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Optic Disc Changes after Pan-Retinal Photocoagulation
for Proliferative Diabetic Retinopathy

A Thesis Submitted to the
Yale University School of Medicine
In Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by

David Jay-Pei Jeng

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ABSTRACT

OPTIC DISC CHANGES AFTER PAN-RETINAL PHOTOCOAGULATION FOR PROLIFERATIVE DIABETIC RETINOPATHY

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This study aims to examine the possible effect that pan-retinal photocoagulation (PRP) performed as treatment for proliferative diabetic retinopathy (PDR) has on the optic disc. In a retrospective study, photographs of the optic disc before and after PRP, as well as photographs of untreated eyes for the control group, were evaluated for cup-to-disc ratio and optic disc pallor as gross measures of optic nerve health. A 5-grade scheme for optic color was devised for this study by selecting representative photographs for each grade, where 5 indicated the healthiest-appearing color.

Kappa statistics indicated strong intra- and inter-observer consistency of observations for both cup-to-disc ratio and optic color (0.61 and 0.79, respectively). A paired t-test showed that change in optic pallor was significant after treatment with PRP ($p = 0.0004$). However, when comparing to the change in the control group using a Student's t-test, no significance was found ($p = 0.387$). No significant change was found in cup-to-disc ratio.

A few conclusions can be made from this study. First, the newly created grading scheme for optic pallor is both reliable and reproducible. While PRP treatment may cause an increase in optic pallor, the change is not significantly different than that seen with merely the passage of time. Lastly, cup-to-disc ratio was not significantly affected by PRP treatment. Further studies may be indicated to elaborate on the effect of PRP on the optic nerve.

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TABLE OF CONTENTS

Introduction	1
Epidemiology	2
Retina	5
Mechanism of pathogenesis	7
Ocular manifestations	12
Clinical presentation and assessment	16
Photography	16
Treatment	17
Specific aim	22
Methods	23
Subjects	23
Grading	23
Statistics	24
Results	24
Epidemiology	24
Optic pallor	25
Cup-to-disc ratio	27
Discussion	28
Statistical analysis	31
Retrospective study	35
Future considerations	38
References	42

INTRODUCTION

Diabetes mellitus is a chronic disease that affects approximately 15-20 million Americans and increases the risk of developing multiple medical complications. Therefore, patients with diabetes require long-term medical observation and management for both preventive and therapeutic reasons. Some complications of diabetes are acute, while others are chronic. One complication sometimes overlooked by the community is that diabetes is the leading cause of blindness in Americans between the ages of 20-74, and indeed, across industrialized nations (1). This remains true even as the impact of diabetes on vision has significantly diminished over the years with improved management of diabetic retinopathy through glucose control, routine ophthalmologic examinations, and proper treatment.

Studies have shown that the risk of severe vision loss is less than 5% if routine fundus exams detect early pathologic changes before the onset of retinopathy and proper treatments are performed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) recommendations (2). Yet, diabetic retinopathy remains a pandemic in the U.S. Such a discrepancy primarily lies in the fact that the majority of patients lose vision because of a delay in medical management rather than a lack of effective therapeutic interventions. Retinal ablation by

photocoagulation remains the mainstay of therapy for established diabetic retinopathy nearly 50 years after its introduction into the medical community.

Epidemiology

In the 1980s, a large series involving over 10,000 diabetic patients called the Wisconsin Epidemiologic Study of Diabetic Retinopathy made several observations about the natural history of diabetic retinopathy, which have been subsequently supported by many other studies (3). These determined that the number one cause of the development of diabetic retinopathy is the duration of the disease (3). It has been shown that patients having type 1 diabetes for less than 5 years rarely show signs of retinopathy. Yet, 27% of patients with the diagnosis of type 1 diabetes for 5-10 years and 71-90% of those who have had the disease for longer than 10 years have diabetic retinopathy (4). The incidence continues to rise to 95% after 20 years. At this stage, up to half of these patients have developed proliferative diabetic retinopathy (PDR). These overall patterns are similar in those with type 2 diabetes, as 23% of those with the disease for 11-13 years had retinopathy, while the prevalence rose to 60% after 16 years of living with the disease (5). At 10 years after diagnosis with type 2 diabetes, 67% of patients had retinopathy and 10% had PDR (4). Indeed, retinopathy was already apparent at the time of diagnosis for some type 2 diabetes patients,

underscoring its insidious onset many years before the diagnosis is clinically established.

Of note, patients were treated with conventional diabetic therapy in the above studies. However, in the mid-1990s, the Diabetes Control and Complications Trial (DCCT) demonstrated that patients with type 1 diabetes with tight control of blood glucose (i.e. four fingerstick measurements per day) had significantly less retinopathy than those with conventional control (i.e. one measurement per day), which was consistent with a decrease in other diabetic complications as well (6). The results of the DCCT were striking as there was a 76% decrease in the development of retinopathy and a 54% reduction in progression of established retinopathy with tight control. Unfortunately, for the most advanced stages of retinopathy, even the tightest control of blood glucose could not prevent progression. A study in the United Kingdom extended tight control to type 2 diabetes patients with similar striking reductions (7).

With the introduction of intensive insulin therapy for tight glycemic control, the prevalence of retinopathy has significantly decreased as observed by a second cohort from Wisconsin. In addition, the stage of retinopathy has been less severe, with only 10% of patients with moderate or severe non-proliferative diabetic retinopathy (NPDR), and only 1 in 474 patients requiring therapy for

proliferative retinopathy at 14 years follow-up, as opposed to 35% at baseline and 25% at 14 years, respectively, in the earlier Wisconsin series (8). Despite this overall drop in incidence, an interesting observation from studies have shown that after the initiation of intensive insulin therapy there is a transient worsening of retinopathy over the first year with increased soft exudates (6). This may be due to changes in intravascular volume secondary to the correction of hyperglycemia or related to increased insulin-like growth factor-1.

A strong correlation exists between retinopathy and renal disease secondary to diabetes, as suggested by proteinuria, elevated blood urea nitrogen (BUN) and/or creatinine (Cre) levels. One study evaluated the correlation between albuminuria and retinopathy in 815 patients with type 2 diabetes (144 Hispanics and 671 whites) (9). The presence of albuminuria, defined as urinary albumin excretion $>200 \mu\text{g}/\text{min}$, was found to be a statistically significant predictor for retinopathy (as detected via stereoscopic retinal fundus photographs) among the Hispanic, but not the white, patients. On the other hand, retinopathy itself is also a strong indicator of other complications of diabetes, where 35% of patients with symptomatic retinopathy will have proteinuria, and elevated BUN/Cre levels (10). The association between the development of diabetic retinopathy and nephropathy, independent of the degree of hyperglycemia and the duration of diabetes, suggests that common pathogenetic factors may underlie the

development of both complications. Additionally, a prospective study of 996 patients with retinopathy followed for 20 years demonstrated increased risk for cardiovascular events and mortality. Therefore, the presence of diabetic retinopathy appears to be a marker of increased cardiovascular and mortality risk (11,12).

Retina

The retina is a very thin sheet of tissue that lines the back of the eye, which is histologically and embryologically a part of the central nervous system (Fig. 1).

The basic structure of the retina will be reviewed and expanded upon to suggest possible reasons for why it is especially susceptible to ischemic and metabolic insults. Briefly, the optic nerve is evident with major blood vessels of retina arising from the center of the nerve. About three disc diameters temporal to the disc, the fovea, which is the focus of vision, lies at the center of the macula. The macula, considered the central retina, spans a diameter of about 6 mm and has the thickest ganglion cell layer with the highest number of horizontal neuronal connections to resolve fine detail (13). Any retina outside of this area is considered peripheral retina.

Histologically, the retina is lamellar in structure, with alternating layers of neuron and plexiform, where synapses and junctions take place (Fig. 2). Due to

embryological development, the photoreceptors are the most posterior cell layer of the retina; therefore, light must travel through all the layers of the retina before activating photoreceptor neurons. The retina is, for the most part, supplied by retinal and choroidal circulations. Within the retina, there are five major cell types: neurons, glial cells, microglia, and vascular endothelial cells. The neurons (i.e. photoreceptors, bipolar, horizontal, amacrine, and ganglion cells), provide the sensory function of the retina and convert photons first into a biochemical message and then an electrical message to be sent to the occipital lobe of the brain. The glial cells, e.g. Muller cells and astrocytes, are support cells that provide nutrition and metabolic regulation for the neurons. The retinal pigment epithelium also provides nutritional and metabolic support for neurons. Microglia are considered the resident macrophages of the CNS, and thus engage in phagocytosis and the inflammatory response to cell death and stress (14,15). The last class of cells includes vascular endothelial cells and pericytes. They also contribute to the nutritional support system for neurons and the removal of waste products. Of all classes, this last class has had the most attention in diabetic retinopathy.

Transparency of the retina must be maintained due to its counterintuitive organization, thus providing a constraint that may, in fact, underlie why the

retina is prone to insult from diabetes. First, retinal axons are unmyelinated, as myelin blocks light transmission through the retina. As such, these neurons require more energy to maintain membrane potentials than myelinated ones (16). Next, because blood vessels are also opaque, the density of vasculature is low, forcing certain parts of the retina farthest from vascular supply (e.g. the inner retina) to be in a constant state of relative hypoxia (17,18). Furthermore, there are fewer mitochondria in the inner retina, as cytochrome proteins can absorb light and block transmission, requiring the reliance on glycolysis for energy production rather than oxidative phosphorylation (18). Even with such a low oxygen tension, the retina remains one of the most metabolically active tissues in the body (19). Therefore, the retina, at baseline, is at a delicate balance between low supply and high demand for energy, and any metabolic stress, such as diabetes, could tip that balance. This theory is supported by the fact that early retinopathy affects the inner, and not the outer, retina, where there is low oxygen supply.

Mechanisms of Pathogenesis

The mechanism of diabetic retinopathy development is as yet unknown.

However, several theories exist.

One theory involves high aldose reductase levels. Sorbitol, a product of aldose reductase, generally does not passively diffuse out of cells. Accumulation of this byproduct generates an osmotic gradient that leads to electrolyte imbalance and eventually cell damage. Diabetic retinopathy may result from such damage because aldose reductase concentrations are high in retinal pericytes and Schwann cells, which are among the first to be affected (20). A polymorphism near the transcription site of the aldose reductase gene has also been observed to be associated with the early onset of retinopathy in some patients with type 2 diabetes, further suggesting the potential role of this pathway in the development of retinopathy (21). Unfortunately, clinical trials, thus far, have failed to show efficacy of aldose reductase inhibitors in preventing or reversing diabetic retinopathy (20).

Alternatively, the prevailing theory regarding retinal neovascularization postulates that the vasoproliferative factors are released by the retina, retinal vessels, and retinal pigment epithelium. Experimental models of retinopathy suggest that neovascularization is mediated in part by the interaction between insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF). In one animal study, an IGF-1 receptor antagonist reduced the degree of retinal revascularization (22). A possible etiological role for IGF-1 is supported by clinical observations that a transient worsening of retinopathy occurs after the

onset of intensive insulin therapy (6,23). Insulin increases serum IGF-1 concentrations while pituitary injury or hypophysectomy lowers serum IGF-1 and reverses the retinopathy (24). Recently, there has been much focus on VEGF as playing a direct role in the vascular abnormalities seen in diabetic retinopathy. Animal models have shown that VEGF expression correlates with the progression of neovascularization (25). It has also been demonstrated that the concentration of VEGF is higher in the vitreous of eyes with PDR than those with NPDR, and intensity of immunostaining for VEGF is proportional to the severity of retinopathy (26,27). Perhaps a good indicator of the validity of this theory is that VEGF inhibitors have been successful in controlling hypoxia-induced neovascularization in certain animal models (28).

Erythropoietin has also been identified as a possible factor affecting retinal angiogenesis in diabetic retinopathy (29,30). Concentrations of erythropoietin were elevated in the vitreous fluid of 73 patients with proliferative diabetic retinopathy when compared to 71 patients without diabetes (erythropoietin concentration 464.0 versus 36.5 mIU per mL, respectively). In fact, erythropoietin has been shown to correlate more strongly with retinopathy than VEGF (30). In experimental models, inhibition of the erythropoietin pathway slowed endothelial cell proliferation *in vitro*, and inhibited retinal neovascularization in a mouse model (31).

Other vasoactive substances may also contribute to progression of the retinal disease, including basic fibroblast growth factor (bFGF) and hepatocyte growth factor (32). The local concentration of bFGF has been found to be much higher in patients with active proliferative retinopathy as compared to those in whom the retinopathy had regressed (33). Although these growth factors are strongly suggested in explaining neovascularization in response to ischemia, the link between hyperglycemia and vascular abnormalities still needs to be illuminated.

One idea suggests that the autoregulation of retinal blood flow is the main culprit behind the release of these vasoactive substances. Normally, retinal blood flow is kept constant through autoregulation until the mean arterial pressure is raised more than 40% above baseline. However, this autoregulatory mechanism is impaired in the presence of hyperglycemia (34). Therefore, even moderate increase in retinal blood flow increases shear stress on the retinal blood vessels, which may then stimulate the production of vasoactive substances.

Genetic factors are also implicated in the severity of retinopathy. In the DCCT, severe retinopathy was three times more frequent among first-degree relatives with retinopathy (35). In some of these patients, platelet abnormalities or alterations in blood viscosity may lead to diabetic retinopathy by occluding focal areas of vasculature, thus leading to ischemia. The ischemia may then incite the

VEGF pathway (36). In a case control study, researchers have found a polymorphism in the platelet membrane glycoprotein Ia/IIa receptor for collagen that significantly increased risk of retinopathy in patients with type 2 diabetes who were homozygous when compared to those who did not have the polymorphism (37). This polymorphism facilitates platelet interaction with glycosylated collagen, leading to more rapid activation of these platelets, and thus retinal vascular injury.

Ethnicity-related genetic susceptibility to retinopathy was also suggested by a study of 105 patients with type 2 diabetes who were followed for four years (38). Retinopathy occurred more often among blacks than whites (50% versus 19%) after accounting for factors such as hemoglobin A1C values, systolic blood pressure, or sex. A cross-sectional study of 778 diabetic patients age 45 to 85 years also found higher prevalence of retinopathy and macular edema in blacks (36.5% and 11.1%) and Hispanics (37.4% and 10.7%) compared to whites (24.8% and 2.7%) (39). However, race was not significantly shown to be an independent predictor of retinopathy in this study. Instead, factors such as longer duration of diabetes, higher fasting glucose, greater waist-hip ratio, and use of insulin or oral hypoglycemic medications arose as independent predictors of diabetic eye disease.

Ocular Manifestations

The retina is one of the most metabolically active tissues in the human body, and is therefore easily affected by changes in substrate equilibrium or oxygen tension (40). At a very early stage, the basement membrane of retinal blood vessels is thickened, akin to any other type of diabetic microangiopathy. While the absolute number of both retinal pericytes and microvascular endothelial cells are diminished, the relative number of pericytes to endothelial cells is also decreased (41). These structural changes in retinal microcirculation are believed to be responsible for a breakdown in the blood-retinal barrier, leading to the formation of microaneurysms and retinal vascular permeability, clinically resulting as hemorrhagic exudates or even macular edema. The presence of microaneurysms and “hard” exudates, i.e. the deposition of lipid and proteinaceous material, are the first clinical signs of diabetic retinopathy. This is referred to as early non-proliferative diabetic retinopathy, or background retinopathy.

Microaneurysms, usually seen in early NPDR as small red dots in the middle retinal layers, may weaken and rupture, causing an intraretinal hemorrhage. Depending on its location within the layers of the retina, a hemorrhage may be shaped differently. Deeper ones are referred to as ‘dot’ or ‘blot’ hemorrhages, while more superficial hemorrhages, such as those in the nerve fiber layer, take on a flame or splinter shape, like those seen in hypertensive retinopathy.

Because hypertension is an independent risk factor for the development of diabetic retinopathy, it is recommended that a diabetic patient with multiple splinter hemorrhages monitor blood pressure regularly (42).

Macular edema is also an important manifestation of NPDR and is actually the leading cause of legal blindness in diabetic patients. The edema is caused by intercellular fluid that is either leaked from microaneurysms or due to vascular abnormalities. Clinically, macular edema presents with the gradual blurring of near and far vision. It is best observed by biomicroscopy, where light is scattered by the edematous retina, leading to a blurred retinal pigment epithelial and choroidal pattern. If the areas of fluid are large enough in the outer plexiform layer, cystoid macular edema can be seen. This is more commonly seen in eyes manifesting with more severe NPDR.

After the initial stage of cell death and increased vascular permeability, cycles of cell death and renewal occur leading to further microvascular destruction, ischemic injury, and eventually uncontrolled angiogenesis (41). As retinopathy progresses, further signs of worsening inner retinal hypoxia are seen. These include multiple retinal hemorrhages, cotton-wool spots, venous beading and loops, intraretinal microvascular abnormalities (IRMA), and large areas of capillary nonperfusion seen on fluorescein angiography. Cotton-wool spots, also

known as “soft” exudates or nerve fiber infarcts, are due to ischemia rather than actual exudation of fluid. Vascular occlusion and local ischemia, possibly due to platelet function abnormalities, erythrocyte aggregation, and high plasma fibrinogen concentrations, leads to obstruction of axoplasmic flow. This blockage causes the nerve fibers to swell and give off a classic white fluffy appearance distal to the occlusion on funduscopy. Fluorescein angiography demonstrates a lack of capillary perfusion surrounding the cotton-wool spot.

Various types of vascular change are noted as well. As a sign of poor retinal circulation, venous beading is observed. Tortuous venous loops are often seen near large areas of capillary nonperfusion. IRMAs are dilated capillaries, which act as collaterals. They may be confused with surface retinal neovascularization. Fluorescein angiography can differentiate the two, as dye does not leak from IRMAs, but will from neovascularization. The ETDRS found that IRMA, multiple retinal hemorrhages, venous beading and loops, widespread capillary nonperfusion, and widespread leakage on fluorescein angiography were significant risk factors for the development of proliferative diabetic retinopathy. However, cotton-wool spots alone were not (43).

While the previously mentioned ophthalmic complications of diabetes may cause legal blindness, ambulatory vision is usually preserved. However, with

proliferative diabetic retinopathy, severe vitreous hemorrhage or retinal detachment is possible, which may lead to hand-movements vision or worse. Nearly 50% of patients with severe NPDR will progress to proliferative diabetic retinopathy within a year (44). Proliferative vessels usually arise from retinal veins and often begin as a locus of fine vessels. When these arise on or within one disc diameter of the optic disc, they are referred to as neovascularization of the disc (NVD). When it develops more than one disc diameter away, they are called neovascularization elsewhere (NVE). Both NVD and NVE leak fluorescein on angiography.

New vessels may proliferate either within retinal layers or push into the vitreous, possibly attaching to the vitreous itself (45). They may also be associated with connective tissue formation, often creating a fragile mesh of fibrous tissue. The main risk of these new vessels is that they are quite prone to rupture, possibly leading to extensive hemorrhages. As PDR progresses, fibrotic tissue may begin to contract. Thickening and organization of the posterior hyaloid face of the vitreous body may pull the retina anteriorly, thus producing a traction retinal detachment. On the other hand, posterior vitreous detachment in diabetic patients is due to a gradual shrinking of the entire vitreous. This pulls the fragile new vessels into the vitreous cavity, possibly leading to vitreous hemorrhage.

Contraction of the vitreous may also pull on the optic disc or the macula, which would further decrease visual acuity.

Clinical Presentation and Assessment

In terms of clinical symptoms, patients with diabetic retinopathy often have poor night vision and poor adaptation under bright lights (46). Blue-yellow discrimination is affected earlier and more severely than red-green discrimination. Color vision deteriorates as retinopathy progresses (47).

Diabetic retinopathy is usually easily diagnosed by ophthalmoscopic exam and can be documented with fundus photography. The typical lesions are microaneurysms. Thus, fluorescein angiography is a good test to help assess the severity of retinopathy, identify areas of leakage and ischemia, and confirm neovascularization. More recently, optical coherence tomography (OCT), a noninvasive imaging technique used to determine retinal thickness, has improved detection of macular edema.

Photography

Stereoscopic fundus photography is currently the most widely used technology to objectively document fundus appearance. Stereoscopic photography offers several advantages including photographs of the disc with its natural color, wide availability of cameras, and modest requirement of technical skill from the

ophthalmic photographer. Additionally, fundus cameras are relatively inexpensive as far as ophthalmic imaging systems are concerned. The major disadvantages of optic nerve head photography are subjective and qualitative assessment and the need for clear media and a dilated pupil. Two methods currently are used to obtain a stereoscopic image: sequential and simultaneous. Sequential stereoscopic photography involves a shift of the camera joystick to opposite sides of a fully dilated pupil either manually or via a mechanical sliding carriage adapter, such as the Allen stereo separator introduced in 1964. Several conditions must be met in order to obtain a consistent stereogram pair. First, constant eye position and fixation must be maintained. In addition, camera-dependent variables such as focus depth, light intensity, stereo base, and exact camera angle must be identical for each session (48). With all the potential for variability, stereoscopic photographs are useful for documentation, but cannot be utilized as the sole diagnostic tool. Simultaneous photography requires two cameras that take pictures at the same time. A single axis allows for fixed camera angles.

Treatment

The most important goal in diabetic retinopathy is tight glycemic control. Both primary and secondary prevention are essential in patients with diabetes. That is, in patients without retinopathy, tight control will prevent, if not delay, the onset

of retinopathy. Those with established retinopathy will slow the progression of the retinopathy with glycemic control. Indeed, the degree of protection seems to be directly related to the level of glycemic control, progressive retinopathy is rare in patients with hemoglobin A1C below 7% (6). However, once advanced retinopathy has set in, there is little to no benefit in attaining good blood glucose control (49,50).

In addition, as previously mentioned, hypertension is an independent risk factor for the progression of diabetic retinopathy, as well as other diabetic complications. The United Kingdom Prospective Diabetes Study (UKPDS) showed that patients with lower blood pressures had a 34% and 47% decrease in significant worsening of retinopathy and visual acuity, respectively (51).

Therefore, it is imperative that diabetic patients are aggressively treated for hypertension. Some evidence indicates that ACE inhibitors may exert a similar benefit in retinopathy as in diabetic nephropathy.

For over twenty years, pan-retinal photocoagulation has been the treatment of choice for advanced retinopathy. In order to understand photocoagulation better, below is a brief explanation of laser usage in ophthalmology. *Laser* is an acronym for *L*ight *a*mplification by *s*timulated *e*mission of *r*adiation. Various active media and activating methods define the different lasers that are available.

Several properties of laser light make it useful to ophthalmologists. Laser energy can be directed at specific target tissue in a controlled manner due to monochromaticity, spatial coherence, temporal coherence, collimation, fast concentration time, and ability to produce nonlinear tissue effects (52).

The choice of optimal wavelength depends on the absorption spectrum of the target tissue. However, light scatter also affects wavelength selection, as blue light, with its shorter wavelength, may scatter more, creating a greater potential for photochemical retinal damage in nearby untreated retina. In general, the most important factor in achieving visible lesions is fundus pigmentation, where the majority of energy absorption occurs. Retinal lesion size, however, is strongly dependent on laser power. Short exposures may lead to photodisruptive effects, whereas exposures of longer duration lead to photocoagulation. Yet, for photocoagulation, increasing the power level affects lesion size more so than extending exposure time. Focal laser treatment is optimal with spots of small size (50–100 μm diameter), whereas pan-retinal photocoagulation covers larger areas of the retina by effectively using larger spots (200–500 μm diameter). Unfortunately, small spots may result in complications such as choroidal rupture and secondary choroidal neovascular membrane (CNVM) when high irradiance levels are used (53).

As with all procedures, patients must be provided with proper preoperative education about the potential risks of laser photocoagulation. Inadvertent photocoagulation of the fovea, cornea, iris, or lens can be minimized using careful technique and appropriate spot size. Yet, this still poses a very real complication. Choroidal effusions are seen most often after extensive pan-retinal photocoagulation, a problem that may be minimized if the treatments are divided into multiple sessions. There also exists a risk for secondary CNVM, believed to result from damage to Bruch's membrane caused by intensive laser treatment. Decreases in intensity and duration, along with the avoidance of smaller spot sizes (50 μ m), may help minimize this complication. It is also possible that contraction of the neovascular membrane as a result of the thermal effects of laser may cause a rip in the retinal pigment epithelium (53).

Even with these risks of laser, PRP was established as an effective treatment for slowing the progression of disease in The Diabetic Retinopathy Study, where 1758 diabetic patients with advanced retinopathy were randomly assigned to pan-retinal photocoagulation in one eye (54). The cumulative risk of developing severe visual loss at six years was reduced by more than 50% in the treated eyes. Studies have shown that good glycemic control is necessary to improve the response to successful PRP. Patients with lower hemoglobin A1C had further regression of retinopathy than those with higher hemoglobin (55). In order to

test whether PRP was effective when used in less severe retinopathy, the ETDRS randomly assigned 3711 patients with mild to severe NPDR or early PDR to photocoagulation in one eye. At 5 years, severe visual loss was low in both the treated and untreated eyes. However, the study showed that there were significant adverse effects such as loss of visual acuity and peripheral vision in those with extensive PRP (44). Therefore, PRP is not recommended for earlier stages of retinopathy.

With regular and frequent screening along with PRP when necessary, visual acuity can be maintained. One study from the ETDRS series showed that only 20% of patients had moderate visual loss and none with severe visual loss when patients were followed closely and treated appropriately over an average of 16.7 years (11). Unfortunately, mortality rates remained high at 61%, which was consistent to what has been seen in other studies of patients with diabetic retinopathy.

When photocoagulation fails to halt neovascularization, severe visual loss may ensue as a result of extensive vitreous hemorrhage or due to fibrous contraction causing retinal detachment or distortion. A vitrectomy to remove vitreous hemorrhage followed by photocoagulation may restore some functional vision, but expedience is crucial. Successful vitrectomy has been shown to induce

regression of neovascularization and prevention of recurrence (56). A randomized trial in 370 eyes with advanced retinopathy demonstrated that the success rate for vitrectomy to restore useful vision was 44% if performed prior to central retinal detachment. In contrast, if vitrectomy was delayed, only 28% of eyes had good visual acuity at four years (56). The importance of aggressive screening in advanced retinopathy and prompt medical attention when there are acute changes in vision is emphasized.

Study Aim

Diabetes is a growing pandemic in the United States, especially with the increasing prevalence of obesity. Careful and aggressive monitoring is necessary to prevent and treat many of the complications of diabetes. The same holds true for diabetic retinopathy. While tight glycemic control is the definitive method to prevent the advent and progression of retinopathy, it is accepted that pan-retinal photocoagulation can slow, if not reverse, worsening of established proliferative diabetic retinopathy. However, the procedure requires the ablation of peripheral retinal tissue. Sadly, this treatment modality has changed little in nearly 50 years. As yet, there has been no literature studying the effects of PRP on the optic nerve itself. Nonetheless, anecdotal evidence has suggested an alteration in the appearance of the optic disc after PRP. It is possible that PRP may induce the production of noxious factors that may indirectly affect the photoreceptors, and

hence the optic nerve. This study examines the possible effect of PRP on the optic disc, using cup-to-disc ratio and optic pallor as gross measures of optic nerve health.

METHODS

Subjects

In a retrospective study, medical records of patients who underwent PRP for PDR specifically at the Yale Eye Center from 1996-2006 were reviewed. Only patients who had Kodachrome photographs of the optic disc before and after PRP were included in this study. Patients who had received PRP prior to photographs, had cataract extraction between pre- and post-photographs, or underwent vitrectomy at any time before either photograph was taken were excluded from the study. For the control group, fellow eyes without PRP or eyes with nonproliferative diabetic retinopathy which did not receive PRP were identified. Only eyes with Kodachrome photographs taken at least 2 months apart were included.

Grading

Photographs of the optic disc were then masked, randomized and individually presented to two experienced ophthalmologists for evaluation. The ophthalmologists used a stereo-viewer to aid in the evaluation. The cup-to-disc

ratio and color of the optic disc were determined and recorded. Cup-to-disc ratio was ascertained according to accepted protocol. For optic disc pallor, five grades were defined and standard photographs were selected representing each grade, where 5 indicated the healthiest-appearing color (Figure 3). Photographs of the optic disc were individually compared to this standard scale for grading. Each ophthalmologist reviewed the photographs twice on separate occasions to minimize bias.

Statistics

For statistics, a kappa test using the SAS statistical package was performed on data from both observers. Along with the kappa statistics, a paired t-test was performed on each individual group. Finally, an independent group Student's t-test was performed to compare the mean change in cup-to-disc ratio and optic disc pallor in our experimental group with the control group.

RESULTS

Epidemiology

Basic demographic data was gathered and analyzed to determine if there were any innate differences between the two patient groups independent of disease presentation (Table 1). Because patients with PDR generally receive PRP soon

after presentation, patients with NPDR accounted for the majority of the control group. Although the size of the PRP group (N=53) was more than twice the size of the control group (N=26), age at presentation, duration of disease, and gender were similar between the two groups. Patients ranged from 22 to 75 years old at presentation, with a mean of 54.2 years and standard deviation of 13.6 years among the PRP group. Similarly, control patients ranged from 29 to 75 years old, with a mean of 57.2 years and standard deviation of 14.5 years. Both groups presented with an average of 17 years of diabetes duration (PRP: 17.6±9.6 years; control: 17.2±13.6 years). Patients were fairly split by gender with males comprising 58% of PRP patients and 54% of control subjects. Racial breakdown of the two groups was comparable as well, but minorities represented a slightly larger proportion in the PRP group.

Optic Pallor

In this study, two parameters were used as an indirect measure of optic nerve health: optic pallor and cup-to-disc ratio. Since no standards for grading optic pallor were available, a *de novo* reference standard was created for this study (Fig. 3). One concern was that this grading scheme would be inconsistent and hence, unreliable. Thus, kappa statistics were calculated to test for grading consistency (Table 2). Two experienced ophthalmologists graded photographs for optic pallor on two separate occasions using the newly-devised standard. First,

internal consistency was tested for each grader with a kappa coefficient of 0.60 being the cutoff for reliability. Kappa coefficients of 0.77 and 0.70 for Observer #1 and #2, respectively, were found, indicating good intra-observer reproducibility. Next, using data from both observers, a kappa coefficient of 0.79 was calculated for inter-observer consistency, again suggesting strong inter-observer consistency.

A battery of statistical tests was then used to evaluate determine whether there was a significant increase in optic pallor after PRP. Again, to ensure that there were no initial differences, mean optic color grade for both the PRP and control groups were calculated prior to PRP. An average grading of 3.95 was found among the PRP patient group while the controls reported a mean grade of 3.88, two values showing no significant difference (Table 3). A paired t-test was then performed using the averaged data from all optic color observations. The PRP group experienced a mean change of 0.488 that was found to be significant with a p -value of 0.0004, whereas the control group had a mean change of 0.231 with a p -value of 0.387 (Table 4). Lastly, to distinguish whether this change was due to the PRP treatment, an independent group Student's t-test was used. This compared the changes in optic color between the PRP and control groups. A p -value of 0.351 was found, indicating no significant difference between the change

in optic color of the PRP group when compared to that of the control group (Table 5).

Cup-to-disc Ratio

The other measure that was used for optic nerve health was cup-to-disc ratio.

Kappa statistics of cup-to-disc ratio were calculated primarily to compare and contrast with those computed for optic pallor (Table 2). Because cup-to-disc ratio is a commonly accepted measurement, it was assumed that kappa coefficients would indicate strong correlation among data sets. The kappa statistics for Observer #1 was 0.82 and for Observer #2, kappa was 0.81; both were strongly consistent. When kappa was calculated for data from both observers, the kappa coefficient for cup-to-disc ratio was 0.62. This data supports the consistency of the generally accepted measure of cup-to-disc ratio.

As with optic color, no statistically significant differences were found between the PRP and control groups for the average cup-to-disc ratio (Table 3). The mean cup-to-disc ratio prior to PRP treatment was 0.39 and increasing to 0.41 after PRP. In the control group, the mean cup-to-disc ratio initially was 0.4, and remained the same at 0.4 as observed in the later photograph. The paired t-test showed that there was a greater average change in the cup-to-disc ratio in the PRP group (0.022) than in the control group (0.004). However, the change was not

significant in either the PRP or control group with p -values of 0.079 and 0.819, respectively. When a Student's t -test was performed to compare the mean difference in cup-to-disc ratio for the PRP group against the mean difference in the control group, no significant difference between the PRP and control groups was found, with a p -value of 0.387. Cup-to-disc ratio did not change after PRP treatment, as there was no significant difference when compared to the control group.

DISCUSSION

Since pan-retinal photocoagulation has been shown to slow and even reverse proliferative diabetic retinopathy, it is considered standard of care to perform PRP in such cases. However, as mentioned, PRP involves the ablation of relatively healthy peripheral retinal tissue via multiple laser burns. The theory of vascular angiogenesis seems to be supported by the success of PRP. Anecdotally, it has been suggested that the optic disc may appear less healthy after PRP. Yet, there have been no published reports that have studied the potential effect of PRP on the optic nerve itself. While it is unlikely that laser treatment of peripheral retinal tissue will directly injure the optic nerve, as only a small percentage of nerve fibers originate from outside of the macula (~30%) (57), it is possible that such destruction of tissue may indirectly damage the optic nerve

perhaps via release of factors that may be harmful to the optic nerve. Because the retina is within a relatively closed environment, these noxious stimuli may remain in the local environs and affect the optic nerve. The final consideration for whether or not to perform PRP may require the weighing of risks versus benefits, i.e. considering the balance between the morbidity due to the disease process itself versus from iatrogenic causes. New treatment modalities are currently being investigated and may bring new hope to patients with diabetic retinopathy.

When evaluating the optic nerve head, common parameters to account for include cup-to-disc ratio, disc and cup area and volume, neuroretinal rim width and area, area of pallor, and cup slope. Since this is a retrospective study analyzing photographs of the optic nerve, cup-to-disc ratio and optic pallor were the two parameters that were most logistically feasible to interpret. Cup-to-disc ratio is a tested and common measure used in the screening for and monitoring of glaucoma, as well as other optic neuropathies. Glaucoma is defined as an optic neuropathy that is often related to elevated intraocular pressure (IOP) diagnosed by visual field loss with no other known causes. However, it is believed that visual field loss occurs when there has already been a significant amount of nerve damage. While increased cup-to-disc ratio is considered a risk factor for developing glaucoma, some believe that it directly represents optic

nerve injury. Optic pallor is another parameter often used when evaluating the optic nerve head for neuropathy. Review of literature revealed no existing grading system for optic pallor. Clinically, the optic disc is determined to have no optic pallor, segmental pallor, or general pallor. However, since some optic discs may look white, others yellow, and normal discs appearing pinkish, it was felt that optic pallor could be graded on an incremental scale ranging from 1, a near white appearance, to 5, a healthy-appearing pink hue. Since this is an artificial and subjective grading system, it is not clear what the clinical significance of such differences in optic color would imply, and future studies may help to establish its clinical correlation.

The results of the study are mixed. Although a statistically significant worsening of optic color was found after PRP, this change over time was not statistically different than that seen in the control group. Additionally, the change in cup-to-disc ratio, a second measure of optic nerve health, did not prove to be significant in either group. These results are difficult to conclusively analyze, but may suggest that PRP has minimal to no effect on the optic nerve. The control group was used to ensure that any change in the appearance of the optic disc was not due to changes over time, but rather directly related to the PRP treatments. This study encountered several obstacles, some of which could be accounted for, and others of which could not.

Statistical Analysis

First, as with any study, statistical analysis of the data must always be considered in the correct context. In an attempt to maintain objectivity and validity, two graders each were presented masked photographs on two separate occasions. Whenever multiple graders are used with relatively subjective determinations, a measure of consistency is needed to strengthen the data.

Therefore, a kappa coefficient was calculated both for inter- and intra-observer agreement. The kappa coefficients were all above 0.6, a value that is accepted as relatively strong reliability. However, there are pitfalls to using kappa statistics.

There is disagreement as to the usefulness of kappa statistics to assess grader agreement. At the least, it can be said that kappa statistics should not be viewed as the unequivocal standard to quantify agreement. There may be two possible uses of kappa: as a way to test rater independence (i.e. as a test statistic), and as a way to quantify the level of agreement (i.e., as an effect-size measure). The first use tests the null hypothesis that there is no more agreement than might occur by random chance. In other words, the kappa test yields a qualitative binary decision about whether raters are independent or not. It is the second use of kappa—quantifying actual levels of agreement—that is occasionally controversial. Kappa is often referred to as a chance-corrected measure of

agreement. However, because the expected agreement in the calculation of kappa is relevant only when the raters are independent, some argue that kappa is not, in fact, chance-corrected. Critics believe that raters are not independent, by definition, because they are grading the same items. Therefore, at best, kappa approximates correlations within a category and should not be used to determine the level of agreement. However, kappa seems well situated in medical literature, and will be used here with caveats (58,59).

Kappa tests were performed on the data sets to determine intra- and inter-observer reliability. Interestingly enough, observations of the optic color had, in general, lower intra-observer kappa coefficients. However, between the two observers, optic color seemed more standardized with a higher kappa coefficient. The fact that cup-to-disc ratio is a tried-and-true measure that is commonplace in everyday clinical practice speaks to the precision of this measurement. Yet, while cup-to-disc ratio determinations were extremely reproducible by an individual observer, when comparing between observers, there seemed to be less agreement. On the other hand, with optic color, although this measure was formulated *de novo* for this study, the standard colors allowed for inter-observer reproducibility, even though intra-observer consistency was slightly lower. In conclusion, there is a correlation between graders that is greater than chance

alone for both optic pallor and cup-to-disc ratio; and evaluation using the *de novo* photographic standard for optic pallor is reproducible and reliable.

While there was concern that the *de novo* standard for optic pallor would be inconsistent and too subjective, this study suggests that having a set reference standard increased the reliability, as determined by the kappa coefficient of inter- and intra-observer grading. For both, the kappa coefficient was higher in optic pallor than in cup-to-disc ratio. This may be due to the uncertainty surrounding such a grading system, and the constant pressure to maintain consistency by continuously comparing to the standard photos. While cup-to-disc ratio is also subjective, the determination of cup-to-disc by ophthalmologists is widely accepted. Perhaps, having standard cup-to-disc photos may increase the consistency in recording cup-to-disc ratio.

In medical research, t-tests are one of the most commonly used statistical tests (60). The purpose of the t-test is to compare the means of a variable in two samples in order to determine whether the difference between the two is greater than the difference expected from chance alone. If the two research samples come from two different groups (for this study, those receiving PRP treatment, and those not receiving PRP), the Student's t-test is used. If the two samples come from the same group over time (for example, pre- and post-PRP values),

the paired t-test is used. Both of these t-tests were employed in this study. In order for these t-tests to be valid, the central limit theorem states that for reasonably large samples (considered to be greater than 30 observations in each sample), the distribution of the means is Gaussian, even though the data in individual samples may be skewed or uneven. Therefore, as long as a sample is considered to be random and of reasonably large size, a t-test may be computed on almost any set of continuous data (61). While the PRP group satisfied the requirement for large samples, the control group had only 26 eyes, which falls short of accepted standards for large sample size. Unfortunately, the study was limited by the number of patients with diabetic retinopathy who had photographs reasonably separated in time.

First, a paired t-test was performed where changes of a continuous variable over time are tested. The variables analyzed were cup-to-disc ratio and optic color before and after PRP treatment. In this comparison, each individual eye serves as its own control. Any variation that is detected in the paired t-test is attributable to the intervention or to changes over time in the same person. In terms of cup-to-disc ratio, no significant difference was found in either group. It is possible that PRP may not injure the optic nerve in the same manner as an optic neuropathy such as glaucoma might. However, the results of this test showed that the optic color statistically decreased after PRP treatment, but no

significant change was found in the non-PRP group. This suggests that the changes in the PRP group may have been due to the laser treatment, rather than merely secondary to time.

Then, an independent group Student's t-test was done to compare means of the same variable between two independent groups. In this study, the mean change in either optic color or cup-to-disc ratio over time was compared between the PRP and control group. Ideally, subjects are randomly selected from a larger population of subjects. Because the variances of each group were unequal in this study, the Satterthwaite method was used, which provides an approximated t-statistic that asymptotically approaches a true t distribution. Although a significant increase in optic pallor in the PRP group was found by the paired t-test, no significance was found when comparing the change of the optic pallor between the PRP group and the non-PRP group. It is possible that the difference would become statistically significant with a larger sample size. As mentioned earlier, the clinical significance of optic pallor is yet to be determined.

Retrospective study

Perhaps one reason why there is no literature looking at this issue is that it is quite difficult to determine the actual health of the optic nerve. In this study, the health of the optic nerve is measured indirectly by the cup-to-disc ratio and optic

pallor. Because photographs of eyes with proliferative diabetic retinopathy are commonly taken for documentation purposes, using these parameters was the most readily accessible method. Both are commonly evaluated parameters in assessing the optic nerve head. Since poor visual acuity stems from multiple causes and more advanced retinopathy is presumed to correlate with poorer vision, VA was not used as an endpoint.

Many factors could not be controlled, because of the nature of a retrospective study. While the majority of issues revolved around not deviating from standard clinical care, the main problem was the number of patients who fit the criteria for the study. While many patients had photographs documenting the degree of retinopathy prior to PRP, it was rare that a patient would have photographs after PRP treatment. Therefore, only a subset of PDR patients had photographs taken before and after treatment. Another factor that limited this study's size was that in 2004, the Yale Eye Center switched from Kodachrome film photographs to digital photographs. The change in exposure and quality prevented reliable comparison of film to digital photographs. Therefore, eyes were included in this study only when both pre- and post-PRP photos were available on film.

A second point of contention is related to the time points at which photographs had been taken. Photographs ideally would have been taken at a set time before

and at regular intervals after PRP treatment. This would allow for observation of longitudinal changes of the optic nerve after PRP treatment. It would also have been preferred if the control group had photographs taken at set time intervals that matched the PRP group's time frame. As there is no evidence that such a practice would improve the disease process, photographs were taken at varied times before and after PRP based on clinical necessity.

Another issue with our study was the selection of the control group. The ideal control group would have consisted of eyes with proliferative diabetic retinopathy that have not received PRP. Unfortunately, standard of care dictates that patients who presents with proliferative diabetic retinopathy should receive PRP soon after. Therefore, very few cases existed where eyes with proliferative diabetic retinopathy did not receive PRP (only 4 in this study) for whatever reason. Instead, non-proliferative diabetic retinopathy eyes were selected. Even so, the population base precluded a high number of severe NPDR eyes for this study (a total of 11). The remainder consisted of either mild or moderate NPDR eyes. The fact that the control group does not perfectly match the treatment group introduces a new variable to the significance of the paired t-test. It is possible that the change in optic pallor may be due to the more advanced disease of the PRP group, rather than the PRP treatment itself.

Future considerations

Future studies should include a prospective study with regular serial photographs, as well as a more objective measure of optic nerve head injury. An acceptable time frame for these diagnostic techniques would be prior to PRP, then 3, 6, and 12 months after the last PRP treatment. For the control group, a time interval of 0, 6, 9, and 15 months might be used. Newer technologies may aid in measuring the health of the optic nerve in a more objective way, such as OCT.

OCT is a relatively new diagnostic imaging technology that uses light wave interference and low-coherence light at a specific wavelength (~840nm) to achieve high-resolution cross-sectional imaging of the eye. The idea behind OCT is similar to ultrasonography, except that light is used instead of sound waves. A probe beam of low-coherence light is split to the tissue of interest and to a reference mirror at a known variable position (reference beam) (62). Reflections of the beams may produce positive interference on a photodetector, leading to the formation of an image. Edges between tissues of different optical densities and layers within a tissue may be differentiated from each other, with a resolution of approximately 5–8 μ m. The retinal nerve fiber layer can be visualized, and thickness measurements are determined by computer analysis of the resultant image. Nerve fiber layer thickness measured by OCT correlates well

with structural (histological nerve fiber layer thickness measurements) and functional (visual fields) parameters in normal individuals and in patients who have glaucoma (63).

Other computer-aided optic nerve head analyzers are being improved upon as well, such as the Heidelberg Retina Tomograph (HRT), which is a confocal scanning laser ophthalmoscope, as well as improved computer software to analyze data and/or stereoscopic photographs more objectively.

While pan-retinal photocoagulation has been the mainstay of treating established diabetic retinopathy for nearly five decades, only in recent years have new treatment modalities been seriously considered. Recently, VEGF inhibitors have been tested to treat proliferative diabetic retinopathy. Pegaptanib is one such inhibitor that has been approved in the United States for the treatment of wet age-related macular degeneration. Retrospective review of the data from those clinical trials suggests that pegaptanib may also induce some regression of neovascularization, with response in 8 of 13 patients in the treatment group and 0 of 3 in the placebo group (64). Bevacizumab, another VEGF inhibitor with a broader spectrum than pegaptanib, showed complete or partial reduction of fluorescein leakage in all 44 eyes treated in a preliminary study of intravitreal bevacizumab administration (65). Recurrence of dye leakage on angiography

occurred in only 1 patient at two week follow-up. Unfortunately, recent reports suggest that anti-VEGF compounds may have a systemic effect of increasing stroke risk in elderly patients (66).

A multitude of other theories abound that address glucose-mediated microvascular damage, such as oxidative stress, activation of advanced glycosylation end products and receptors, and protein kinase C activation (67-69). Of these, so far PKC inhibition has been shown to be effective in randomized clinical trials (70). However, theories that address other mechanisms of pathogenesis are being suggested. One such idea emphasizes the fact that the retina is a neural tissue, and thus, therapies using neuroprotective agents may be useful (71). Another hypothesis argues that inflammation may be a critical component in the progression of diabetic retinopathy (72). Therefore, controlling the inflammatory process may limit the amount of tissue damage. More broadly, there has also been much study involving the role of insulin as having a direct effect on the retina in the development of diabetic retinopathy (14). Many of these theories may only have weak supporting evidence, but they provide clinician scientists with a slew of new therapeutic targets in the fight to control diabetic retinopathy.

While this study suggests that pan-retinal photocoagulation has no significant effects on the optic nerve, the structure of the study imposed certain constraints that could not be accounted for by other means. The overall clinical significance of increased optic pallor is not clear. Therefore, future studies using a more objective measure in evaluating the optic nerve would be helpful. Furthermore, a prospective study would be able to standardize of time intervals between optic nerve evaluations and allow for matched controls. Although PRP is the accepted treatment for established diabetic retinopathy, it is not without its own side effects ranging from decreased peripheral vision to the rare complication of retinal pigment epithelium tear. In theory, PRP is considered by some to be crude with the “burning” of retinal tissue. With the proposition of many alternative models of pathogenesis, more targets for drug therapy are being tested, some closer to clinical use than others. Perhaps with the advent of these drugs, PRP may not be as heavily relied upon to control diabetic retinopathy. This study suggests that PRP does not have a significant effect on the optic nerve; however, PRP is not without its own risks, and alternative therapies must be pursued.

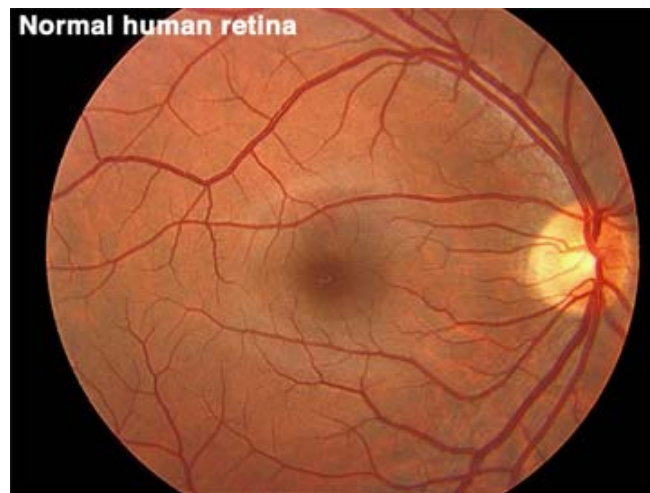


Figure 1. Normal human retina. The optic nerve emerges from the retina as the optic disc, where major retinal blood vessels also arise. Temporal to the disc, there is a reddish spot, called the fovea, located at the center of the macula. Any retina outside of the macula is generally considered peripheral retina. (Photograph courtesy of <http://retina.umh.es/Webvision/>)

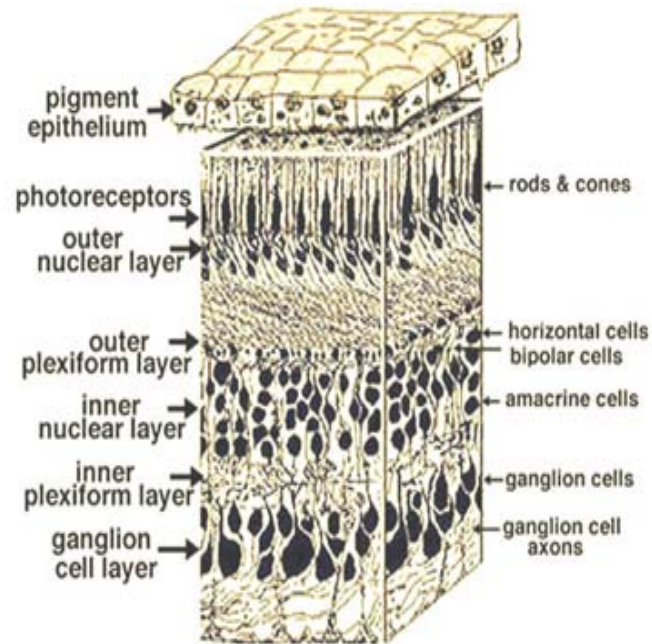


Figure 2. Schematic diagram of the layers of the retina. This represents a cross-section of the retina, oriented with the most posterior layer (i.e. the pigment epithelium) on top. The retina is organized in a lamellar fashion with alternating neuronal layers with axonal, or plexiform, layers. Light must travel through all of these layers before being absorbed and processed by the photoreceptors. (Image courtesy of <http://retina.umh.es/Webvision/>)

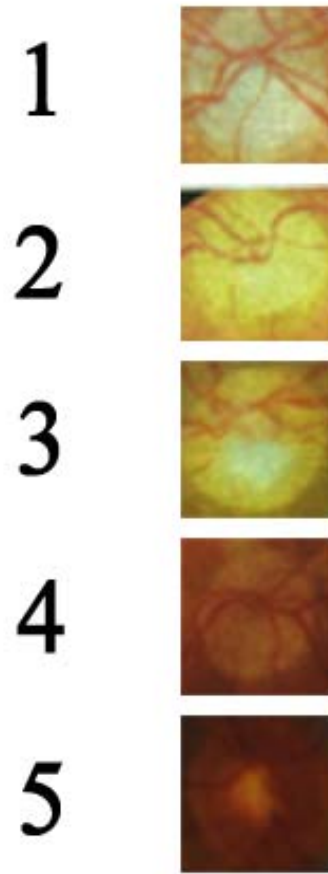


Figure 3. Representative photos of *de novo* reference standard devised to grade optic pallor. Five photographs were selected to create a scale with equal gradations in color of optic disc. A score of 5 represents the healthiest-appearing optic disc, while 1 represents the least healthy-appearing disc.

	PRP	Control
Number of Patients	53	26
Age (years)		
Mean	54.2	57.2
S.D.	13.6	14.5
Duration of Disease (years)		
Mean	17.6	17.2
S.D.	9.6	13.6
Gender (%)		
Male	58	54
Female	42	46
Race (%)		
Caucasian	34	54
Hispanic	21	12
African American	42	31
other	4	4

Table 1. Demographics of the subject groups, PRP treatment group and non-treatment control group, were analyzed. No significant differences were seen in terms of age, duration of disease, or gender. There was a slightly higher percentage of minority groups in the PRP group than in the control group.

	Cup-to-Disc Ratio	Optic Color
Observer #1	0.82	0.77
Observer #2	0.81	0.67
Inter-Observer	0.61	0.79

Table 2. Kappa statistics were used to measure consistency in ratings by an observer over time (intra-observer) and in comparison to each other (inter-observer). The kappa coefficient was calculated for each individual grader and between graders for both cup-to-disc ratio and optic color. A kappa coefficient greater than 0.6 is accepted in medical literature as being strongly consistent.

	Pre-PRP		Post-PRP	
	Mean	SD	Mean	SD
Cup-to-Disc Ratio				
PRP	0.39	0.016	0.41	0.016
Control	0.40	0.05	0.40	0.05
Optic Color				
PRP	3.95	0.10	3.46	0.11
Control	3.88	0.17	3.65	0.11

Table 3. Summary statistics for all observations are presented. The mean and standard deviation (SD) were calculated for cup-to-disc ratio and optic color at two time points. The pre-PRP data was obtained from evaluation of initial photographs prior to any PRP treatment. As indicated, the post-PRP data for the PRP group derived from evaluation of photographs after PRP treatment. For the control group, the post-PRP data was extracted from photographs taken at a date subsequent to initial evaluation since no PRP was performed. No significant differences are noted between initial measurements of cup-to-disc ratio or optic color.

	d	SE	t	p-value
Cup-to-Disc Ratio				
PRP	0.022	0.012	1.80	0.079
Control	0.004	0.017	0.23	0.819
Optic Color				
PRP	0.488	0.129	3.77	0.0004*
Control	0.231	0.337	0.68	0.387

Table 4. Paired t-tests analyzed the change of the cup-to-disc ratio and optic color measurements in post-PRP and control patients over time. For the PRP group, pre- and post-PRP measurements were compared, while for the control group, earlier versus later measurements were compared. The mean change of each parameter over time (d) is presented with the standard error (SE). The change in optic color in the PRP group is significant while other parameters did not change significantly over time.

	Difference in Mean Changes over time	SE	<i>t</i>	<i>p</i>-value
Cup-to-Disc Ratio	0.018	0.025	0.88	0.387
Optic Color	0.274	0.288	0.96	0.351

Table 5. Independent group Student's *t*-test was used to evaluate differences in the change of cup-to-disc ratio and optic color measurements between post-PRP versus control patients. This comparison allows for a measure of whether the change in each parameter was specifically due to PRP treatment. The mean difference in the change of each parameter between the two groups was calculated. Since the variances of the two groups were unequal (see Table 4), the Satterthwaite method was utilized to approximate a *t*-statistic, which indicates that there was no significant difference found between the groups in the change of either parameter over time.

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