverse outcomes for the infant are listed in Supplemental Table 1. Duration of diabetes was defined as the period between the reported age at diagnosis and the baseline visit. A participant was considered a current smoker if she had smoked >100 cigarettes in her lifetime and was currently smoking. The presence of DR in an eye was defined as level 20 or higher on the modified Airlie House classification scale. Incidence of DR was defined as an increase from level 10 at baseline to level 20 or higher by the follow-up examination. Progression of DR was defined as an increase of one or more step on the modified Airlie House classification scale between baseline and the follow-up examination. Concatenated retinopathy score was based on the maximum retinopathy level in the two eyes, assigning a higher score when a person's

Table 1
Characteristics of pregnant and non-pregnant participants at the baseline examination.

Measure	Pregnant		Non-pregnant		
	N	Mean (SD) or N (%)	N	Mean (SD) or N (%)	P
Duration of diabetes, yrs	158	11.99 (7.15)	279	14.34 (8.07)	0.002
Age, yrs	158	26.87 (4.66)	279	29.06 (7.54)	<0.001
Systolic blood pressure, mm Hg	148	116.82 (14.80)	274	119.49 (16.90)	0.105
Diastolic blood pressure, mm Hg	148	69.13 (10.86)	274	79.06 (11.37)	<0.001
Total Glycated hemoglobin, %	136	8.27 (2.06)	264	11.70 (2.80)	<0.001
Present proteinuria	136	25 (18.38%)	259	52 (20.08%)	0.644
Mean CRAE in both eyes, μm	135	165.41 (14.88)	241	166.35 (16.04)	0.574
Mean CRVE in both eyes, μm	135	247.64 (25.28)	243	250.07 (23.72)	0.351
Present DR in one or both eyes	143	106 (74.13%)	273	209 (76.56%)	0.487

other eye was at the same level and a lower score when the other eye was at a lower level (e.g. concatenated scores −10/10, 20/<20, 20/20, 30/<30, 30/30 etc.).[15]

2.4. Statistical analyses

Baseline characteristics of pregnant and non-pregnant women were compared using standard univariate logistic regression for binary characteristics and linear regression for continuous characteristics. The study data are derived from study visits approximately one year apart, during the first trimester (baseline) and post-partum in the pregnant group and at two visits a year apart for the non-pregnant group.

Two primary analyses were performed. For each, a series of standard logistic regression models were fit. An initial model was built including the risk factor of interest and covariates chosen by backward selection criteria. Covariates which were not significant predictors but altered the modeled relationship between the factor of interest and the outcome were included as confounders. Retinal vessel diameters were then added as part of a second model. Where appropriate, baseline retinopathy level was added as part of a third model.

The first primary analysis examined the association of pregnancy with incidence and progression of DR, considering duration of diabetes, glycated hemoglobin level, and systolic blood pressure as potential covariates. Each eye was modeled using generalized estimating equations to account for correlation between the eyes. A secondary analysis was performed in which progression of DR was redefined as a two-level increase on the modified Airlie House classification scale.

The second primary analysis examined the association of retinopathy in the mother with severe adverse outcome in the infant, considering maternal characteristics at the baseline examination (age, duration of diabetes, smoking status, total glycated hemoglobin level, and systolic blood pressure) as possible covariates. Concatenated baseline retinopathy score was used to represent retinopathy in the mother, and CRVE and CRAE were each averaged across the right and left eye to represent retinal vessel diameters in the mother. Procedures were performed using SAS software (SAS Institute Inc., Cary, NC, Version 9.4).

3. Results

3.1. Retinopathy outcomes

Of the 437 women considered for analysis (158 pregnant, 279 non-pregnant), 23 were excluded due to missing baseline retinopathy data in both eyes, 66 because both eyes were already at level 60 on the modified Airlie House classification scale and were therefore ineligible for change (either incidence or progression) of DR, and 5 more due to missing retinal vessel diameter data. Of the 343 remaining participants, 36 were excluded due to missing retinopathy data at the follow-up examination. This left 307 participants for analysis, of which 106 (34.5%) were pregnant. These 307 women contributed up to 583 eye-intervals for analysis.

3.2. Incidence of DR

Of the 583 eye-intervals considered for analysis, 382 were excluded because the eye in question was already at level 20 or higher on the modified Airlie House classification scale at baseline and thus ineligible for incidence of DR. Of the remaining 201 eye-intervals, 74 belonged to pregnant women, 18 of which developed DR (24.3%), and 127 belonged to non-pregnant women, 32 of which developed DR (25.2%). While adjusting for duration of diabetes and glycated hemoglobin (Table 2, Model 1), pregnancy was significantly associated with increased odds of incidence of DR (OR = 4.13, CI = 1.31–12.98). When retinal vessel diameters were added (Table 2, Model 2), the association of pregnancy with incidence of DR was modestly strengthened (OR = 4.43, CI = 1.42–13.79). This amounted to a 4.9% change in the log odds. Retinal vessel diameters were not themselves associated with incidence of DR.

3.3. Progression of DR

All 583 eye-intervals were included when modeling progression of DR, of which 205 belonged to pregnant women, with 65 experiencing progression of DR (31.7%), and 378 belonged to non-pregnant women, with 114 experiencing progression of DR (30.2%). While adjusting for duration of diabetes and glycated hemoglobin (Table 3, Model 1), pregnancy was significantly associated with increased odds of progression of DR (OR = 2.38, CI = 1.37–4.12). When retinal vessel diameters were added to the model (Table 3, Model 2), the association of pregnancy with progression of DR was strengthened (OR = 2.62, CI = 1.52–4.51), amounting to an 11.2% change in the log odds. When baseline retinopathy level was added (3, Model 3), the association was further strengthened (OR = 3.09, CI = 1.68–5.69).

In a secondary analysis, progression of DR was redefined as an increase of two or more levels on the modified Airlie House classification scale (Supplemental Table 2). Of the 583 eye-intervals considered for analysis, 581 were eligible for progression under this stricter definition, and progression was experienced in only 28 (13.7%) eyes among pregnant women and 44 (11.7%) eyes among non-pregnant women. Despite having fewer cases of progression in both groups, the model estimates were similar to those for the one-step definition of progression, with an OR of 2.88 in pregnant women while adjusting for duration of diabetes and glycated hemoglobin and a strengthening of the association while also adjusting for retinal vessel diameters and baseline retinopathy level.

3.4. Infant outcomes

Of the 158 well-documented pregnancies considered for analyses, 17 pregnancies were excluded for missing or ungradable baseline concatenated retinopathy data and 9 were excluded for missing retinal vessel diameter data. Among the 132 pregnancies contributing to analysis, 22 (16.7%) resulted in still births, spontaneous abortions, or babies with severe adverse outcomes. Mean CRVE was wider in mothers of infants with severe anomalies, but in this reduced sample the difference

Table 2
Multivariate models for incidence of DR.

Predictor	Model 1: adjusted for significant covariates and confounders		Model 2: adjusted for retinal vessel diameters	
	OR (95% CI)	P	OR (95% CI)	P
Pregnancy status	4.13 (1.31, 12.98)	0.015	4.43 (1.42, 13.79)	0.010
Duration of diabetes, per 5 yrs	2.49 (1.48, 4.20)	<0.001	2.44 (1.44, 4.14)	<0.001
Total Glycated hemoglobin, per 1%	1.28 (1.11, 1.47)	<0.001	1.30 (1.13, 1.49)	<0.001
CRAE, per 10 μm			1.21 (0.86, 1.71)	0.267
CRVE, per 10 μm			0.98 (0.78, 1.24)	0.897

Table 3
Multivariate models for progression of DR.

Predictor	Model 1: adjusted for significant covariates and confounders		Model 2: adjusted for retinal vessel diameters		Model 3: adjusted for baseline retinopathy level	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Pregnancy status	2.38 (1.37, 4.12)	0.002	2.62 (1.52, 4.51)	<0.001	3.09 (1.68, 5.69)	<0.001
Duration of diabetes, per 5 yrs	1.14 (0.98, 1.33)	0.078	1.13 (0.97, 1.32)	0.115	1.39 (1.12, 1.71)	0.002
Total Glycated hemoglobin, per 1%	1.22 (1.13, 1.33)	<0.001	1.23 (1.13, 1.34)	<0.001	1.25 (1.14, 1.37)	<0.001
CRAE, per 10 μm			1.11 (0.93, 1.32)	0.247	1.05 (0.89, 1.25)	0.551
CRVE, per 10 μm			1.06 (0.95, 1.18)	0.295	1.11 (1.00, 1.24)	0.051
Baseline retinopathy, per 1 level					0.63 (0.48, 0.82)	<0.001

between the two groups of mothers was not significant. Higher baseline concatenated retinopathy level was associated with increased odds of having a severe adverse outcome in the infant (OR = 1.27, CI = 1.06–1.52). When retinal vessel diameters were added to that model this relationship was largely unaffected (Table 4).

4. Discussion

This investigation builds upon a prior study of the association of pregnancy in women with T1D to DR in the mother and of baseline retinopathy level to severe adverse outcomes in the fetus.[5,16] The purpose of these analyses is to expand that investigation to include additional characteristics of the retinal vasculature in women with T1D to the same outcomes as originally described. Specifically, the current aims are to determine whether retinal vessel diameters provide additional markers of incidence or progression of DR during pregnancy and to evaluate whether retinal vessel diameters influence the odds of a severe adverse outcome in infants of diabetic mothers.

While adjusting for important risk factors such as duration of diabetes and glycated hemoglobin, there were differences in incidence and progression of DR between pregnant and non-pregnant women. There was some evidence that retinal vessel diameters provided additional information about risk. Namely, retinal vessel diameters were found to be modest confounders of the relationship between pregnancy and progression of DR. Measurement of retinal vessel diameters, currently performed by specially designed computer programs with trained grader oversight, may be further refined to minimize human oversight while improving efficiency and thus reducing cost and permitting improved estimates of risk to pregnant T1D women.

In our study, retinal vessel diameters do not provide independent information beyond baseline retinopathy status for risk of adverse outcomes in the infant. However our sample size to test this possibility was small. A larger sample would be desirable to further test the utility of the measure of retinal vessel diameters to quantitate the use of these measures for prognosis of infants of mothers with T1D.Thus, the findings from the investigations described here are in concert with previously reported findings, which were done prior to the measurement of central retinal arteriolar and venular equivalents.

This study has several limitations. Care patterns were not uniform throughout the pregnancy and in the postpartum follow-up period. This likely affected our information about retinopathy as well as the infant outcome. Participants were mainly of European descent, and this ethnic uniformity may affect generalizability. In addition, there may have been differences in health care between the pregnant and non-pregnant groups. Guidelines, based on information from the Diabetes Control and complications Study and from the Wisconsin Epidemiologic Study of Diabetic Retinopathy, for levels of glycemia are currently lower than they were when this study was undertaken in all persons with T1D. Nevertheless there are more current data to suggest that severe adverse infant outcomes in infants of mothers with T1D are still relatively common.[17] Further studies on women with T1D aimed at decreased microvascular and other complications of T1D should be a health care priority for future medical research.

Acknowledgements

Author contributions

B.E.K.K. and K.M.L.H. analyzed the data and wrote the manuscript. S.M.M. supervised a component of the data collection and reviewed and revised manuscript. A.E.M. and A.F.E. scanned the images, performed the vessel measurements and graded image quality. L.G.D. provided data management review and oversight. K.E.L. assisted with data analysis and reviewed and revised the manuscript. R.K. reviewed and revised the manuscript. B.E.K.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Will Kelly of the Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison School of Medicine and Public Health, assisted with the technical editing and preparation of the manuscript. He received no additional compensation beyond his normal wages as an employee of the University of Wisconsin-Madison for his assistance.

Disclaimer

The content is solely the responsibility of the authors. The funding organizations had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Table 4
Multivariate models for infant outcome.

Predictor	Model 1: adjusted for significant covariates and confounders		Model 2: adjusted for retinal vessel diameters	
	OR (95% CI)	P	OR (95% CI)	P
Concatenated retinopathy score, per 1 unit	1.27 (1.06, 1.52)	0.011	1.28 (1.05, 1.57)	0.016
Duration of diabetes, per 5 yrs	1.18 (0.74, 1.87)	0.484	1.19 (0.74, 1.92)	0.465
Total Glycated hemoglobin, per 1%	1.15 (0.87, 1.53)	0.320	1.16 (0.87, 1.54)	0.311
Mean CRAE in both eyes, per 10 um			1.19 (0.69, 2.06)	0.534
Mean CRVE in both eyes, per 10 um			1.01 (0.74, 1.38)	0.961

Funding/financial support

This study was supported by grant EY03843 and EY016379 (to B.K., and R.K.) from the National Institutes of Health, Bethesda, MD, and an unrestricted grant from Research to Prevent Blindness, New York, NY. The sponsors or funding organizations had no role in the design or conduct of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2019.05.012.

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