THE RELIABILITY OF ANTERIOR SEGMENT LESIONS AS INDICATORS OF ONCHOCERCAL EYE DISEASE IN GUATEMALA

KEVIN L. WINTHROP,* ROBERTO PROAÑO, ORLANDO OLIVA, BYRON ARANA, CARLOS MENDOZA, ALFREDO DOMINGUEZ, JOSEF AMANN, GEORGE PUNKOSDY, CARLOS BLANCO, ROBERT KLEIN, MAURICIO SAUERBREY, AND FRANK RICHARDS

Division of Parasitic Diseases, US Centers for Disease Control and Prevention, Atlanta, Georgia; Casey Eye Institute, Oregon Health Sciences University, Portland, Oregon; Medical Entomology Research Unit, US Centers for Disease Control and Prevention and Universidad del Valle, Guatemala City, Guatemala; Program for the Elimination of Onchocerciasis in the Americas, Guatemala City, Guatemala

Abstract. World Health Organization certification criteria for onchoceriasis elimination use anterior segment eye lesion prevalence as an indicator of mass ivermectin treatment program success. Lesions either contain visible microfilaria (noninflammatory punctate keratitis [PK] or microfilariae in anterior chamber [MFAC]), or microfilaria obscured by inflammation (inflammatory PK). To assess the utility of these disease indicators, two experienced ophthalmologists independently examined persons from endemic (N = 325) and nonendemic (N = 348) Guatemalan communities. Thirty-six (11.1%) and nine (2.6%) persons from endemic and nonendemic areas respectively had lesions found by either ophthalmologist (prevalence ratio = 4.3, 95% CI 2.1–8.8, P < 0.001). All lesions in nonendemic areas were inflammatory PK in whom no persons were seropositive for onchocerciasis. Overall, observer agreement was moderate (Kappa = 0.49), and most (61%) discordance occurred with inflammatory PK lesions. Our findings suggest that inflammatory punctate keratitis is neither a specific nor a reliable indicator of onchocercal eye disease. Future prevalence surveys should rely upon noninflammatory lesions as disease indicators.

INTRODUCTION

Onchocerca volvulus causes skin and eye lesions in humans (onchocerciasis). The parasitic worms are encased in subcutaneous nodules, and female worms can release large numbers of pre-larval organisms (microfilariae) that migrate to, and damage, corneal and retinal tissues. Regular administration of ivermectin (Mectizan, donated by Merck & Co., Haarlem, The Netherlands) kills the microfilariae, temporarily diminishes the fecundity of adult worms, and prevents the development of ocular lesions in infected persons.1–4 The Onchocerciasis Elimination Program for the Americas (OEPA) is a regional initiative with the goals of eliminating the ocular morbidity and transmission of onchocerciasis throughout the region.5,6 OEPA estimates that about 500,000 people reside in areas at risk of infection in six countries in the Americas: Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela.7 The OEPA strategy is to encourage and strengthen ministries of health in the six endemic countries to provide sustained mass ivermectin treatment every 6 months. National treatment programs report having reached at least 85% of persons eligible for treatment who reside in all communities known to be endemic for onchocerciasis for the past 3 years.

To certify the elimination of onchocerciasis ocular morbidity from a geographic focus, the World Health Organization (WHO) requires programs to demonstrate the “absence of reversible lesions in the anterior segment of the eye (punctate keratitis, microfilariae in the anterior chamber).” The “absence of reversible lesions” is defined by WHO as a lesion prevalence of less than 0.1% (1 case per thousand).8 Onchocercal punctate keratitis (PK) and microfilaria in the anterior chamber (MFAC) of the eye are transient or “reversible” lesions in the anterior segment of the eye that should become less frequent in the onchocerciasis endemic areas over time if adequate population coverage with ivermectin is achieved.9 Microfilariae in the anterior chamber are seen as small mobile worms swimming in the convection currents of aqueous humor. PK can be seen in a progression of stages ranging from early noninflammatory lesions in which clearly visible microfilaria are present within the corneal stroma, to later stage lesions in which microfilariae are obscured by nummular, stromal opacities containing numerous “snowflake-like” inflammatory infiltrates. The OEPA initiative, in consultation with regional experts, has developed a staging system for PK that has been used in surveys of sentinel villages around the region that are monitored ophthalmologically every 3 years to demonstrate impact of the mass treatment programs. MFAC and PK of stages A and B are considered noninflammatory lesions with clearly visible microfilariae. PK of stages C, D, and E are inflammatory lesions in which microfilaria are engulfed by inflammatory cells.

Recent surveys in Guatemalan sentinel villages suggest that PK prevalence has remained unexpectedly elevated despite high reported ivermectin treatment coverage.9 These findings prompted OEPA consultant ophthalmologists to question the specificity of the PK lesion and its use as a morbidity indicator.9 Because of these concerns, we undertook a study to evaluate the specificity of the various anterior segment manifestations of onchocerciasis in Guatemala. In addition, we sought to better understand how interobserver variability might impact the reproducibility of PK and MFAC prevalence measures in OEPA ocular morbidity prevalence surveys, and to determine if new recommendations should be made to WHO and OEPA pertaining to the certification of Onchocerciasis morbidity elimination in the Americas.

METHODS

The study was approved by the Ministry of Health of Guatemala and deemed non-human subject research by the institutional review board of the Centers for Disease Control and Prevention, the Universidad del Valle de Guatemala, and the Ministry of Health of Guatemala.
Study site and subject recruitment. We selected eight communities for study. The four ‘endemic’ communities were located in the central endemic zone (CEZ) for onchocerciasis in Guatemala, near Lake Atitlan. These were coffee-growing ‘fincas’ (farms) located at an elevation between 900 and 1200 meters. Four ‘nonendemic’ communities, also coffee farming communities, were selected that were similar in size, elevation, ethnic, and socioeconomic status as the endemic communities. We conducted anterior segment ophthalmologic surveys in these eight communities during 2 weeks in October 2004. The study was not blinded and the ophthalmologists conducting the anterior segment examinations knew if they were in an endemic or nonendemic area.

In each community, local leaders were asked to identify and encourage all permanent, non-migratory residents over the age of 5 years to participate. Informed consent was obtained for interested participants, and in the case of children, parents were asked to provide consent. All subjects were interviewed using a standardized form to collect information regarding age, sex, length of residence, and place of birth.

Examinations. Each participant was examined twice, once by each ophthalmologist (A and B), both experts in ocular onchocerciasis and longtime consultants to OEPA. Prior to the study, the two observers were standardized and asked to grade corneal and anterior chamber exams according to the standard PK grading system used by OEPA as described later in this article.10 The examinations were performed independently in separate, darkened, indoor examination areas and the order of examination was allowed to vary between the two observers. Observers A and B each brought a personal slit-lamp for use; a maximum slit-lamp magnification of 32X was used by observer A and 25X by observer B. Before examination with each ophthalmologist, patients lowered their heads between their knees for 5 minutes to facilitate microfilariae descent to their most visible location in the anterior chamber. Using the slit-lamp, examiners assessed the anterior chamber of each eye for the presence of microfilariae and the corneal surface for PK. No fluorescein, Rose-Bengal, or other corneal stains were used. Patient pupils were not dilated and a retina examination was not performed.

Observers A and B recorded the numbers and location of MFAC and PK on identical but separate data collection forms, and there was no discussion of results until after they had been recorded. PK lesions were classified by stage used in OEPA sentinel village examinations10. Stage A (microfilariae live/coiled without inflammation); Stage B (microfilariae straightened/dying without inflammation); Stage C (inflammation around complete microfilariae); D (inflammation around fragmented microfilariae); and Stage E (inflammation only with absence of visible microfilariae fragments). Stage E inflammation was specified as being nummular, in the corneal stroma, with numerous small, soft, “snowflake-like” points of inflammation. Stages A/B (noninflammatory lesions) and Stages C/D (inflammatory lesions) were recorded as a combined result.

Serology. All patients gave 0.25 mL of blood for serology testing. Blood was collected by finger stick and placed on filter paper. Filter paper samples were dried and stored at room temperature until reaching the Medical Entomology Research Unit laboratory (Guatemala City, Guatemala). Two 6-mm punches of saturated filter paper were placed in a PBS-Tween 0.05% and BSA 5% buffer and eluted overnight at 4°C. The elution was then run in duplicate in a standard ELISA to detect IgG4 antibodies against the OV-16 recombinant antigen.11 Positive controls included serum from patients with a parasitological diagnosis of onchocerciasis.

Analysis. Data were double entered into EPI INFO (version 3.2, Atlanta, GA). Prevalence of anterior segment onchocercal eye disease was determined for endemic and nonendemic areas using two case definitions:

Case-patient definition (1), Inflammatory or noninflammatory onchocercal eye lesions: case-patients must have at least one eye with either inflammatory lesions PK stages C/D or E, or noninflammatory lesions MFAC and PK stages A/B.

Case-patient definition (2), Noninflammatory onchocercal eye lesions: case-patients must have at least one eye with noninflammatory lesions MFAC or PK stages A/B (i.e. inflammatory lesions not accepted as disease).

Case-patient prevalence using both case definitions were calculated for each ophthalmologist independently, as well as together taking into account observer agreement or disagreement. For instance, calculating a given prevalence using a case definition where either observer diagnosed onchocerciasis offered the highest prevalence estimate. Conversely, using a case definition that necessitated that both observers diagnose onchocerciasis in a given patient offered the most conservative prevalence measure. Case-patient prevalences were compared between endemic and nonendemic zones using STATCALC (EPI INFO, version 3.2). Yates corrected two-tail P values were used for the comparisons. In cases of small cell sizes (< 5), Fisher exact P values were used.

Interoobserver variability with respect to case-patient diagnosis was analyzed with a standard Cohen Kappa measurement. To further assess discordance, all patients diagnosed with onchocerciasis by either observer A or B were identified. In those cases in which the observers did not agree on the diagnosis, the individual eye findings of both observers were reviewed and the lesion of disagreement was recorded.

RESULTS

A total of 673 persons were enrolled; 325 from endemic communities and 348 from nonendemic communities. Subjects from endemic and nonendemic communities were similar with respect to age and sex. The median age of subjects was 23 years in both endemic (range 7–80 years) and nonendemic communities (range 7–89 years). One hundred and sixty (49.2%) of endemic patients were male, compared with 150 (43.1%) of nonendemic study subjects (P = 0.065).

Endemicity in the communities based on serology. OV-16 serologic studies confirmed the difference in onchocerciasis endemicity in the two study areas. In the endemic communities, 113 (34.8%) of patients had serologic evidence of Onchocerciasis whereas only one (0.3%) person in the nonendemic communities was seropositive (P < 0.001).

Prevalence of patients with anterior segment lesions. The prevalence of persons with anterior segment lesions was significantly higher in endemic areas. Using case-patient definition (1), 36 (11.1%) persons in the endemic zone were found to have lesions by at least one observer, compared with 9 (2.6%) persons in nonendemic areas (prevalence ratio = 4.3, 95% CI 2.1–8.8, P = < 0.001) (Figure 1). Prevalence varied by ophthalmologist. In the endemic area, observer A found 31 (9.5%) persons with anterior segment lesions versus 17 (5.2%) for observer B (prevalence ratio = 1.8, 95% CI 1.03–
The percent of patients diagnosed by each observer independently, diagnosed by at least one observer (observer A or B), or diagnosed by both observers in agreement (observers A and B).

3.23, \( P = 0.05 \). The difference in prevalence measures in the endemic zone between the two observers was attributable to a much higher number of patients diagnosed with PK stage E by observer A (Figure 2A).

Using case-patient definition (2), prevalence of anterior segment disease was zero (0%) in nonendemic areas, and in endemic areas was similar between observers: 4 (1.2%) patients for observer A and 4 (1.2%) for observer B.

Microfilariae in anterior chamber and lesion stage prevalence. Microfilariae were observed in the anterior chamber in just four subjects and only in the endemic area. Similarly, PK lesions of type A/B were found only in the endemic area. PK lesions of type C/D and E, while much more prevalent in the endemic areas (Figure 2A) were also found in nonendemic areas (Figure 2B). The difference in lesion prevalence between endemic and nonendemic zone reached statistical significance (\( P < 0.05 \)) in only three instances: persons with E lesions diagnosed by observer A, 24 (7.4%) patients in the endemic zone versus 5 (1.4%) in the nonendemic zone; persons with E lesions diagnosed by observer B, 9 (2.8%) patients in the endemic zone versus 2 (0.6%) in nonendemic zone; persons with C/D lesions diagnosed by observer A, 7 (2.1%) patients in the endemic area versus 0 (0%) in the nonendemic zone.

In nonendemic areas, only 9 subjects were found to have PK lesions, all of which were inflammatory in nature. Observer A diagnosed five patients with E lesions, and observer B diagnosed three patients with C/D lesions, and two with E lesions. (Note: observers agreed on only 1 patient, hence 9 total patients were found to have lesions.) All of these persons denied a history of living in endemic areas or of having skin nodules, and all were seronegative for onchocercal infection.

Interobserver reliability. Concordance between observers was high no matter which case definition was used. For the case definition (1), 641 of 673 (95.2%) of patients were classified similarly (presence or absence of anterior segment disease) by both observers, although a Kappa measurement of 0.42 [95% CI 0.26–0.59] indicated only moderate agreement beyond chance. The high concordance was driven by the fact that most patients had negative exams (93.3%). Interobserver agreement was similar when the case-patient definition (2) was considered (kappa = 0.49 [95% CI 0.07–0.92], concordance = 98.8%).

In persons with onchocercal lesions identified, disagreement between the expert ophthalmologists was common, regardless of whether the lesions were inflammatory or not (Table 1). Among the total of 45 patients diagnosed with anterior segment disease by either observer A or B using the broadest case definition (1), a total of 69 eyes were found to have PK lesions; however, in 49 (71%) of these eyes, the two observers disagreed with regard to the staging of the corneal pathology or in noting the presence of MFAC. Most often, disagreement occurred when there were inflammatory lesions, Stages C/D or E. In some cases, this meant disagreement between distinguishing a C/D lesion from an E lesion, or an E lesion from a non-oncho “other” lesion. In other cases, this disagreement represented the failure of one observer to observe the lesion. Similarly, the likelihood of disagreeing upon the presence of the noninflammatory lesions MFAC and PK stage A/B was also high.

Serology results in patients with eye lesions. Of the seropositive patients, 19 (17%) had onchocercal eye lesions (as seen by either observer) at the time of examination. Among endemic community subjects, persons with lesions were significantly more likely to be seropositive than seronegative.

### Table 1

<table>
<thead>
<tr>
<th>Observer</th>
<th>MFAC (n = 4)</th>
<th>A/B (n = 2)</th>
<th>C/D (n = 8)</th>
<th>E (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFAC (n = 3)</td>
<td>1 (17%)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (n = 3)</td>
<td></td>
<td>2 (67%)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/D (n = 12)</td>
<td></td>
<td></td>
<td>3 (19%)†</td>
<td></td>
</tr>
<tr>
<td>E (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td>5 (13%)†</td>
</tr>
</tbody>
</table>

† The number of eyes with each type of anterior segment lesion as diagnosed by observers A (first row) and B (first column). Total eyes examined n = 1345 (673 patients).

†† Number (percent) of eyes with concordant diagnosis for each lesion type. Percent calculated as the number of eyes with an anterior segment lesion agreed upon by both observers divided by the total number of eyes with lesions diagnosed by either observer alone.
(for observer A, RR = 2.9, 95% CI 2.0–4.4, P < 0.01; for observer B, RR = 2.3, 95% CI 1.3–4.0, P = 0.01; in cases of observer agreement, RR = 2.9, 95% CI 1.6–5.1, P < 0.01). However, a substantial percentage of case-patients in endemic areas were found to be seronegative, including one of the four patients with MFAC, and nearly half of patients with other lesion types. Age was a significant predictor of seropositivity: the median age of seropositive and seronegative persons was 34 and 15 years, respectively (P < 0.01). Among endemic community members, in a logistic model controlling for the effect of age, having PK of any stage or MFAC found by at least one observer remained significantly associated with seropositivity (adjusted OR = 2.4, 95% CI 1.2–5.0, P = 0.02).

DISCUSSION

We examined patients from similar communities in endemic and nonendemic areas for onchocerciasis in the highlands of Guatemala. In endemic areas, we found serologic evidence of infection in over one third of persons and inflammatory or noninflammatory microfilarial lesions in as many as 11% of subjects. Not surprisingly, nonendemic areas were virtually devoid of seropositive persons, yet a small number of persons (2.6%) were found to have inflammatory lesions in these areas, suggesting the possibility that inflammatory PK lesions could be associated with disease processes other than onchocerciasis. In addition, our study demonstrated a high degree of interobserver disagreement in diagnosing onchocercal lesions, particularly inflammatory PK lesions. For these reasons, we believe inflammatory PK lesions as currently defined and graded by our observers are not a reliable indicator of onchocercal eye disease, and therefore should not be used to assess the impact of OEPA.

Given the visibility of the complete microfilaria in MFAC and noninflammatory PK, it is easy to conclude that these lesions are caused by onchocerciasis. The etiology of inflammatory PK stages C/D and E, however, is less clear. Although we found inflammatory PK lesions to be far more prevalent in endemic areas, they were the only type of PK lesion found in nonendemic area. In each instance, the patient had no serologic evidence of onchocerciasis and no history of dermal nodules or previous exposure to onchocerciasis. Given the low sensitivity of our serologic test, we could not completely rule out onchocercal infection in these individuals, but it was likely that some or all of these inflammatory lesions represented false-positive eye findings for onchocerciasis. Further, the observed 2.6% background prevalence of these inflammatory PK lesions in nonendemic areas suggests that the WHO certification criteria for disease elimination of one new case per 1000 patients is not achievable if inflammatory PK lesions are considered anterior segment sequelae of onchocerciasis.

To evaluate the impact of ivermectin treatment, sentinel communities within onchocerciasis endemic areas undergo repeated cross-sectional ophthalmologic surveys at three yearly intervals. Because these surveys frequently use different examiners, our study suggests that repeated prevalence assessments could vary widely due to observer disagreement. Such variability would complicate the assessment of treatment program impact. In our study, agreement beyond chance between observers was not high. In persons with a lesion diagnosed by one observer, the other observer was more likely to disagree than agree on the presence or staging of that lesion. Most of this disagreement involved inflammatory PK lesions (i.e., distinguishing a C/D lesion from an E lesion, or an E lesion from a non-onchocercal punctate opacity). To minimize the potential variability in future surveys that could occur due to such observer disagreement, sentinel regions should attempt to use the same observer over time. In addition, the more prevalent, but less specific, inflammatory PK lesions should be removed from the case definition of onchocercal anterior segment disease. Although removing these lesions from the case definition in our study did not significantly improve observer agreement, it did cause prevalence estimations to be lower and similar for each observer.

We used the OV-16 serology to both define our endemic and nonendemic areas, and to investigate OV-16 as a diagnostic tool for the eye findings in our study. The near absence of seropositivity in nonendemic areas, combined with the high prevalence of seropositivity in endemic areas, suggested this assay to be a highly specific indicator of onchocercal infection. This is consistent with previous OV-16 studies. However, the high specificity of this assay apparently came at a cost with regard to sensitivity. A large and similar percentage of persons with MFAC and PK of all stages in the endemic area were found to be seronegative. Thus, OV-16 is not a reliable test for distinguishing patients with onchocercal eye disease from other etiologies in Guatemala. It is unclear why the OV-16 appeared so poorly sensitive in our study, although repeated treatments with ivermectin might diminish the assay's sensitivity.

Our study was potentially limited by observer bias, as both ophthalmologists were aware of when they were examining patients inside and outside of the endemic zones. This potential bias, however, should have made it less likely that observers would diagnose corneal lesions as onchocercal PK in nonendemic areas. We were also potentially limited in our ability to compare results between observers, as the examination conditions were not completely identical. The difference in maximum slit-lamp magnification used by the two ophthalmologists could have contributed to some interobserver discordance, particularly between D and E lesions where it was often difficult to distinguish microfilarial fragments from surrounding inflammatory infiltrate. Lastly, combining PK stages C and D lesions into a single recorded category prevented us from assessing any potential difference in specificity or interobserver agreement between these two lesion types. This was unfortunate, because stage C lesions are morphologically quite distinct with microfilariae intact and much more visible than in stage D lesions making it a potentially more reliable indicator of disease activity.

In summary, our study suggests that only MFAC and noninflammatory PK lesions should be used in onchocerciasis eye disease prevalence surveys. Restricting the definition of PK to the noninflammatory stages A and B would offer a more specific and reproducible indicator of onchocercal ocular disease activity, thereby improving PK's usefulness as an indicator tool in the elimination process.

Future surveys assessing elimination progress should use observers well trained in the recognition of PK and MFAC, and in areas where repeated surveys will be undertaken, efforts should be made to use the same observer.
Received February 17, 2006. Accepted for publication August 10, 2006.

Authors’ addresses: Kevin L. Winthrop, Casey Eye Institute, Oregon Health Sciences University, 3375 Terwilliger Blvd., Portland, Oregon. Roberto Proaño˜o, Orlando Oliva, Alfredo Domínguez, and Mauricio Sauerbrey, Programa para la Eliminación de la Oncocercosis en las Américas (OEPA), 14 Calle 3-51, Zona 10, Edificio Murano Center, Oficina 1401, Ciudad de Guatemala 01010, Guatemala. Byron Arana, Carlos Mendoza, and Robert Klein, Medical Entomology Research Unit (MERTU), AMEMB/HHS/MERTU/G, Unit 3321, APO AA 34024-3321, Miami, FL. Josef Amann, George Punkosdy, and Frank Richards, US Centers for Disease Control and Prevention, Centers for Infectious Disease, Division of Parasitic Diseases, 4770 Buford Hwy., MS F-22, Atlanta, GA 30341. Carlos Blanco, Ministerio de Salud Pública y Asistencia Social, 6ª Calle 3-45, Zona 11, Guatemala City, Guatemala.

Reprint requests: Kevin L. Winthrop, Casey Eye Institute, Oregon Health Sciences University, 3375 S.W. Terwilliger Boulevard, Portland, OR 97239-4197, E-mail: winthrop@ohsu.edu

REFERENCES