Prediction of Proliferative Diabetic Retinopathy With Hemoglobin Level

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**Objective:** To investigate the role of hemoglobin (HGB) level in predicting proliferative diabetic retinopathy (PDR).

**Methods:** We assessed 426 individuals without PDR at baseline (213 men; 213 women) from the Pittsburgh Epidemiology of Diabetes Complications Study, an 18-year prospective cohort study of childhood-onset type 1 diabetes. Presence of PDR was determined by stereo fundus photography. Cox proportional hazards modeling with stepwise regression was used to determine the independent association of HGB level with PDR. Analyses were sex specific.

**Results:** There were 206 events. Although the incidence of PDR did not vary by sex (48% in both men and women), in men, HGB exhibited a positive linear relationship with 18-year incidence of PDR (hazard ratio, 1.33; 95% confidence interval, 1.10-1.60; \( P = .003 \)), while in women, HGB level exhibited a quadratic relationship with PDR (\( P < .001 \)). After multivariable adjustment for univariately significant covariates, HGB level remained significantly predictive of PDR in both men (\( P = .004 \)) and women (\( P = .04 \)).

**Conclusion:** Higher HGB level predicts the incidence of PDR in type 1 diabetes mellitus, though the association varies by sex, being linear and positive in men and quadratic in women.


**PROLIFERATIVE DIABETIC RETINOPATHY (PDR) is a leading cause of blindness in the United States and has been linked to retinal hypoxia and characterized by small blood vessel proliferation, retinal microaneurysms, and vitreous hemorrhaging.** Lower hematocrit (HCT) levels have been associated with the progression of diabetic kidney disease and with the incidence of PDR, suggesting that anemia may increase complication risk. In contrast, however, stabilization of retinopathy has also been reported in those with end-stage renal disease, a condition associated with anemia. The association between hemoglobin (HGB) and retinopathy is likely, therefore, to be complex, particularly given the recent observation of unusually high HGB levels, reaching 18.8 g/dL, in study participants with nephropathic type 1 diabetes mellitus.

Hemorheological factors are altered in diabetes, and there is some evidence that these disturbances may also be associated with diabetic retinopathy. Increased HCT and blood viscosity have been observed cross-sectionally in those with diabetic retinopathy. Elevated erythropoietin has also been observed in the vitreous fluid of those with diabetic retinopathy and found to be an angiogenic promoter of PDR.

Given these observations, we decided to investigate the association of HGB with the incidence of PDR in type 1 diabetes mellitus.

**METHODS**

The Pittsburgh Epidemiology of Diabetes Complications (EDC) study is an 18-year prospective study based on a well-defined cohort with childhood-onset (age <17 years) type 1 diabetes living within 100 miles of the University of Pittsburgh at study baseline. The 658 participants (325 female, 333 male; 67% of all those eligible) were diagnosed between January 1, 1950, and May 30, 1980, at Children’s Hospital of Pittsburgh, and first seen in EDC between 1986 and 1988, when the mean age and diabetes duration were 28 and 19 years, respectively. They were then seen biennially for 10 years, after which they were followed up by survey with examinations limited to certain subgroups. Between 2004 and 2007, however, an 18-year follow-up was conducted for all participants. The design and methods of the study have been previously described.

Before attending each cycle of examinations, information was collected by questionnaire concerning demographic characteristics.
Fasting blood samples were assayed for lipids, lipoproteins, glycosylated hemoglobin (HbA1c) and HGB. Hemoglobin was measured using the Coulter Counter Model S-Plus IV (Coulter Electronics, Hialeah, Florida) automated blood cell counter. High-density lipoprotein cholesterol level was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method.\(^9\) Cholesterol was measured enzymatically. Glomerular filtration rate was estimated using the modified Arlie House System,\(^17\) and clinically significant retinopathy was diagnosed and documented by a retinal specialist if required. Those with panretinal photocoagulation (as a quadratic term also remained significantly predictive of PDR after 18 years. The median time to follow-up was 14.6 years. In men, however, the Wald test showed a significant deviation from linearity (Wald test of linearity \(P = .15\)); therefore, HGB was entered into Cox models as a continuous linear covariate. In women, however, the Wald test showed a significant deviation from linearity (\(P = .02\)) and a graph of the restricted cubic spline suggested a quadratic relationship between HGB and the log hazard ratio (Figure); therefore, HGB was entered into Cox models as a quadratic term. Univariately, HGB showed a significant linear association with the risk of developing PDR in men (\(P = .003\)) and a significant nonlinear association in women (\(P = .008\), quadratic term).

In multivariable analyses allowing for univariately significant covariates, HGB remained significantly predictive of PDR in men (hazard ratio,\(1.29; 95\%\) confidence interval, 1.08-1.54; \(P = .004\)) (Table 3). In women, HGB as a quadratic term also remained significantly predictive of PDR (\(P = .04\)). Other significant multivariable predictors included HbA1c, and diastolic blood pressure in both sexes, albumin excretion rate in men, and systolic blood pressure and fibrinogen in women. Finally, the association of HGB with other components (markers) of retinopathy was also explored. A significant prediction of both macular edema (\(P < .001\) and \(P = .03\) for men and women, respectively) and 2-step progression (\(P = .03\) and \(P < .001\) for men and women, respectively) was observed.

The cumulative incidence of PDR in this population was 48% after 18 years. The EDC incidence rates are similar to those observed in the DCCT/Epidemiology of Diabetes Interventions and Complications conventional treatment group of similar diabetes duration (49.7% at 30 years...
of diabetes duration\textsuperscript{11} and the Wisconsin Epidemiological Study of Diabetic Retinopathy population (37\% at approximately 26.6 years of diabetes duration\textsuperscript{18}). Although much attention has been given in recent years to anemia and low levels of HGB in diabetes, particularly as it relates to kidney disease,\textsuperscript{22-28} this is the first study, to our knowledge, to show high HGB levels to be predictive of the long-term incidence of PDR. We have also shown that the relationship of HGB with PDR varies by sex, with HGB demonstrating a linear relationship in men, but a nonlinear quadratic ($P = .008$) relationship in women. These results, if confirmed, may have important clinical relevance, as they may both identify new pathogenetic pathways to PDR and influence clinical treatment. There are several potential explanations for these findings.

First, the HGB levels in this population were relatively high, ranging from 9.2 to 20.0 g/dL. We have observed that HGB levels in our population, including the 120 participants with PDR and 228 with overt nephropathy at baseline, were approximately 1 g/dL higher in both men and women compared with the National Health and Nutrition Examination Survey III white population limited to the same age range.\textsuperscript{29} Higher HGB levels in type 1 diabetes were also noted by Pichler et al.\textsuperscript{30} who observed a higher (0.8 g/dL) difference in HGB levels between children and adolescents with type 1 diabetes compared with controls. Factors related to increased HGB such as testosterone, hypoxia, growth factors, and viscosity may be responsible for the PDR associated with increased HGB in this population.

Androgens, in particular testosterone, are a known stimulant of erythropoiesis,\textsuperscript{31} and testosterone has been fairly consistently shown to be elevated in type 1 diabetes.\textsuperscript{32-34} Haffner et al\textsuperscript{32} found higher testosterone levels and lower sex hormone-binding globulin\textsuperscript{33} in male participants with type 1 diabetes and PDR compared with those without PDR. Chaurasia et al\textsuperscript{34} also found higher testosterone levels in type 1 diabetes and PDR compared with controls. Factors related to increased HGB such as testosterone, hypoxia, growth factors, and viscosity may be responsible for the PDR associated with increased HGB in this population.

Erythropoietin is a glycoprotein produced by the peritubular fibroblasts of the kidney, and its production is primarily determined by tissue hypoxia in those with...
normal renal function. Erythropoietin stimulates angiogenesis and has been found to be associated with the retinopathy of prematurity. In diabetes, Watanebe et al found both erythropoietin and vascular endothelial growth factor to be independently associated with PDR cross-sectionally, while blocking of erythropoietin was found to inhibit retinal neovascularization. In preterm infants, Romagnoli et al observed that infants treated with recombinant human erythropoietin to reduce the need for blood transfusions were at an increased risk of retinopathy of prematurity, with no change in the need for transfusion. Brown et al also noted an increased risk of prematurity in preterm infants given recombinant erythropoietin. In diabetic patients receiving hemodialysis, Diskin et al observed that recombinant erythropoietin dose per week and HCT were positively associated with deterioration of retinopathy. Finally, recent trials have also observed an increase in adverse events, ie, a deterioration of kidney disease and more cardiovascular events and mortality, associated with the correction of anemia by erythropoietin therapy in those with kidney disease. Because these target treatment HGB levels were relatively low, this seems to suggest that growth factors stimulated by erythropoietin are more likely to be associated with adverse outcomes rather than high HGB per se.

Another potential related mechanism is that the HGB association with PDR reflects increased erythropoietin production stimulated by general hypoxia, which may contribute to the microvascular disease seen in both the retina and the kidneys in type 1 diabetes mellitus. Some recent studies have shown increased levels of anemia in type 1 diabetes to be associated with complications of diabetes, including retinopathy, renal impairment, and macrovascular disease. However, these patients were from tertiary care clinics and were older than our participants. In contrast, other literature suggests increased blood viscosity in diabetes, which may lead to tissue ischemia. Vekasi et al found higher levels of HCT, plasma fibrinogen, and higher plasma and whole blood viscosity in those with diabetic retinopathy compared with nondiabetic controls. Blood viscosity is directly related to HCT. As higher HCT and blood viscosity have also been observed in those with retinal vein occlusion, and the HGB levels associated with PDR in our population were quite high, it is quite possible that our PDR association may be partly due to increased viscosity and associated sludging. Unfortunately, direct viscosity measures are not available.

The sex differences, eg, HGB demonstrating a non-linear relationship with PDR in women and a linear relationship in men, are of interest. However, this is likely to just reflect the small sample sizes at the lower ends of the distribution in men (n=5) at levels (≤ 13.4 g/dL) where an increased risk was seen in women (n=46). A U-shaped relationship is thus likely to be true, and many U-shaped relationships exist between risk states and outcomes (eg, weight and mortality), with different pathological pathways operating at each end of the spectrum. Interestingly, a very recent study of type 1 diabetes found a genetic variant promoter of the EPO gene to be associated with both PDR and end-stage renal disease.
other limitation of our study was that we could not adequately stratify by renal disease status, a complication associated with both HGB levels and diabetic retinopathy, as only 14% (n = 56) of those free of PDR at baseline had overt nephropathy. However, we did try to account for kidney disease by controlling for albumin excretion rate, which, while predictive of PDR, did not account for HGB’s association with PDR incidence.

In conclusion, these data suggest that high HGB levels may be associated with increased risk of PDR. Furthermore, these PDR findings are complimented by observations that HGB also predicts 2-step progression and macula edema. Increased testosterone in type 1 diabetics, growth factors, viscosity, and/or compensation for generalized ischemia/hypoxia may account for the association of high HGB levels with PDR. Taken as a whole, these results suggest that further evaluation of the potential adverse role of high HGB in those with type 1 diabetes is warranted.

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C ongratulations to the winner of our June quiz, Sachindra Laishram, MD, Consultant Cornea & Glaucoma Services, Shija Eye Care Foundation, Langol, Manipur, India. The correct answer to our June challenge was congenital melanocytoma. For a complete discussion of this case, see the Letters: Research Letters section in the July Archives (Bajaj MS, Khuraijam N, Sen S, Pushker N. Congenital melanocytoma manifesting as proptosis with multiple cutaneous melanocytic nevi and oculodermal melanosis. Arch Ophthalmol. 2009;127[7]:937-939).

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