

A Study of Histopathological Features of Latanoprost-Treated Irides With or Without Darkening Compared With Non-Latanoprost-Treated Irides

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Objectives: To study the histopathological features of latanoprost-treated irides with or without darkening, compared with non-latanoprost-treated irides.

Methods: Iridectomy specimens and patient history forms were independently examined by 3 ophthalmic pathologists in a masked fashion. Specimens were evaluated for premalignant changes and for differences in level of pigmentation and degrees of cellularity, inflammation, and vascular abnormalities.

Results: The specimens consisted of 22 latanoprost-treated darkened irides, 35 latanoprost-treated irides without darkening, and 35 non-latanoprost-treated irides. There was a statistically significant decrease in the number of nuclear invaginations and prominent nucleoli in latanoprost-treated darkened irides compared with the other 2 groups ($P = .004$

and $P = .005$, respectively). The average thickness and pigmentation of the anterior border layer was greater in the latanoprost-treated darkened irides than in the other 2 groups ($P = .03$ and $P = .02$, respectively). The latanoprost-treated darkened irides had increased pigmentation of the stroma ($P < .001$), stromal fibroblasts ($P < .001$), melanocytes ($P = .005$), vascular endothelium ($P = .02$), and adventitia ($P < .001$) relative to the other 2 groups.

Conclusions: There is no histopathological evidence of premalignant changes in latanoprost-treated darkened irides. The latanoprost-induced iris color changes are due to a thickening of the anterior border layer and an increased amount of melanin in the anterior border layer and within the stromal melanocytes.

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PROSTAGLANDIN ANALOGUES constitute one of the most effective classes of antiglaucoma drugs and include various congeners such as latanoprost, unoprostone isopropyl, travoprost, and bimatoprost.¹ In various clinical studies, these drugs often have been demonstrated to cause increased pigmentation of the iris.² A small number of studies have consisted of histopathological evaluations to determine the mechanism of iris darkening.³⁻⁵ In 2004, we performed the first large study to evaluate, in a systematic manner, key histopathological findings in iris specimens obtained from patients with ($n = 449$) or without ($n = 142$) a history of latanoprost therapy.³ That study included latanoprost-treated and control or nontreated irides but did not specifically look at irides with darkening as a separate group. The study investigated the safety aspects of treatment, including evidence of malignant change or cell proliferation, such as the presence of melanoma, the presence of mitoses, and an increased number of cells. The investigators concluded that latanoprost treatment caused increased amounts of melanin within the iris stromal melanocytes but was not as-

sociated with an increase in melanocyte number. That study found no evidence of adverse histopathological effects in latanoprost-treated irides compared with controls.

A recent study by Arranz-Marquez et al⁴ compared iridectomy specimens from patients with latanoprost-induced darkening ($n = 22$) and those from untreated controls ($n = 8$). The authors found that latanoprost-induced darkened irides had an increased number of melanocytes with intranuclear inclusions, nuclear invaginations, prominent nucleoli, and mitotic figures suggestive of atypia. Furthermore, they found more granules of melanin in the vascular walls and an increased number of melanocytes and free melanin granules in the stroma compared with controls. To evaluate these findings, we repeated the study and compared latanoprost-treated irides with darkening, latanoprost-treated irides without darkening, and non-latanoprost-treated control irides.

METHODS

Iris specimens were obtained at the time of iridectomy during glaucoma surgery. The source of the specimens has been previously cited.³ The

Table 1. Melanocytic Cellular Atypia

Atypia	Mean (SD) No. of Features			P Value ^a
	Latanoprost-Treated Irides With Darkening	Latanoprost-Treated Irides With No Darkening	Control Irides	
Intranuclear inclusions	1.3 (1.6)	1.1 (0.9)	1.3 (1.1)	.67
Nuclear invaginations	2.5 (1.7)	4.0 (1.6)	3.4 (1.4)	.004
Prominent nucleoli	1.9 (1.3)	3.1 (1.5)	2.7 (1.1)	.005

^aBased on the *F* test from analysis of variance models for continuous and ordinal variables and Pearson χ^2 test for categorical variables.

tissue was received in 10% neutral-buffered formalin at the Latanoprost Pathology Center (LPC), University of Wisconsin, Madison, and labeled by the LPC coordinator with a unique study accession number. The formalin-fixed tissue specimens were processed overnight on an automated tissue processor, embedded in paraffin, serially sectioned at 5 μ m, and mounted on treated slides. The first 3 slides were stained with hematoxylin-eosin, the next 3 slides were bleached with potassium permanganate before staining with hematoxylin-eosin, and the next 7 slides were left unstained. All slides were labeled with the unique study accession number assigned to the specimen. If any tissue remained after sectioning, the paraffin block was archived. Also included were the slides of 11 of the 22 cases with latanoprost-induced darkening from the study by Arranz-Marquez et al.⁴

Sets of microscopic slides (1 hematoxylin-eosin-stained slide, 1 bleached hematoxylin-eosin-stained slide, and 1 unstained slide) were sent to the 3 reviewing pathologists (D.M.A., H.E.G., and W.R.G.), who examined the sets independently and with no knowledge of therapy history. They completed the accompanying pathology grading form, which included an assessment of the quality of the specimens, their location, and their orientation. The specimens were graded by level of pigmentation as none, mild, moderate, and heavy. The thickness of the anterior border layer was ascertained by measuring the average number of flattened cells on the iris surface, oriented parallel to the iris surface, and overlying the loose connective tissue of the stroma proper. These were counted in 3 separate areas of the anterior border layer, and the average was determined by each of the 3 examiners. The number given represents the further average of these 3 determinations. These forms were returned to the LPC coordinator, who completed a composite grading form using specific rules for combining data from the 3 grading forms. Specimens were adjudicated using a protocol formulated by the 3 pathologists before the beginning of the study.

The patient history form that accompanied each specimen included iris color (blue, hazel, or brown); type of glaucoma; ocular history; type of surgery; treatment history, including the start of medical treatment; medication history, including specific medications used; duration of latanoprost therapy (<3 months, 3 months to 3 years, or >3 years); other glaucoma medications used; and presence or absence of increased pigmentation after glaucoma medication. The latanoprost group was defined by a history of latanoprost therapy. It was further divided into irides with and without latanoprost-induced darkening. The control group was defined as having no history of therapy with latanoprost or any other prostaglandin analogue.

After review by the LPC coordinator, the patient history form and the composite grading form (identified only by the unique accession number) were sent to the Statistical Data Analysis Center (Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison) for data entry (Oracle database; Oracle, Redwood Shores, California) and statistical analysis. This study

received institutional review board approval from the University of Wisconsin Health Sciences Human Subjects Committee.

Comparisons of groups were based on the *F* test from analysis of variance models for continuous and ordinal variables and Pearson χ^2 test for categorical variables. Ordinal variables were converted to integer equivalents for analysis. Statistical significance was set at $P < .05$. No formal adjustment for multiple comparisons was used.

RESULTS

From May 1, 2005, through March 31, 2007, 92 iris specimens from 92 unique patients were evaluated by the 3 reviewing pathologists. The specimens consisted of 22 latanoprost-treated darkened irides, 35 latanoprost-treated irides without darkening, and 35 non-latanoprost-treated irides. The 22 latanoprost-treated darkened irides included 11 cases obtained from Arranz-Marquez et al⁴ and 11 other cases from the LPC. Evaluation of the composite pathology grading forms indicated that the latanoprost-treated groups with or without darkening and the control group were comparable in terms of quality, location, and orientation of the iridectomy specimens. All specimens were obtained from the peripheral iris.

No melanomas were reported on the pathology grading form in either of the latanoprost-treated groups or in the control group. The iris specimens in all of the groups were evaluated for melanocytic atypia (**Table 1**). The various features suggestive of melanocytic atypia included intranuclear inclusions, nuclear invaginations, prominent nucleoli, and mitotic figures. No mitotic figures were noted in any of the iris specimens. There were no statistically significant differences in the number of intranuclear inclusions among the 3 groups. There was a statistically significant decrease in the number of nuclear invaginations and prominent nucleoli in latanoprost-treated darkened irides compared with the other groups ($P = .004$ and $P = .005$, respectively).

The 22 latanoprost-treated darkened irides were further examined in terms of the following 2 subgroups: 11 cases obtained from the series studied by Arranz-Marquez et al⁴ and 11 other cases from the LPC. There were more intranuclear inclusions in the cases obtained from Arranz-Marquez et al than in those from the LPC (mean, 2.2 vs 1.1; $P = .01$). However, there were fewer nuclear invaginations (mean, 1.7 vs 3.4; $P = .01$) and prominent nuclei (mean, 1.1 vs 2.7; $P = .003$) in the cases obtained from Arranz-Marquez et al compared with those from the LPC.

The average (SD) thickness of the anterior border layer was greater in the latanoprost-treated darkened irides (2.2 [0.9] cells) than in the latanoprost-treated irides without darkening (1.8 [0.7] cells) and the control group (1.7 [0.7] cells) ($P=.03$). There was also a statistically significant in-

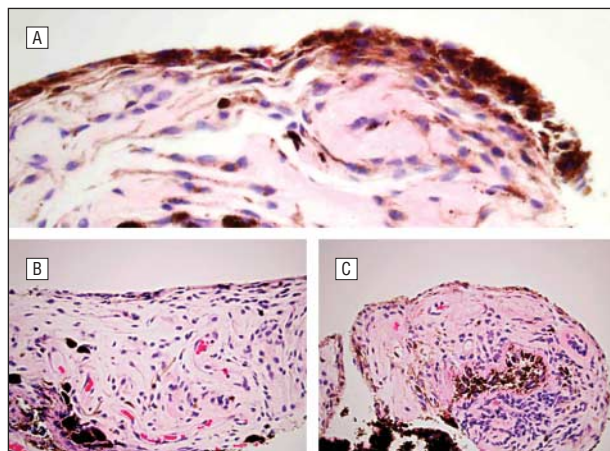


Figure 1. Hematoxylin-eosin–stained sections of iridectomy specimens showing increased thickness of the anterior border layer in latanoprost-treated darkened irides (A) compared with latanoprost-treated irides without darkening (B) and control irides (C) (original magnification $\times 400$).

crease in pigmentation of the anterior border layer in the latanoprost-treated darkened irides (21 of 22 irides [95%] with moderate or heavy pigmentation) compared with the latanoprost-treated irides without darkening (20 of 35 irides [57%] with moderate or heavy pigmentation) and the controls (21 of 34 irides [62%] with moderate or heavy pigmentation) ($P=.02$; **Figure 1**). Similarly, the latanoprost-treated darkened irides had increased pigmentation of the stroma ($P<.001$), stromal fibroblasts ($P<.001$), melanocytes ($P=.005$), vascular endothelium ($P=.02$), and adventitia ($P<.001$) relative to the latanoprost-treated irides without darkening and the controls (**Table 2** and **Figure 2**). In the areas of increased pigmentation, the melanocytes were differentiated from pigmented fibroblasts by the plump, fusiform cytoplasm in melanocytes, compared with fibroblasts, which have wispy cytoplasm and narrow, spindle-shaped nuclei.

A subset analysis was performed for the extent of pigmentation in the various layers of iris in the latanoprost-treated darkened irides (11 cases obtained from Arranz-Marquez et al⁴ and 11 cases from the LPC). Arranz-Marquez et al also noted a thickening of the anterior border layer, but this was given as a measurement in millimeters rather than in terms of the number of cells. However, in the LPC cases,

Table 2. Constituent Cells Graded with Heavy (Maximum) Pigmentation According to Location and Treatment

Level of Pigmentation	No. (%) of Cases ^a			P Value ^b
	Latanoprost-Treated Irides With Darkening (n=22)	Latanoprost-Treated Irides With No Darkening (n=35)	Control Irides (n=35)	
Anterior border layer ^c				.02
None	0	0	0	
Minimal	1 (5)	15 (43)	13 (38)	
Moderate	14 (64)	14 (40)	15 (44)	
Heavy	7 (32)	6 (17)	6 (18)	
Stroma				<.001
None	1 (5)	8 (23)	15 (43)	
Minimal	5 (23)	19 (54)	10 (29)	
Moderate	15 (68)	7 (20)	9 (26)	
Heavy	1 (5)	1 (3)	1 (3)	
Fibroblasts				<.001
None	1 (5)	3 (9)	6 (17)	
Minimal	2 (9)	24 (69)	18 (51)	
Moderate	14 (64)	6 (17)	8 (23)	
Heavy	5 (23)	2 (6)	3 (9)	
Melanocytes				.005
None	1 (5)	2 (6)	1 (3)	
Minimal	1 (5)	20 (57)	16 (46)	
Moderate	15 (68)	10 (29)	12 (34)	
Heavy	5 (23)	3 (9)	6 (17)	
Endothelium ^c				.02
None	12 (55)	30 (86)	29 (85)	
Minimal	10 (45)	5 (14)	4 (12)	
Moderate	0	0	1 (3)	
Heavy	0	0	0	
Adventitia ^c				<.001
None	2 (9)	24 (69)	21 (62)	
Minimal	17 (77)	11 (31)	11 (32)	
Moderate	3 (14)	0	2 (6)	
Heavy	0	0	0	

^aPercentages have been rounded and may not total 100.

^bBased on the *F* test from analysis of variance models for continuous and ordinal variables and Pearson χ^2 test for categorical variables.

^cOnly 34 samples of control irides were available for this determination.

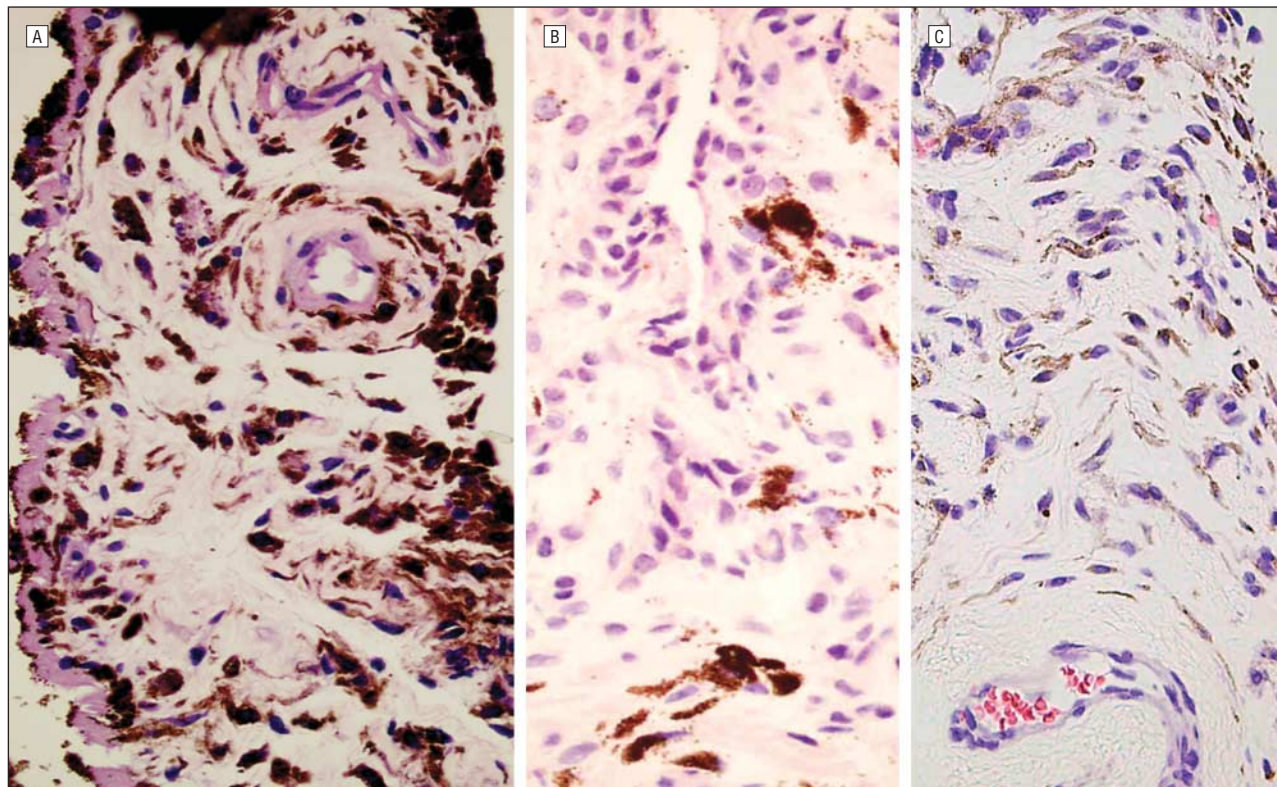


Figure 2. Hematoxylin-eosin–stained sections of iridectomy specimens showing increased pigmentation of the stroma, stromal fibroblasts, melanocytes, vascular endothelium, and adventitia in latanoprost-treated darkened irides (A) compared with latanoprost-treated irides without darkening (B) and control irides (C) (original magnification $\times 400$).

there was no statistically significant difference in pigmentation of the anterior border layer (11 of 11 [100%] vs 10 of 11 [91%] with heavy or moderate pigmentation; $P = .45$), stroma (9 [82%] vs 7 [64%]; $P = .51$), stromal fibroblasts (11 [100%] vs 8 [73%]; $P = .39$), melanocytes (11 [100%] vs 9 [82%]; $P = .22$), vascular endothelium (0 vs 0; $P > .99$), and adventitia (1 [9%] vs 2 [18%]; $P = .67$).

A detailed evaluation of all the iris specimens also found no evidence of stromal inflammation or abnormal appearance of stromal blood vessels in any irides. The posterior iris pigment epithelium was also unremarkable in all specimens. There was no evidence of rubeosis, pseudoexfoliation material, synechiae, descemetization, or endothelialization in any of the iris specimens. Two specimens, one from the latanoprost-treated darkened irides group and the other from the control group, showed attached Descemet membrane. Three specimens, including 1 latanoprost-treated iris without darkening and 2 controls, had hemorrhage on the anterior border layer and/or the posterior iris pigment epithelium.

COMMENT

The differences in the iris color of normal eyes can be attributed to the variable amounts of melanin granules within a constant number of melanocytes in the superficial stroma of the iris.^{6,7} The amount of melanin is genetically predetermined and reaches its maximum concentration during early childhood.^{8,9} Thereafter, it usually remains constant throughout life unless affected by certain ocular disor-

ders, which can lead to hypopigmentation or hyperpigmentation.¹⁰ Latanoprost and various other prostaglandin analogues used in the medical management of glaucoma have been demonstrated to cause iris darkening in clinical trials in the United States.^{2,11,12} This iris darkening is typically noted after 6 months of drug therapy, and the degree of increased pigmentation varies from 5% to 70%.¹³⁻¹⁵ Furthermore, in a recent study, the darkening of the iris after 6 months of latanoprost treatment was noted more frequently in patients older than 75 years compared with patients younger than 60 years.¹⁶

The pathogenesis of iris darkening in latanoprost-treated eyes has been studied in various *in vitro* and histopathological studies.¹⁷⁻²¹ The *in vitro* studies have documented elevated tyrosinase activity in human uveal cell lines and iris melanocyte cultures.²²⁻²⁶ The light and electron microscopic studies indicate that latanoprost-induced eye color change is due to an increased amount of melanin within the iris stromal melanocytes rather than any increase in melanocyte number.²⁷⁻³⁰ As stated in the Introduction, our previous histopathological study of iris specimens obtained from patients with and without a history of latanoprost therapy³ observed no evidence of malignant or premalignant changes in either group. This conclusion was based on the absence of melanomas, atypical nevi, cells with an atypical appearance, or increased numbers of mitotic figures. There was an increased prevalence of freckles in the latanoprost-treated group. We proposed that the increased number of freckles was a manifestation of focal increased tyrosinase expression and

that the same pathogenesis exists for the more diffuse darkening. We did not believe that the increase in iris freckles had malignant potential.

The study performed by Arranz-Marquez et al⁴ noted a statistically significant increase in atypia in melanocytes in latanoprost-treated darkened irides. There was also a thickening of the anterior border layer and a higher number of melanin granules within the stroma.⁴ Therefore, we designed a new study to evaluate latanoprost-treated eyes with or without iris darkening and compared those groups with non-prostaglandin-treated controls.

The anterior border layer and stroma of the latanoprost-treated darkened irides showed a statistically significant increase in melanin pigment granules compared with the other groups. There was also a thickening of the anterior border layer. These findings are consistent with the findings of other studies in the literature^{4,5} and may be responsible for darkening of the iris in latanoprost-treated eyes. However, in contrast to the series by Arranz-Marquez et al,⁴ there was no evidence of melanocyte atypia. On the contrary, we found that the numbers of nuclear invaginations and prominent nucleoli were significantly decreased in latanoprost-treated darkened irides. This unexpected finding may be related to the effects of prostaglandin analogues on cell growth and differentiation.³¹⁻³³

Thus, our results indicate that the latanoprost-induced iris darkening is due to a thickening of the anterior border layer and to an increased amount of melanin in the anterior border layer. The iris stromal melanocytes also show an increase in the amount of melanin granules, but there is no evidence of melanocyte atypia. Although not entirely in agreement with the study by Arranz-Marquez et al,⁴ these findings are consistent with those of previous studies.⁵ The differences between the 2 subgroups in the latanoprost-treated darkened irides may reflect ethnic differences^{13,34,35} or may be related to the duration³⁶ of latanoprost treatment and the dose³⁷ of the medication used.

In conclusion, there is no histopathological evidence of precancerous lesions in latanoprost-treated darkened irides. This finding is consistent with the absence of documented iris melanomas in reports on patients treated with latanoprost during the past decade.

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Disclaimer: Dr Albert, the journal's chief editor, was not involved in the editorial review or the decision to publish this article.

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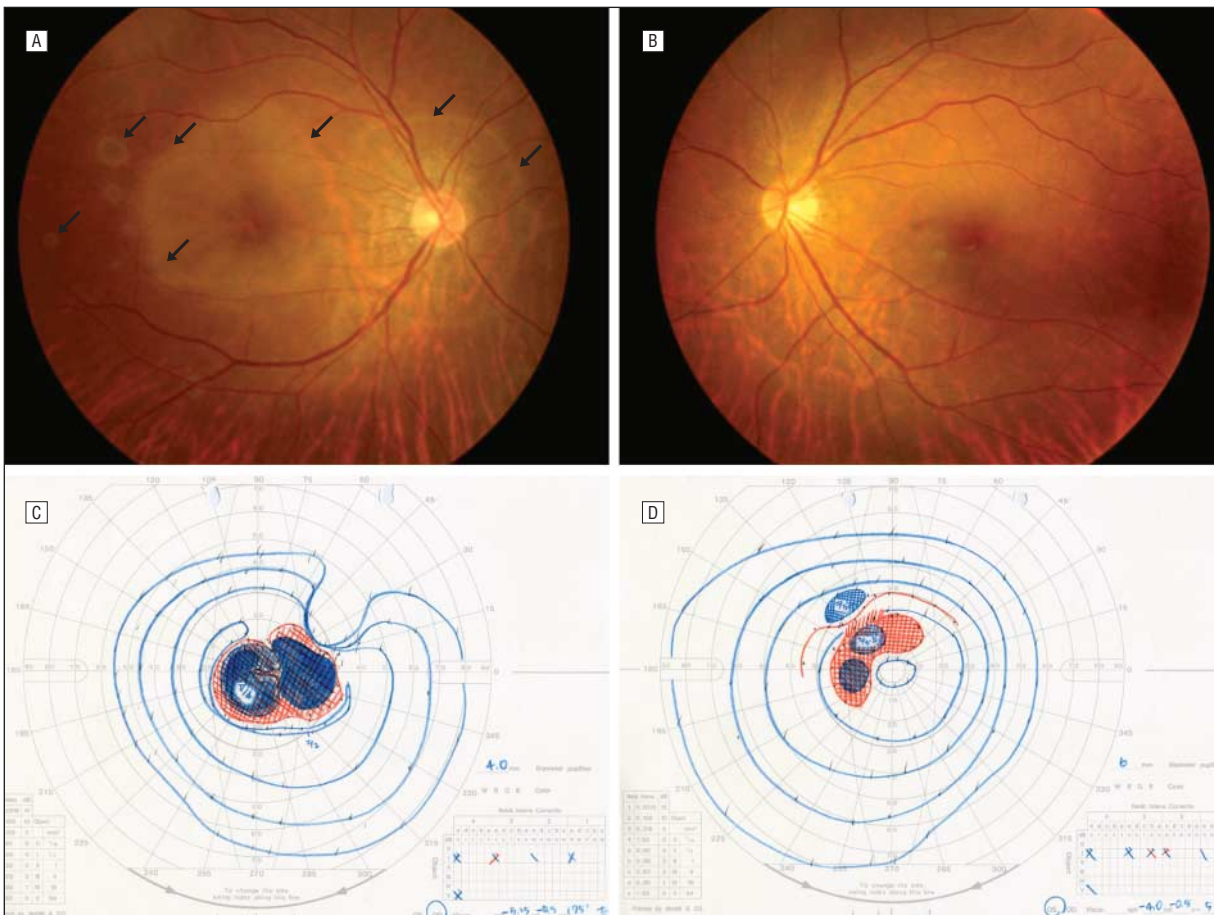
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Archives Web Quiz Winner

Archives Web Quiz Winner

Congratulations to the winner of our December quiz, Peykan Turkcuoglu, MD, Department of Ophthalmology, Inonu University School of Medicine, Malatya, Turkey. The correct answer to our December challenge was acute annular outer retinopathy. For a complete discussion of this case, see the Clinicopathologic Reports, Case Reports, and Small Case Series section in the January *Archives* (Tang J, Stevens RA, Okada AA, Chin M, Nussenblatt RB, Chan C-C. Association of antiretinal antibodies in acute annular outer retinopathy. *Arch Ophthalmol*. 2008;126[1]:130-132).



Be sure to visit the *Archives of Ophthalmology* Web site (<http://www.archophthalmol.com>) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month's print edition of the *Archives*. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also be able to choose one of the following books published by AMA Press: *Clinical Eye Atlas*, *Clinical Retina*, or *Users' Guides to the Medical Literature*.

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Correction

Errors in Text. In the Clinical Sciences article titled “A Study of Histopathological Features of Latanoprost-Treated Irides With or Without Darkening Compared With Non-Latanoprost Treated Irides” by Albert et al, published in the May issue of the *Archives* (2008;126[5]:626-631), there were errors in quoted data of the study by Arranz-Marquez et al. The number of iridectomy specimens from patients with latanoprost-induced darkening should have been listed as 14 instead of 22. No mitotic figures or prominent nucleoli were present in any specimens. Therefore, on page 626, the first 2 sentences of the Introduction’s second paragraph should have read as follows: “A recent study by Arranz-Marquez et al⁴ compared iridectomy specimens from patients with latanoprost-induced darkening (n=14) and those from untreated controls (n=8). The authors found that latanoprost-induced darkened irides had an increased number of melanocytes with intranuclear inclusions and nuclear invaginations suggestive of atypia.”