The Relationship Between Melanin and Glaucoma: A Case-control Study

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Precis: In a case-control study, skin pigmentation was not statistically significantly different when comparing glaucoma patients to those without glaucoma.

Purpose: Darker skin color has been implicated as a risk factor for glaucoma based on previous studies’ subjective assessments of skin pigmentation. This study used objective measurements to determine whether cutaneous pigmentation is a risk factor for glaucoma.

Methods: This case-control study was conducted at Menelik II Tertiary Referral Hospital in Addis Ababa, Ethiopia. Patients aged 40 years or older from the glaucoma clinic who were being scheduled for trabeculectomy were enrolled as cases and age-matched patients without glaucoma from other clinics at Menelik II Tertiary Referral Hospital were enrolled as controls. A Dermacatch device was used to capture melanin measurements in triplicate from the inner arm of each participant. The exposure variable of interest was the median of the triplicate skin melanin measurements, in arbitrary units. The outcome of interest was presence of glaucoma.

Results: Agreement between the triplicate inner arm melanin measurements was high, with an intraclass correlation of 0.99 (95% confidence interval, 0.98-0.99). Mean melanin values were 704 units (SD 94) in 76 cases and 694 units (SD 93) in 152 controls. Melanin was not statistically significantly associated with glaucoma after adjusting for sex and season of measurement (ie, dry vs. rainy), with an odds ratio of 1.15 (95% confidence interval, 0.59-2.24) per 100 units of inner arm melanin.

Conclusion: This study failed to find a significant association between skin pigmentation and glaucoma using an objective and reproducible assessment of pigmentation.

Key Words: glaucoma, open-angle, skin pigmentation, ocular hypertension, melanin, Ethiopia

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African ancestry is a well-known risk factor for glaucoma. Although the reasons for the increased risk are not clear, previous studies have implicated iris or skin pigmentation levels as possible mediating risk factors, with darker pigmentation associated with higher intraocular pressures and increased risk of glaucoma.2,4 An association between melanin and glaucoma is not inconceivable given its role in other glaucomatous conditions such as pigment dispersion glaucoma and exfoliation glaucoma. For example, aqueous melanin has been shown to be positively correlated with intraocular pressure in both conditions, and trabecular meshwork pigmentation has been shown to be associated with more severe pigment dispersion glaucoma.5-8 The previous studies assessing the relationship between skin pigmentation and glaucoma were conducted over 2 decades ago and relied on subjective assessments of skin pigmentation. In the meantime, skin pigment analysis devices have been developed to allow more precise and objective measurements of skin pigmentation.9-12

To further explore the relationship between melanin and glaucoma, we conducted a case-control study in Addis Ababa, Ethiopia. We chose a sample of patients with and without glaucoma and compared skin melanin levels using a colorimeter device. We hypothesized that the group of glaucoma patients would have increased levels of skin pigmentation.

METHODS

Study Design

A cumulative case-control study was conducted from September 2016 to July 2018 in Menelik II Tertiary Referral Hospital, Addis Ababa, Ethiopia.

Study Population

Cases were defined as patients seen in the glaucoma clinic aged 40 years or older who had a clinical diagnosis of glaucoma and were being scheduled for trabeculectomy. All cases of glaucoma were diagnosed by a fellowship-trained glaucoma specialist. Controls were age-matched (ie, ± 3 y of the case) patients without a clinical diagnosis of glaucoma, drawn from other eye clinics at the same hospital. Patients seeking ophthalmologic care at this tertiary care medical center were posited to come from the same study base and were thus expected to have a similar distribution of exposures. Two controls were drawn for each case. The original design called for sampling controls on the same day as cases, but this proved logistically challenging due to staff shortages.

Pigment Measurements

Estimates of skin pigmentation were taken in triplicate with a Dermacatch colorimeter (Neuchâtel, Switzerland). The device was calibrated each day before use. Two anatomic locations were chosen: (1) the central forehead, to estimate pigmentation in an area with high exposure to sun,
and (2) the inner upper arm, for an area with low exposure to sun and presumably less skin pigment variability between seasons.

The Dermacatch provides readings of melanin and erythema, both reported as continuous integers. Colormeters have been shown to classify skin pigmentation more precisely than subjective assessments, and the Dermacatch appears to more reproducibly differentiate skin melanin from skin erythema compared with other devices. The distinction of melanin from erythema is important since erythema measurements are subject to short term variability based on external stimuli and the physical pressure of the device on the skin, whereas melanin measurements should have much less fluctuation over the short term. All data were recorded using REDCap mobile application tools.

**Statistical Analysis**

Measurements of each anatomic site were taken in triplicate; intrarater reliability of the 3 measurements was assessed by computing the intraclass correlation coefficient (ICC) (consistency type, single-rater, 2-way random-effects model). For remaining analyses, the median of the 3 measurements was used. The main analyses assessed skin melanin estimates and not erythema, with the rationale that melanin provided a more accurate representation of underlying skin pigmentation that was not subject to short term vasomotor alterations the way that the erythema estimates would be. The relationship between skin pigmentation and glaucoma was assessed in conditional logistic regression models adjusted for dichotomous terms for sex and calendar season, with season classified according to the time of skin pigment measurement as either the dry season (ie, September through May) or wet season (ie, June through August). Sample size calculations were based on the number of glaucoma patients that could feasibly be enrolled; including 75 glaucoma patients and 150 controls provided ~80% power to determine of a 20-unit difference between the 2 groups assuming a SD of 50 units (based on a previous study) and an α of 0.05. All statistical analysis for this study was performed in the statistical package R version 3.5.2 (R for Statistical Computing, Vienna, Austria).

**Ethics**

The study was approved by ethical review boards at the University of California, San Francisco and Addis Ababa University. Written informed consent was obtained from all participants. The described research adhered to the tenets of the Declaration of Helsinki.

**RESULTS**

During the study period 76 glaucoma cases and 152 controls without glaucoma were enrolled. As shown in Table 1, the demographic characteristics were similar in the 2 groups, but more control patients were enrolled during the rainy season. Most control patients were being seen for cataract (N=89; 59%) or external disease (N=27; 18%). Cases consisted mostly of exfoliative glaucoma (N=35/73 with available data; 48%), primary open-angle glaucoma (28/73; 38%) or primary angle closure glaucoma (6/73; 8%).

Intrarater reliability between triplicate measurements was high for melanin readings of the inner arm [ICC 0.99, 95% confidence interval (CI), 0.98-0.99] and forehead [ICC 0.97, 95% CI, 0.96-0.97], but slightly lower for erythema estimates (inner arm ICC 0.93, 95% CI, 0.91-0.94; forehead ICC 0.91, 95% CI, 0.89-0.92). A plot of the mean versus SD of the triplicate melanin measurements showed that while there was some variability between the 3 measurements, the amount of measurement error was not affected by the magnitude of pigment present (Fig. 1A). The median inner arm melanin estimate was moderately correlated with the median value from the forehead (Pearson R=0.66; Fig. 1B). Median values were used for all subsequent analyses.

Mean melanin measurements of the inner arm were 704 (SD 94) for cases and 694 (SD 93) for controls, and mean melanin measurements of the forehead were 778 (SD 50) for cases and 753 (SD 82) for controls (Table 2). In analyses adjusted for sex and season, melanin of the inner arm was not significantly associated with glaucoma (adjusted odds ratio, 1.15; 95% CI, 0.59-2.24, P=0.69) nor was melanin of the forehead (adjusted odds ratio, 1.24, 95% CI, 0.52-2.94, P=0.63) (Table 2). Subgroup analyses stratified by type of glaucoma revealed slight differences in the magnitude of association, but none of these secondary analyses was statistically significant (Table 2).

**DISCUSSION**

Population-based studies have shown that African descent is a risk factor for open-angle glaucoma. Those of African descent are more likely than Whites to have a variety of risk factors for glaucoma, including thinner central corneal thickness, beta-zone parapapillary atrophy, and higher intraocular pressure, and have also been shown to have faster progression of glaucoma. The reasons for the increased risk and severity of glaucoma among those of African descent remain unclear, but several studies have suggested that darker skin complexion may be associated with increased intraocular pressure—though these studies used a subjective assessment of skin color.

This study had several strengths. We used an objective assessment of skin melanin to more accurately determine whether skin melanin might be a risk factor for glaucoma. We restricted the study population to Ethiopian nationals, thereby limiting the likelihood that any relationship between skin pigment and glaucoma would be confounded by other
factors associated with skin complexion. We enrolled 2 controls per case to improve the statistical power of the study.

The main finding of the study was a lack of a significant association between skin melanin levels and glaucoma. Although the regression coefficients suggested that on average, individuals with higher levels of skin pigmentation were more likely to have glaucoma, the wide CIs meant that the observed results could also be consistent with the opposite conclusion. Subgroup analyses did not provide compelling evidence that any relationship between skin pigment and glaucoma might differ based on type of glaucoma (ie, primary open glaucoma or exfoliative glaucoma), although the relatively small numbers in each group made it difficult to show statistical significance.

The optimal way to assess for an individual’s pigmentation is unclear. We decided to measure skin melanin instead of iris melanin, rationalizing that we would observe a wider range of values in the skin and that skin measurements should be correlated with iris measurements. We tested 2 anatomic locations that we reasoned would have varying amount of sun exposure: the forehead and inner arm. We found that melanin measurements taken from the inner arm were more repeatable, which we speculate could have been due to the curvature of the forehead preventing close apposition of the Dermacatch sensor in some cases. The Dermacatch device captures both a melanin reading and an erythema reading; the erythema readings were less repeatable perhaps because the amount of pressure applied to the skin could result in changes to local blood vessels and resulting redness.

Limitations of this study should be noted. As in all case-control studies, bias could have resulted from the way controls were selected. Although we intended to enroll the matched controls within several days of the case to limit any temporal confounding, this proved difficult in practice due to constraints in manpower at the hospital. Instead, most of the controls were enrolled over a few-month period in the rainy season, meaning that the date of assessment could be a confounder in the analysis. However, the possibility of bias should be lower for the inner arm measurements since the lack of sun exposure at this anatomic site likely limits the amount of skin pigmentation variability throughout the year. The CIs of the primary analyses were fairly wide, suggesting that the study was too small to determine definitively whether skin pigmentation was associated with glaucoma, especially for the subgroups. Our regression

| TABLE 2. Comparison of Skin Melanin in Cases and Controls |
|----------------------------------------------------------|
| Melanin Measurement                                      |
| Skinsite        | Glaucoma Cases | Controls | Odds Ratio (95% CI)* | Crude | Adjusted† |
| All cases       | N              | Mean ± SD | N              | Mean ± SD |          |          |
| Inner arm       | 76             | 704 ± 94  | 152            | 694 ± 93  | 1.12    | 1.15 |
| Forehead        | 76             | 778 ± 50  | 152            | 753 ± 82  | 1.84    | 1.24 |
| POAG            | N              | Mean ± SD | N              | Mean ± SD |          |          |
| Inner arm       | 28             | 709 ± 63  | 56             | 685 ± 107 | 1.34    | 1.31 |
| Forehead        | 28             | 776 ± 43  | 56             | 744 ± 101 | 1.77    | 1.01 |
| Exfoliative     | N              | Mean ± SD | N              | Mean ± SD |          |          |
| Inner arm       | 35             | 714 ± 79  | 70             | 700 ± 81  | 1.26    | 1.03 |
| Forehead        | 35             | 775 ± 51  | 70             | 755 ± 63  | 2.06    | 1.13 |

*Conditional logistic regression grouped by age-matched group, expressed for each additional 100 units of melanin; positive values indicate increased skin melanin associated with increased odds of glaucoma.
†Adjusted for sex and season (ie, dry season vs. rainy season).
CI indicates confidence interval; Exfoliative, exfoliative glaucoma; POAG, primary open-angle glaucoma.
findings may be attenuated from the use of conditional logistic regression on the matched triads; however, this direction of bias is preferable to anticonservative bias that would have resulted from ordinary logistic regression. We did not perform subjective assessments of skin pigmentation, preventing any comparison of subjective and objective methods of skin pigmentation estimation in this particular patient population. However, previous studies have demonstrated similar devices to be more repeatable than subjective pigment assessments, which likely applies to the device used in this study. We were unable to find studies using the study colorimeter in other African populations, so it is difficult to place our findings in context. Finally, the generalizability of the study outside of this particular population is not clear. Patients with glaucoma enrolled in this study typically had advanced disease, since their glaucoma had to be severe enough to be scheduled for trabeculectomy. Moreover, Ethiopians are genetically distinct from other African populations, and previous population-based studies suggest a higher prevalence of glaucoma in west African populations than east African populations. The conclusions from this study may not apply to a non-Ethiopian population or those with less advanced glaucoma.

In conclusion, this study found the Dermacatch device to provide repeatable estimates of skin melanin, especially of the inner arm. The study failed to confirm earlier studies that had reported a positive correlation between darker skin complexion and glaucoma using subjective skin assessments. A larger study in a population with many types of glaucoma (eg, primary open-angle, primary angle closure, exfoliation glaucoma) would have more statistical power to determine the relationship between skin pigment and glaucoma.

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