Reliability of clinical diagnosis in identifying infectious trachoma in a low-prevalence area of Nepal

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The WHO Alliance for Global Elimination of Trachoma by 2020 has increased the need to identify ocular chlamydial infections by clinical examination in areas of both high and low prevalence. The relationship between clinically active trachoma (as defined by clinical examination) and chlamydial infection is known for areas with hyperendemic trachoma, but not for areas with a low prevalence of the clinical disease. In the present study, we examined, photographed, and DNA tested the conjunctivae of children in the Surkhet district of mid-western Nepal, an area known to have a low prevalence of clinically active trachoma. Although 6% of the children aged 10 years and under were found to have clinically active trachoma, none were found to have chlamydia infection by the most sensitive DNA amplification tests available. A very low prevalence of clinically active trachoma is not necessarily evidence of the presence of chlamydial infection. Therefore, the WHO policy of not recommending an intensive trachoma control effort when the prevalence of clinically active trachoma is less than 10% in children is appropriate for this area of Nepal.

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Introduction

Trachoma is the leading cause of preventable infectious blindness worldwide and is endemic in poor, dry regions of Africa, Australia, South America, and South-East Asia. Children in endemic areas are repeatedly infected with ocular serovars of *Chlamydia trachomatis*, the causative agent of trachoma. Progressive scarring from these infections causes a cascade of effects: entropion, trichiasis, corneal infections, corneal scarring and, ultimately, blindness (*1*). WHO has recently launched the initiative Alliance for Global Elimination of Trachoma by the year 2020 (*2*). This programme is dependent on identification of communities where blinding trachoma is present and individuals within these communities in need of treatment. Although novel DNA amplification techniques such as the polymerase chain reaction (PCR) and the ligase chain reaction (LCR) are the most sensitive tests for diagnosing infection (*3*), they are relatively expensive to perform, do not give immediate results, and are not readily available in areas of the world where trachoma is prevalent (*4*). Thus, the need to accurately identify ocular chlamydial infection by clinical examination is very important.

Studies in the United Republic of Tanzania and the Gambia have provided some data on the relationship between clinically active trachoma, as identified by examination according to WHO criteria, and chlamydial infection, as determined by DNA amplification tests. Infection without clinically active disease, and clinically active disease without infection are relatively common. In one area of the United Republic of Tanzania where the prevalence of clinically active trachoma among 1–7-year-olds was 48%, the sensitivity and specificity of clinical examination in identifying infection were 79% and 61%, respectively (*5*). In an area of the Gambia where the prevalence of clinically active trachoma among children aged $\leqslant$10 years was 31%, clinical examination was 77% sensitive and 89% specific in identifying infection (*6, 7*; R. Bailey, personal communication, 1998). Since both the sensitivity and the specificity of a given test can vary with the prevalence of the disease (*8*), this relationship

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between clinically active trachoma and chlamydial infection may be different in areas with a significantly lower prevalence of clinical disease.

WHO has declared Nepal to be a country of high priority for trachoma control (*2*). An extensive epidemiological survey carried out in 1981 revealed that trachoma was the second leading cause of blindness in Nepal and that 31% of the children aged ⩽10 years in the Bheri zone had clinically active trachoma (*9*). Subsequently, the prevalence of active trachoma has been markedly reduced to 9.7% and 7.0%, respectively, in the Banke and Bardia districts of the Bheri zone. This decrease is presumably due to economic development and to the intensive community-based education and treatment programmes of the Nepal Netra Jhoti Sangh, the Nepalese Red Cross, and the Swiss Red Cross (*10*). The Surkhet district of Bheri zone has not been surveyed recently, but correction of trichiasis from trachoma is the most frequent outpatient surgery performed at the regional eye care centre in Birendranagar (B. Shreshta, personal communication, 1997); thus infectious trachoma has previously been prevalent. Small-scale, rapid assessment surveys of the area have revealed that clinically active trachoma is now present but at a low prevalence. This article reports on a study undertaken to determine whether conjunctival examination predicts the presence of *C. trachomatis* in an area with a low prevalence of active clinical disease.

Methods

Population

Six villages, chosen arbitrarily, but with consideration for their geographical diversity, were selected in the Surkhet district of Bheri zone. The inhabitants of five of the villages were predominantly Tharu, an ethnic group found to have the highest prevalence of trachoma by the 1981 Nepal Blindness Survey (*9*). The inhabitants from the sixth village were predominantly from the ethnic group known as the "hilly people". The chief of each village was contacted and the risks and benefits to children entering the study were explained.

All children aged 1–10 years in each of the six villages were traced and requested to undergo a clinical examination by at least one examiner. The parents or guardians of each child were contacted for their consent, and examinations were performed between 17 November and 1 December 1997. All children with clinically active trachoma (see definition below) and one-eighth of the children without active disease were evaluated further by a second observer, as well as by photographing their conjunctiva and by collecting a conjunctival specimen for chlamydia testing. All examinations and laboratory tests were performed independently and without knowledge of the results of other examinations or laboratory tests. The children without active disease were chosen for further evaluation by assigning to each child a computer-generated 3-digit random number drawn inclusively from 000 to 999. If the number assigned was less than 125, the child was further evaluated, whether or not he or she had clinically active disease.

Procedures

Clinical examination. An ophthalmic assistant (*B.S.* or *C.R.P.),* who had considerable experience using the WHO simplified trachoma grading scale (*11*), examined each child using a 1.8–2.5 × binocular loupe. The tarsal conjunctival area of the upper lid was examined in both eyes, although only the results of the examination of the right eye were used in this study. Conjunctival inflammation was graded as follicular trachoma ((TF): the presence of five or more follicles in the lower two-thirds of the upper palpebral conjunctiva) or intense trachoma ((TI): greater than one-half of the underlying upper palpebral conjunctival blood vessels obscured by pronounced inflammation) (*11*).

Photography. Two photographs of the tarsal conjunctiva of the right upper lid were taken for each child. These were later graded, masked, by an ophthalmologist (*T.L.*), again using the WHO simplified grading system for trachoma (*11*).

LCR testing. A swab was taken of the right conjunctiva and placed in LCR transport medium. Samples were immediately put in an ice-chest in the field, and kept on ice or refrigerated (4 °C) until being analysed at the University of California San Francisco (UCSF). LCR was used to determine the presence of *C. trachomatis* DNA (LCx probe system, Abbott Laboratories, Abbott Park, IL, USA). Further analyses were undertaken on 12 LCR-negative specimens taken from children with clinically active disease; tests included repeat LCR, LCR of diluted samples, and LCR for target DNA within the chlamydial major outer membrane protein gene (*omp1*) (performed by Abbott Laboratories, Abbott Park, IL, USA).

Giemsa staining. Giemsa staining was performed on an arbitrary subset (15) of the clinically active children. Conjunctival scrapings of the right eye were collected using a Kimura spatula. Specimens were immediately fixed with ethanol, and later analysed with Giemsa staining at UCSF (*12–14*).

Definitions of clinically active trachoma and chlamydial infection. Clinically active trachoma was defined by the initial examiner as either TF or TI in the right eye. Chlamydial infection was defined as a positive LCR test for *C. trachomatis*.

It should be noted that clinical diagnosis of active trachoma does not necessarily indicate the presence of *C. trachomatis,* the causative agent of trachoma; nor does diagnosis of chlamydial infection necessarily imply clinically active disease.

Results

Of the 765 children identified in the six villages, 726 (95%) were examined, with the number of children

examined in each village ranging from 24 to 197. Of those examined, 125 (17%) were further evaluated. All 125 children underwent conjunctival photography and a conjunctival swab was collected from them, but five children refused a second examination. Clinically active disease was found in the right eye of 46 children of the 726 seen by the first examiner (6%, 95% confidence interval (CI) = 5–8%). Photographic evaluation indicated that 32 (70%) of these 46 children had clinically active disease, as had 14 (18%) of 79 children found not to have clinically active disease on examination. The concordance rates and correlation coefficients between clinical examiners 1 and 2 and the photographic examiner are shown in Table 1.

A total of 90 specimens (all 46 specimens from clinically active children and a randomly chosen 44 specimens from clinically inactive children) were tested by LCR for the presence of *C. trachomatis*, and all were found to be negative (0% prevalence, 95% CI = 0–3%). Thus none of the 46 children found to be clinically active on photographic examination and none of 44 found to be normal on examination were found to be positive by LCR. Repeat LCR testing of samples from 12 of the clinically active cases for *C. trachomatis* DNA was still negative. These 12 samples were then diluted to minimize the effect of any potential LCR inhibitors; all diluted samples were also LCR negative. In addition, LCR targeted towards the chlamydial *omp1* gene did not amplify any chlamydial DNA from the specimens.

Conjunctival specimens taken from 15 children with clinically active trachoma were examined by Giemsa staining: no chlamydial inclusion bodies were found. Bacteria were found on three slides: Gram-negative diplobacilli consistent with *Moraxella* sp. on two; and Gram-positive diplococci consistent with *Streptococcus pneumoniae* on one. There was no significant collection of eosinophils.

Table 1. **Concordance rates and correlation coefficients (*r*) between clinical and photographic examiners for the 125 children who underwent further evaluation**

	Concordance rate (%)	
	Clinical examiner 2	**Photographic examiner**
Clinical examiner 1	76	78
	$r = 0.49$	$r = 0.53$
Clinical examiner 2	—	78
		$r = 0.50$

Discussion

The results of the study revealed that there is currently a low prevalence of active conjunctival disease in Surkhet district, Nepal; 6% of the children were identified as clinically active by clinical examination. Clinical examinations were confirmed by a second examiner and by photographic evaluation, with a reasonable degree of concordance, indicating that the findings were not a misinterpretation of the WHO grading system. Interestingly, using the most sensitive diagnostic test available, no evidence of chlamydial infection was found, even among those who were identified as clinically active by both clinical examination and photography. There are inhibitors to LCR (*4*), but further testing designed to dilute their effect failed to reveal the presence of chlamydial DNA. LCR testing that was targeted to the *omp1* gene of chlamydia also failed to amplify any chlamydial DNA.

Other causes of conjunctivitis may sometimes be confused with trachoma. However, trachoma causes a characteristic pattern of regularly spaced follicles in the upper tarsal conjunctiva (*1*). Viral conjunctivitis caused by adenovirus, echovirus or coxsackievirus (acute haemorrhagic conjunctivitis), picornavirus (Newcastle disease), herpes simplex, and *Molluscum contagiosum* can also result in a follicular conjunctivitis (*15, 16*). No periocular molluscum or herpetic skin lesions were observed on clinical examination or on careful examination of the photographs. The giant papillae observed in cases of vernal conjunctivitis, atopic conjunctivitis and contact-lens-associated giant papillary conjunctivitis can mimic upper tarsal follicles (*14*). However, no eosinophils were seen on Giemsa staining of conjunctival scrapings from the 15 cases tested, and contact lenses were not used by the villagers. Other bacteria, in particular *S. aureus* and *M. lacunata,* can cause a follicular reaction (*15*). It is interesting to note that two of the 15 Giemsa stainings performed in the study revealed evidence of *Moraxella* sp., supporting the evidence that bacteria other than chlamydia may now be playing a role in the pseudo-trachoma seen in Surkhet (Fig.1). Stimuli other than chlamydia may

Fig. 1. **Upper palpebral conjunctiva of a 3-year-old girl, consistent with follicular trachoma (TF). Repeated ligase chain reaction (LCR) testing failed to reveal the presence of *Chlamydia trachomatis*. Giemsa staining revaled the presence of Gram-negative diplobacilli consistent with *Moraxella* sp.**

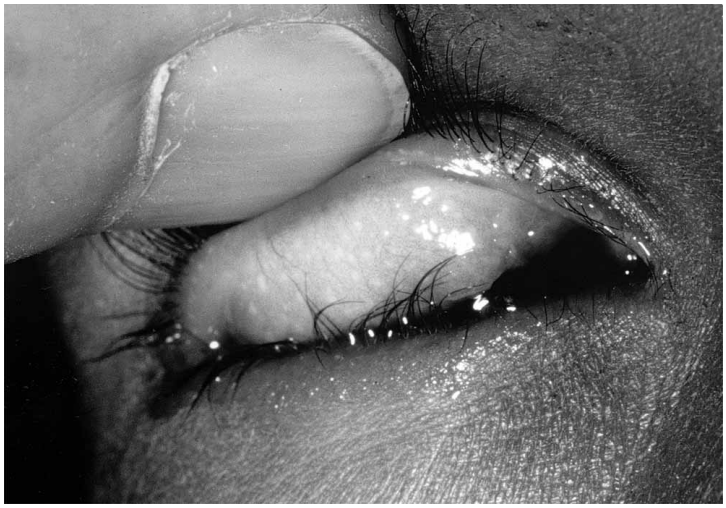

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Table 2. **Specificity and positive predictive value of the clinical examination in detecting *Chlamydia trachomatis* infection in areas with different prevalences of clinically active trachoma**

Area	Prevalence of clinically active trachoma in children (%)	Specificity of clinical examination in detecting infection (%)	Positive predictive value of clinically active trachoma for infection (%)
United Republic of Tanzania (*5*)	48[a]	61	65
Gambia (*6*)	31[b]	89	75
Surkhet, Nepal	6[b]	94	0

[a] Children aged ⩽ 7 years.

[b] Children aged ⩽ 10 years.

elicit this characteristic follicular pattern in conjunctivae that have previously had active trachoma.

The positive predictive value of a diagnostic test typically decreases with the prevalence of disease (*8*). If clinical examination is used as a test for chlamydial infection, the present study indicates that the positive predictive value of clinically active trachoma is zero. Interestingly, the specificity of clinical examination for detecting infection may actually improve as the prevalence decreases. In a hyperendemic area of the United Republic of Tanzania, clinical examination was 61% specific for determining trachoma (*5*). In the area of Surkhet with a very low prevalence of clinically active trachoma, the specificity of clinical examination was 94% (Table 2). Even with this specificity, clinical examination may have limited value in identifying infected individuals in an area with a low prevalence of infection.

WHO has recommended different treatment strategies depending on the prevalence of clinically active trachoma. If the prevalence of active disease is below 10% in children, an intensive control effort is not recommended (*2*). In an area that previously had endemic blinding trachoma, and still has a 6% prevalence of clinically active conjunctival disease that meets the clinical criteria for active trachoma, we were unable to isolate any chlamydia agent even using the most sensitive tests available. The WHO policy of not recommending an intensive trachoma control effort when the prevalence of clinically active trachoma is less than 10% in children is therefore appropriate in this area of Nepal.

In many settings, clinical examination is the only feasible way of estimating the prevalence of infection; however, it should be kept in mind that a very low prevalence of clinically active trachoma is not necessarily evidence for the presence of chlamydial infection. ■

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Résumé

Fiabilité du diagnostic clinique pour l'identification du trachome infectieux dans une région de faible prévalence au Népal

Il est devenu d'autant plus nécessaire d'identifier les infections oculaires à chlamydia dans les régions de faible comme de forte prévalence, que l'OMS a pris l'initiative d'éliminer le trachome dans le monde d'ici 2020. Les techniques d'amplification génique telles que la PCR et la LCR sont les épreuves les plus sensibles pour le diagnostic de l'infection, mais elles sont relativement coûteuses à exécuter, ne donnent pas de résultat immédiat et ne sont pas disponibles dans les régions du monde où sévit le trachome. Il est donc absolument nécessaire de pouvoir identifier avec précision les infections oculaires à chlamydia.

Si la relation entre trachome évolutif et infection à chlamydia a été bien étudiée dans les zones où la maladie est hyperendémique, il n'en va pas de même dans les régions où sa prévalence est faible. Une enquête réalisée en 1981 a révélé que c'était dans la région de Bheri au Népal que la prévalence du trachome était la plus élevée, mais une autre enquête, récemment menée en vue d'une évaluation rapide, a montré que dans cette même région, la prévalence du trachome évolutif était désormais faible sur le territoire du district de Surkhet.

La présente étude a été effectuée dans six villages de ce district, où l'effectif total des moins de 10 ans était

de 765. Parmi ceux-ci, 726 (95%) ont été examinés et 46 (6%) trouvés porteurs d'un trachome cliniquement évolutif de l'œil droit (en utilisant le système de stadification simplifié de l'OMS). Ces 46 enfants, auxquels on a ensuite adjoint un autre groupe de 79 autres enfants sans trachome évolutif, ont fait l'objet d'une évaluation clinique plus poussée par un deuxième observateur, avec photographie de la conjonctive et recherche des chlamydia au moyen d'une amplification génique par la LCR.

Les 6% d'enfants trouvés porteurs d'un trachome, bien que présentant des manifestations cliniques évolutives, n'avaient pas d'infection à chlamydia selon le test d'amplification de l'ADN. Un second test par la LCR, puis l'amplification d'une autre région du génome chlamydial et enfin, l'exécution du test LCR sur des échantillons dilués (pour réduire l'effet d'éventuels inhibiteurs), n'ont pas révélé non plus la présence de chlamydia. La coloration au Giemsa de 15 échantillons a révélé deux cas d'infection à *Moraxella* sp. et une infection à *Pneumococcus* sp.

Dans les zones d'hyperendémicité trachomateuse, l'examen clinique est d'une relativement grande valeur pour la mise en évidence d'une infection à chlamydia, même si nombre d'enfants atteints d'un trachome cliniquement évolutif ne sont pas porteurs de chlamydia. A mesure que la prévalence du trachome diminue, il en va de même de la valeur prédictive de l'examen clinique dans la recherche d'une infection à chlamydia. Toutefois, dans de nombreuses circonstances, l'examen clinique est le seul qui soit praticable pour l'estimation de la prévalence de l'infection. Cette étude montre qu'un trachome cliniquement évolutif n'est pas forcément l'indicateur d'une infection à chlamydia lorsque sa prévalence est très faible. Dans ces conditions, on peut considérer que la politique de l'OMS, qui consiste à ne pas recommander un effort soutenu de lutte contre le trachome lorsque la prévalence des manifestations cliniques évolutives est inférieure à 10% chez les enfants, convient bien à cette région du Népal.

Resumen

Fiabilidad del diagnóstico clínico en la identificación del tracoma infeccioso en una zona de prevalencia baja de Nepal

La iniciativa OMS de Eliminación Mundial del Tracoma para 2020 ha acentuado la necesidad de identificar las infecciones oculares clamidianas mediante examen clínico en zonas tanto de alta como de baja prevalencia. Las nuevas técnicas de amplificación del ADN, como la reacción en cadena de la polimerasa (RCP) y la reacción en cadena por la ligasa (RCL) son las pruebas más sensibles para diagnosticar la infección, pero son también relativamente costosas, sus resultados no son inmediatos y no están fácilmente disponibles en las zonas donde el tracoma es frecuente. Así pues, la identificación precisa de la infección ocular clamidiana mediante exploración clínica reviste gran importancia.

Se ha estudiado la relación entre el tracoma clínicamente activo y la infección clamidiana en zonas con tracoma hiperendémico, pero no en zonas de baja prevalencia de la enfermedad clínica. Una encuesta llevada a cabo en 1981 reveló que la región nepalí de Bheri presentaba la prevalencia más alta de tracoma del país, pero una encuesta reciente de evaluación rápida en el distrito Surkhet de la zona de Bheri ha revelado que la prevalencia de tracoma activo es ahora baja en esa zona.

El presente estudio se llevó a cabo en seis aldeas de Surkhet, con una población de 765 menores de 10 años. Se examinó a 726 de ellos (95%), hallándose que 46 (6%) presentaban signos de tracoma activo en el ojo derecho (según el sistema simplificado de clasificación de la OMS). Esos 46 niños, junto con un grupo de 79 niños sin manifestaciones clínicas seleccionados aleatoriamente, fueron objeto de una ulterior evaluación que incluyó otro examen por un segundo observador, el análisis fotográfico de la conjuntiva y pruebas de RCL para detectar la presencia de clamidia.

Aunque el 6% de los menores de 10 años presentaban tracoma clínicamente activo, según la prueba de amplificación del ADN ninguno de ellos estaba infectado por clamidia. La repetición de la prueba de RCL, la aplicación de dicha prueba a un tramo diferente del genoma clamidiano y el análisis por RCL de muestras diluidas (a fin de reducir el efecto de posibles inhibidores) tampoco revelaron indicios de presencia de clamidia. La tinción con Giemsa de 15 muestras reveló dos casos de infección por *Moraxella sp*. y otro por *Pneumococcus sp*.

En las zonas con tracoma hiperendémico el valor predictivo positivo del examen clínico para la detección de la infección clamidiana es relativamente alto, aun cuando muchos niños con manifestaciones clínicas no están infectados . A medida que disminuye la prevalencia del tracoma, el valor predictivo positivo del examen clínico para la identificación de la infección también disminuye, y puede no ser fiable para detectar la infección. Sin embargo, en muchas circunstancias el examen clínico es la única opción viable para estimar la prevalencia de la infección. Este estudio mostró que una prevalencia muy baja de tracoma clínicamente activo no refleja necesariamente la presencia de infección clamidiana. Por consiguiente, la política de la OMS de no recomendar un control intensivo del tracoma cuando la prevalencia de tracoma clínicamente activo es inferior al 10% entre los niños es válida para esta zona de Nepal.

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