A Correlation of Retinal Lesion Appearance and Distribution to CD4 Counts of Patients with Human Immunodeficiency Virus Using Ultrawide Field Scanning Laser Ophthalmoscope Images

Anna Maria F. Payawal-Lucero, MD[1], Paolo S. Silva, MD[2,3]

[1]Eye and Vision Institute, The Medical City, Pasig City, Metro Manila, Philippines
[2]Beetham Eye Institute, Joslin Diabetes Center, Boston, Massachusetts, USA.
[3]Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA

Correspondence: Anna Maria F. Payawal-Lucero, MD
Office Address: Department of Ophthalmology, The Medical City, Ortigas Avenue, Pasig City 1604, Metro Manila, Philippines
Office Number: +6329881000 local 6252
Email Address: annapie.payawal@gmail.com

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ABSTRACT

Objective: To identify retinal lesions through ultrawide field (UWF) images and correlate their presence, size, location, and number with the immunologic status of individuals living with human immunodeficiency virus (HIV)

Methods: This retrospective study reviewed UWF retinal images and CD4 counts of adult patients diagnosed with HIV. ImageJ software was used to annotate lesions and create heat maps. The distribution of lesions (hemorrhages, cotton wool spots, cytomegalovirus [CMV] lesions) was evaluated across 3 retinal zones: posterior pole, mid-periphery and far periphery. Statistical analyses were conducted using SAS version 9.4.

Results: The study included 44 eyes of 23 male HIV patients, with a mean age of 35 ± 9.3 years, and a mean CD4 count of 74 ± 145 cells/mm³. HIV retinopathy was present in 24 (54.5%) eyes and CMV retinitis in 6 (13.6%) eyes. Among eyes with HIV-related findings (N=30), 8 (26.7%) had hemorrhages, 19 (63.3%) had cotton wool spots, and 7 (23.3%) had both. Eyes with HIV retinopathy had significantly low CD4 counts (17 vs. 25 cells/mm³, p=0.0398), and eyes with CMV retinitis had even lower CD4 counts (9 vs. 22 cells/mm³, p=0.0133). Lesion annotations showed that the mean area covered by hemorrhages was 0.47 mm² (97.9% in the posterior pole), cotton wool spots was 0.73 mm² (96.0% in the posterior pole), and CMV lesions was 22.89 mm² (37.9% in the posterior pole, 35.9% in the mid-periphery, and 26.1% in the far periphery).

Conclusion: HIV retinopathy findings are predominantly located within 10 mm of the foveal center, while over 62% of CMV lesions are present outside this zone. This highlights the importance of evaluating the retinal periphery in high-risk patients. Regular monitoring using UWF imaging is recommended for HIV-infected individuals with low CD4 counts, to detect vision-threatening conditions like CMV retinitis.

Keywords: HIV; HIV retinopathy; CD4+; CMV retinitis; cotton wool spots; retinal hemorrhages; ultrawide field images

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Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) have been significant public health issues since their emergence in the 1980s. Despite advancements in antiretroviral therapy (ART), HIV continues to be a major global health challenge. Southeast Asia is the second most affected region, with the Philippines having one of the fastest-growing HIV epidemics.[1] According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), an estimated 68,000 Filipinos were living with HIV/AIDS in 2017. The Philippine National AIDS Council reported a dramatic increase in new diagnoses, with 32 new cases daily in 2018. The cumulative number of cases from January 1984 to July 2018 reached 57,134 representing a 3,147% increase over the past decade. The HIV/AIDS and ART Registry of the Philippines (HARP) recorded 8,533 new infections from January to September 2018, predominantly in males.[2]

HIV, a retrovirus that targets CD4+ T-lymphocytes, is transmitted through blood and body fluids. It causes widespread immunosuppression, leading to various complications across multiple organ systems, including the eyes. Ocular manifestations can be the first indication of systemic infection and become more prevalent as CD4+ T cell counts decrease.[3]

Approximately 70-80% of adults with HIV/AIDS experience ocular complications, which may be either infectious or non-infectious.[4] These complications can result from direct viral effects or opportunistic infections, leading to conditions such as microvascular retinopathy, cotton-wool spots, retinal hemorrhages, and cytomegalovirus (CMV) retinitis. These ocular conditions can significantly impact vision, with blindness being a severe consequence for some patients.[5]

This study aimed to identify retinal lesions through ultrawide field (UWF) imaging and correlate these findings with the immunologic status or CD4 counts of individuals living with HIV. We sought to determine if there was a specific pattern in the appearance, location, and number of lesions relative to CD4 count levels, providing insights into the relationship between ocular manifestations and immune status in HIV patients.

METHODS

This was a retrospective, cross-sectional study of consecutive adult patients diagnosed with HIV, who had both UWF fundus photographs and corresponding CD4 T-lymphocyte counts from May 1, 2017 to July 31, 2019. Demographic and CD4 counts were collected following manual review of patient charts. Approximate interval between CD4 T-lymphocyte count and UWF fundus photos was 3 months. Patients included in the study were 19 years old and above. The reading center evaluated all images and identified HIV retinopathy or CMV retinitis as the primary cause of the retinal findings. Eyes with other causes of uveitis or retinal vascular disease were excluded in the analysis. Additional exclusion criteria included ungradable UWF images due to dense media opacity.

The calculated sample size for this study was 44 eyes, determined using a one-sample inference reference table. This calculation was based on a significance level of 0.05, a power of 0.80, and an anticipated effect size of 0.50. These parameters were selected to ensure that the study would have adequate power to detect clinically meaningful differences in retinal outcomes associated with the conditions under investigation.

This study was approved by The Medical City Institutional Review Board.

Imaging and Image Evaluation

All UWF images were obtained by certified retinal photographers using the Optos California system (Dunfermline, Fife, UK; V5.1.11.118750) which provides mydriatic, simultaneous, stereoscopic, on-axis, non-steered 200-degree retinal images. Images were de-identified, labeled with study numbers linked to patient demographics and medical history, and evaluated at a centralized reading center under controlled standardized conditions. Monitors used for evaluation were calibrated according to the reading center's standards.

Images were projected stereographically using proprietary software from the manufacturer (Optos plc), based on ray tracing of each pixel using a

combined optical model of the Optos California and a Navarro UWF model eye. The foveal center was aligned to the center of each image, and a template of the retinal fundus was digitally overlaid based on fovea and optic nerve head locations. The extent and distribution of lesions were determined using concentric rings around the fovea, defining the posterior pole as <10 mm, midperiphery as 10-15 mm, and far periphery as >15 mm., as seen in **Figure 1**.

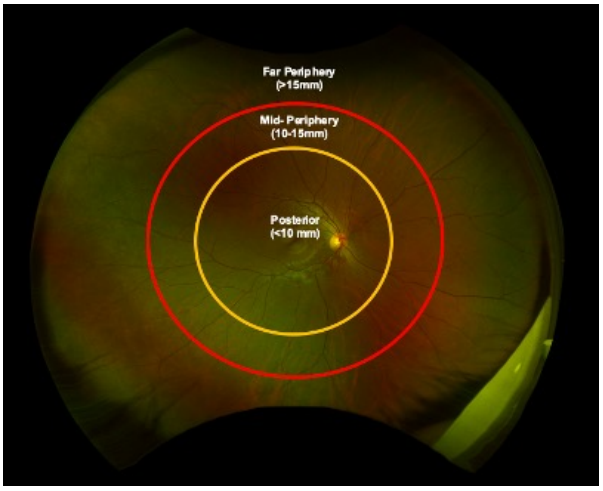


Figure 1. Zones for determination of retinal lesion distribution (all centered on the fovea): posterior (10 mm), mid-periphery (10-15mm), far periphery (>15mm), central macula (5mm) ([9])

Lesion Annotation[6]

Each UWF image was evaluated for hemorrhages, cotton wool spots, and CMV retinitis abnormalities using the Fiji distribution of ImageJ software version 1.48 (NIH, Bethesda, MD, USA). A masked, board certified grader (A.P.L.), experienced in evaluating UWF images, independently performed the evaluations and annotations, overseen by a retina specialist (P.S.S.) with expertise in HIV-related retinal changes. Lesions were annotated by drawing free-hand lines demarcating their extent and number. Annotated images were saved as projected and registered image masks, segmented using exact pixel coordinates corresponding to a normal retinal image.

The total area of lesions for each eye was calculated in square millimeters by summing the size of all pixels in the mask. This measurement used a tool implementing DICOM supplement 173 provided by Optos plc, defining individual pixel sizes by their image location and calculating retinal area using spherical trigonometry, ensuring accuracy independent of visual distortion.[7]

Statistical Analysis

Nonparametric analyses, specifically Wilcoxon rank-sum tests, were used to compare continuous variables between groups with and without HIV-related lesions and CD4 count level. Additionally, applied logistic multivariate regression models were created to adjust for potential confounders. Repeated measures were used to account for correlations between eyes from individual participants. Statistical significance was set at $p < 0.05$. All analyses were performed using SAS software version 9.4 (SAS Inc, Cary, NC, USA).

RESULTS

This study included 44 eyes of 23 HIV-positive patients. The demographic characteristics are reported in **Table 1**. The mean age was 35 ± 9.3 years (range 26-75), and all patients were male (100.0%). The mean CD4 count was 74 ± 145 cells/mm³. HIV retinopathy was present in 24 (54.5%) eyes, and CMV retinitis was present in 6 (13.6%) eyes.

Table 1. Age and CD4 count in eyes with and without HIV-related ocular findings

Characteristic	All retinal lesions	HIV Retinopathy	CMV Retinitis	No HIV-related Retinal Findings
Mean age ± SD, in years	35.26 ± 10.39	33.43 ± 8.11	41.83 ± 16.36	35.21 ± 7.07
Range	26-75	26-56	33-75	26-48
Mean CD4 count ± SD, in cells/mm³	43.88 ± 22.54	19 ± 132.02	3.33 ± 5.12	130.28 ± 180.58
Range	0-500	0-500	0-13	0-500

HIV – human immunodeficiency virus; CMV – cytomegalovirus; SD – standard deviation

We conducted the analysis at the eye level, recognizing that patients could present findings in only one eye. In this cohort, 30 eyes (68.2%) from 17 patients (74.0%) exhibited HIV-related findings. Among the 30 eyes with HIV-related findings, 8 eyes (26.7%) had hemorrhages, 19 eyes (63.3%) had cotton wool spots, and 7 eyes (23.3%) displayed both hemorrhages and cotton wool spots. Additionally, 14

eyes (31.8%) showed no retinal abnormalities upon review of the ultrawide field (UWF) images.

Comparison of CD4 Counts

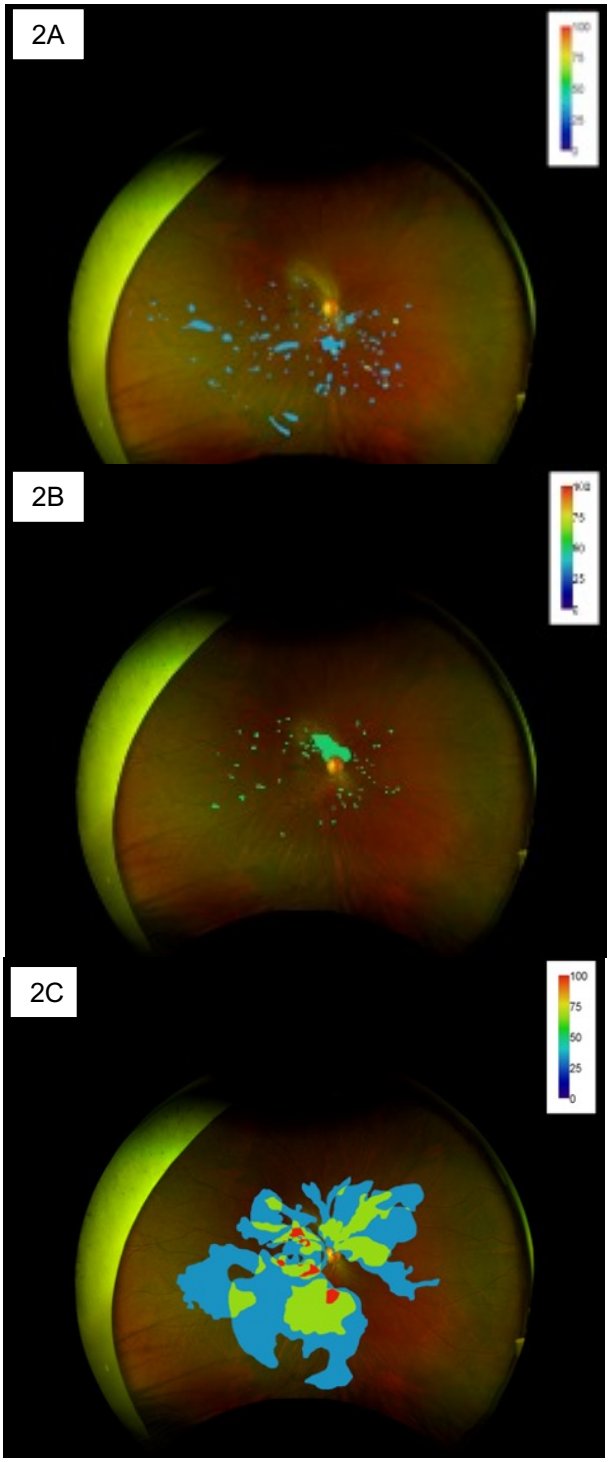
Eyes with HIV retinopathy had significantly lower CD4 counts compared to those without retinal changes (17 vs. 25 cells/mm³, p = 0.0398). Similarly, eyes with CMV retinitis had even lower CD4 counts compared to those without retinal changes (9 vs. 22 cells/mm³, p = 0.0133).

Lesion Area Analysis

The distribution of the individual lesions are reported in **Table 2**. Locations of individual retinal lesions are seen in **Figure 2**. The mean area covered by hemorrhages was 0.47 mm², predominantly in the posterior pole (0.45 mm² or 97.9%), with minimal presence in the mid-periphery (0.02 mm² or 2.0%) and none in the far periphery. Cotton-wool spots covered a mean area of 0.73 mm², with 0.65 mm² (96.0%) in the posterior pole, 0.07 mm² (3.4%) in the mid-periphery, and 0.01 mm² (0.6%) in the far periphery. CMV lesions covered a mean area of 22.89 mm², distributed across the posterior pole (11.13 mm² or 37.9%), mid-periphery (8.14 mm² or 35.9%), and far periphery (3.62 mm² or 26.1%). No statistically significant correlation was found between lesion size and CD4 counts (p=0.36). Larger lesion sizes were associated with lower CD4 counts, particularly in cases of CMV retinitis (Hemorrhages r = -0.14557, p = 0.5521; Cotton-wool Spots r = -0.32095, p = 0.1803; CMV lesions r = -0.45358, p = 0.3663) but these findings were not statistically significant.

Table 2. Mean areas of lesion distribution over each retinal zone

Lesion Type	Total area of lesions in mm²	Posterior Pole 10mm	Mid Periphery 10-15mm	Far Periphery >15mm	Central Macula 5mm
All lesions	24.88	13.05 (84.6%)	8.20 (9.6%)	3.62 (5.6%)	2.87 (19.9%)
Hemorrhages	0.47	0.45 (97.9%)	0.02 (2.0%)	0	0
Cotton-wool spots	0.73	0.65 (96.0%)	0.07 (3.4%)	0.01 (0.6%)	0.26 (24.10%)
CMV lesions	22.89	11.13 (37.9%)	8.14 (35.9%)	3.62 (2.61%)	2.04 (5.6%)



Figures 2. Annotated Heat Maps of Retinal Lesions, (A) Retinal cotton-wool spots, (B) Retinal hemorrhages, (C) Composite of retina exudates and areas of retinitis. Color scale represents frequency within the area.

DISCUSSION

This retrospective study analyzed 44 eyes from 23 male patients diagnosed with HIV, exploring the correlation between retinal lesion characteristics and CD4+ T-lymphocyte counts using UWF scanning laser ophthalmoscope images. The study results contribute valuable insights into the ophthalmic manifestations of HIV and their association with the immune status of affected individuals.

The study reports that 54.5% of the eyes show fundus photographic signs of HIV retinopathy, while 13.6% showed signs of CMV retinitis. The significant presence of these conditions underscores the critical need for regular ocular examinations in HIV-infected individuals. The findings align with previous research indicating that ocular manifestations of HIV become more prevalent as CD4+ T-cell counts decrease.[8] Notably, eyes with HIV retinopathy and CMV retinitis had substantially lower CD4 counts compared to those without retinal changes, emphasizing the importance of monitoring immunologic status in managing ocular health in HIV patients.

The study found that HIV retinopathy lesions, such as cotton-wool spots and hemorrhages, were predominantly located within 10 mm of the foveal center. This distribution pattern suggests that these microvascular changes are more likely to occur in the central retina, possibly due to its higher vascular density and metabolic demand.[9] In contrast, CMV lesions were more widely distributed, with over 62% located outside the 10 mm zone from the foveal center. This peripheral distribution highlights the importance of comprehensive retinal examination, including the periphery, to detect potentially sight-threatening conditions.

The findings of this study underscore the necessity of regular ophthalmic screenings for HIV-infected individuals, particularly those with low CD4 counts. UWF imaging, which captures up to 200 degrees of the retina, is an invaluable tool in identifying both central and peripheral retinal lesions. Early detection and treatment of retinal complications can prevent significant visual impairment, improving the quality of life for HIV patients.

The study also highlights the potential benefit of using smaller angle fundus cameras (i.e. 40-60 degrees) in community screening programs where UWF imaging may not be available. These tools can provide a sufficient assessment of the retina, facilitating early detection and intervention in resource-limited settings.

The pathogenesis of HIV retinopathy remains multifactorial, involving direct viral damage, immune complex deposition, and microvascular abnormalities. The predominance of cotton-wool spots and hemorrhages in the posterior pole may be attributed to ischemic damage resulting from impaired retinal blood flow and increased blood viscosity.[10] In contrast, CMV retinitis follows retinal vasculature, leading to a more widespread distribution of lesions, reflecting the systemic dissemination of the virus in severely immunocompromised patients.

Future research with larger sample sizes is warranted to validate these findings and further elucidate the relationship between retinal lesion characteristics and immunologic status. Strengthening screening policies and ensuring access to advanced imaging techniques will enhance the early detection and treatment of ocular manifestations in HIV-infected individuals, ultimately improving their visual and overall health outcomes.

The findings of this study must be considered in light of our main limitation which is a small sample size. If we were to extend this study or conduct a similar one, more eyes would mean a more favorable representation of our population, for stronger statistical analyses.

In summary, this study emphasizes the critical role of fundus imaging in the management of HIV-infected individuals. Hemorrhages and cotton-wool spots are predominantly found within the central 10 mm of the retina, whereas CMV lesions are more peripherally distributed, necessitating comprehensive retinal evaluations. Given the inverse correlation between CD4 counts and the severity of retinal lesions, regular ophthalmic evaluations should be an integral part of the clinical management of HIV patients, especially those with low CD4 counts.

Implementing thorough screening guidelines and follow-up protocols, in collaboration with primary care physicians and ophthalmologists, is essential to prevent vision-threatening complications.

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