Evaluation of the Eiken latex agglutination test for anti-*Toxoplasma* antibodies and seroprevalence of *Toxoplasma* infection among factory workers in Addis Ababa, Ethiopia

Tilahun Woldemichael[1], Arnaud L. Fontanet[2], Tefera Sahlu[2], Henk Gilis[3], Tsehaynesh Messele[2], Tobias F. Rinke de Wit[2], Hailu Yeneneh[2], Roel A. Coutinho[4] and Tom Van Gool[3] *[1]Department of Infectious and Non-Infectious Diseases and [2]Ethiopian–Netherlands AIDS Research Project, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia; [3]Department of Medical Microbiology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; [4]Department of Public Health, Municipal Health Services, Amsterdam, The Netherlands*

Abstract

Sera from 170 factory workers aged 18–45 years enrolled in a pilot study of human immunodeficiency virus 1 (HIV-1) infection in Addis Ababa, Ethiopia, were screened for anti-*Toxoplasma* immunoglobulin G antibodies by the Sabin–Feldman test (reference standard) and the Eiken latex agglutination test (under evaluation for use in developing countries). Based on the Sabin–Feldman test, the prevalence of anti-*Toxoplasma* antibodies was 80·0% (95% confidence interval 73·9–86·1%). The sensitivity and specificity of the Eiken latex agglutination test were 96·3% and 97·1%, respectively, showing its validity for the detection of anti-*Toxoplasma* antibodies. The prevalence of antibodies did not differ between individuals infected and uninfected with HIV-1 (74·2% versus 83·3%, $P>0·05$). However, antibody titres were higher in HIV-infected persons than in those who were uninfected ($P<0·001$). Based on these findings, we expect that toxoplasmic encephalitis will be a common opportunistic infection among HIV-infected Ethiopians, and chemoprophylaxis with co-trimoxazole may be beneficial to those with low CD4+ T cell counts. The prognostic significance of high titres of anti-*Toxoplasma* antibodies remains to be established among Ethiopian HIV-infected individuals.

Keywords: toxoplasmosis, *Toxoplasma gondii*, seroprevalence, chemoprophylaxis, human immunodeficiency virus 1, Ethiopia

Introduction

Since the advent of the acquired immunodeficiency syndrome (AIDS) in the early 1980s, many immunocompromised patients have suffered from the reactivation of chronic latent *Toxoplasma* infection. The incidence rate of toxoplasmic encephalitis in individuals infected with the human immunodeficiency virus (HIV) and with positive *Toxoplasma* serology was estimated at 15·3 per 100 person-years when CD4+ T cell counts were $<200\times10^6$/L (DEROUIN *et al.*, 1996), and 35·0 per 100 person-years when CD4+ T cell counts were $<100\times10^6$/L (OKSENHENDLER *et al.*, 1994).

We studied the prevalence of anti-*Toxoplasma* antibodies among factory workers enrolled in a pilot study of HIV infection near Addis Ababa, Ethiopia. A wide range of prevalence of *Toxoplasma* infection has been reported in Ethiopia, from 8% to 75% (ROEVER-BONNET, 1972; TSEGA & BELIHU, 1980; MENGESHA *et al.*, 1984; GUEBRE-XABIER *et al.*, 1993; ESHETE *et al.*, 1994). These variations may be explained by the different population groups included in these surveys and the different serological assays used. We therefore decided to base our prevalence estimate on the best method available, the Sabin–Feldman (SF) test, and to compare it with the Eiken latex agglutination (ELA) test, which would be easier to use in a developing country laboratory.

Materials and Methods

From November 1995 to April 1996, a pilot study was carried out in 4 factories in Akaki, an industrial suburban area south of Addis Ababa in Ethiopia, in order to identify a site suitable for a long-term cohort study on the natural history of HIV infection. Among 956 individuals aged 18–45 years randomly selected from the list of factory workers, 879 (92%) were available, and 689 (78%, 444 males and 245 females) agreed to participate in the study. Study participants underwent the following procedures: pre-test counselling and obtaining informed consent for HIV testing and study participation; interview on socio-demographic characteristics and sexual behaviour; blood collection in ethylenediaminetetraacetic acid and in plain Vacutainer™ tubes (Becton–Dickinson) for examination for HIV-1 antibodies by Vironostika™ enzyme-linked immunosorbent assay (ELISA; Organon, Boxtel, The Netherlands). All sera giving positive reactions were confirmed with a Western blot test (HIV Blot 2·2™, Genelabs, USA). The study protocol was reviewed and approved by the Ethiopian Health and Nutrition Research Institute's ethical committee.

A subset of 170 sera was selected for anti-*Toxoplasma* antibody testing, including sera from 59 HIV-positive males (overall HIV prevalence among the 444 male subjects was 13%), 3 HIV-positive females in 2 small factories (overall HIV prevalence among the 245 female subjects was 9%; other sera were kept for possible use in a study on HIV and progesterone), and 108 randomly selected HIV-negative individuals (96 males and 12 females). The SF method was used as described (SABIN & FELDMAN, 1948), with some modifications. In short, 10 µL of serum were diluted two-fold in phosphate-buffered saline in a flat-bottom microtitre plate. To each well, 25µL of complement source and 10 µL of parasite suspension were added. After incubation for 1 h at 37°C, 10 µL of methylene blue/borax in carbonate buffer at pH 11 were added to visualize the reaction (killed parasites not taking up the stain appeared white, unaffected parasites stained blue). Plates were examined with an inverted microscope (400×), the titre being the serum dilution in which 50% of the parasites were killed. Sera with a titre ≥1 iu/mL were regarded as positive. The ELA test was used according to the manufacturer's guidelines (Toxoreagent-MT™; Eiken Chemical Company, Japan) and samples with a titre >1:32 were regarded as positive (as recommended by the manufacturer). Each serum sample was tested in duplicate using both methods at the Academic Medical Centre in Amsterdam, The Netherlands.

Statistical analysis

Data were analysed using the Stata™ statistical package (Stata Corporation, College Station, Texas, USA). Exact 95% confidence intervals (95% CI) were calcu-

Address for correspondence: Tilahun Woldemichael, Department of Infectious and Non-Infectious Diseases, Ethiopian Health and Nutrition Research Institute, P.O. Box 1242, Addis Ababa, Ethiopia.

lated for binomial proportions. Means and proportions were compared using the Mann–Whitney U test and χ^2 tests as appropriate. Correlations were assessed by the method of Spearman.

Results

A total of 170 sera was examined by both the SF and ELA tests; 131 gave positive results, and 33 gave negative results, by both techniques, a qualitative diagnostic agreement of 96·5%. Five of the remaining sera were positive in the SF test only, and one in the ELA test only. Based on the SF test, the overall prevalence of anti-*Toxoplasma* antibodies was 80·0% (136/170; 95% CI 73·9–86·1%).

Relative to the SF reference standard test, the sensitivity and specificity of the ELA test were 96·3% (131/136; 95% CI 91·6–98·8%) and 97·1% (33/34; 95% CI 84·7–99·9%), respectively. The positive and negative predictive values for the ELA test results were 99·2% (131/132; 95% CI 95·9–100%) and 86·8% (33/38; 95% CI 71·9–95·6%), respectively.

The serological titres obtained with both techniques are shown in the Figure. The correlation between the titres obtained with the 2 methods was high (Spearman's rank correlation coefficient=0·75).

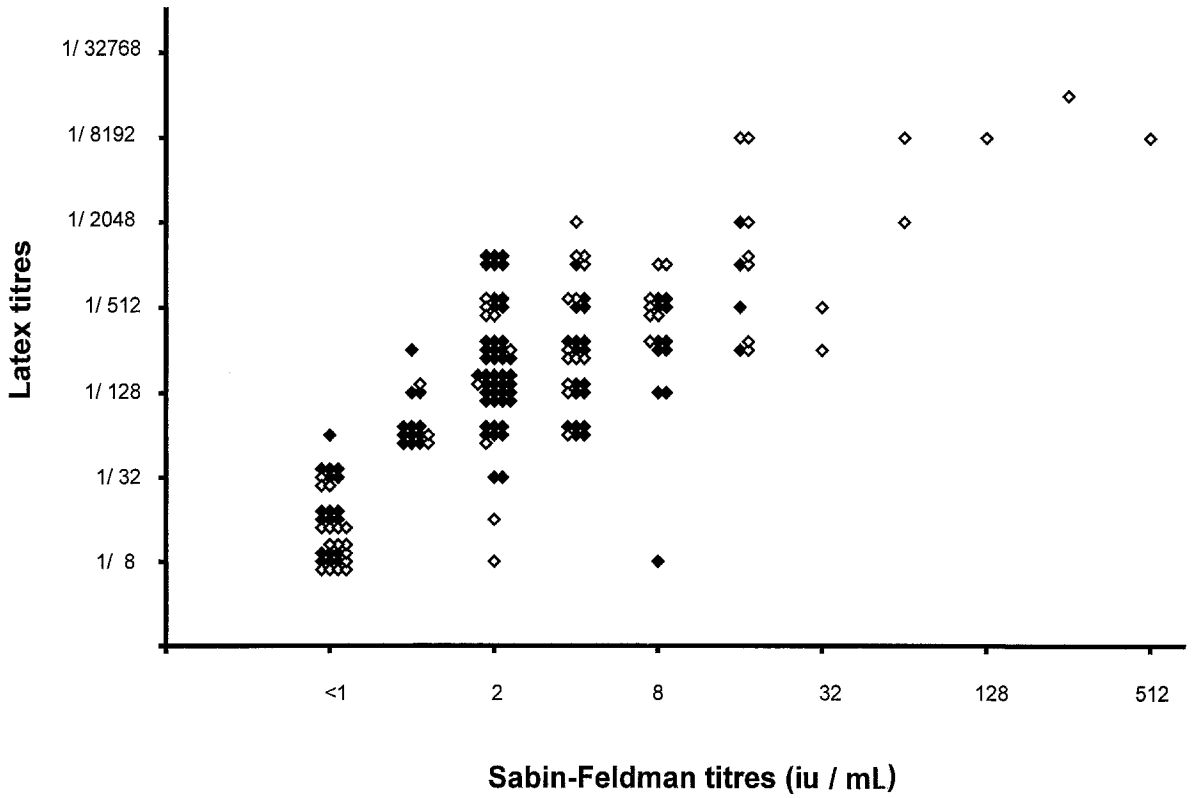

Figure. Anti-*Toxoplasma gondii* antibody titres obtained by the Sabin–Feldman and the Eiken latex agglutination methods with sera from donors infected (◇) and uninfected (◆) with human immunodeficiency virus 1.

The prevalence of anti-*Toxoplasma* antibodies did not differ significantly between persons with and without HIV-1 infection (46/62=74·2% versus 90/108=83·3%; $P>0·05$). However, among those in whom antibodies were detected, the mean titre was higher in those infected with HIV than in those who were uninfected (Mann–Whitney U test, $P<0·0001$). Of the 62 HIV-positive individuals, 5 had titres greater than 64 iu/mL, compared to none of the 108 HIV-negative individuals ($P=0·0006$).

Discussion

The high prevalence of anti-*Toxoplasma* antibodies observed among the study subjects is in accordance with the results of the most comprehensive study carried out so far in Ethiopia on the prevalence of *Toxoplasma* infection (GUEBRE-XABIER *et al.*, 1993). The over-representation of HIV-positive sera in the collection tested for anti-*Toxoplasma* antibodies did not significantly bias the overall prevalence, since we did not find any association between the presence of anti-HIV-1 and anti-*Toxoplasma* antibodies. It seems reasonable to attribute the high transmission rate of *Toxoplasma* in Ethiopia to the widespread habit of consuming raw or under-cooked meat.

This study demonstrated that the ELA test provides a suitable alternative to the SF test. This is shown by its high sensitivity (96·3%) and specificity (97·1%) relative to the standard SF test results which are in good agreement with other published values (KOBAYASHI *et al.*, 1977; LESTER, 1983; WALLS & REMINGTON, 1983). The strong positive correlation between the titres obtained with the 2 tests adds further evidence of their comparability. The ELA test is technically straightforward, requiring minimal equipment, making it appropriate for middle-level diagnostic laboratories in developing countries (BALFOUR *et al.*, 1982; WALKER *et al.*, 1992). One limitation of the ELA test is that it requires at least 12 h before agglutination reactions are read as opposed to a couple of hours for the SF test. However, the SF test requires live parasites, passaged in mice, and complement, making it impractical in most laboratories.

We found higher antibody titres in persons infected with HIV than in those who were uninfected, as has already been documented (GRANT *et al.*, 1990; DEROUIN *et al.*, 1991; CANDOLFI *et al.*, 1992), although this was not found by DOEHRING *et al.* (1995). High titres of anti-*Toxoplasma* antibodies were found to be predictive of the occurrence of toxoplasmic encephalitis in HIV-infected individuals with CD4+ T cells counts less than 200×10^6/L (DEROUIN *et al.*, 1996). Also, a few prospective studies have indicated a significant rise in anti-*Toxo-*

plasma antibody titres in HIV-infected patients (DEROUIN *et al.*, 1991; ISRAELSKI *et al.*, 1993), followed by symptoms of toxoplasmic encephalitis (DEROUIN *et al.*, 1991). However, no antibody increase may be observed in patients with profound immunodeficiency, sometimes at the onset of clinical symptoms of toxoplasmic encephalitis (DEROUIN *et al.*, 1996).

The high prevalence of anti-*Toxoplasma* antibodies in our study population (80%) indicates a need for action to prevent toxoplasmic encephalitis developing. The current recommendation for primary prophylaxis concerns HIV-infected individuals with positive anti-*Toxoplasma* serology and CD4+ cell counts less than 100×10^6/L (USPHS/IDSA, 1995). One drug recommended for prophylaxis which is available in Ethiopia is co-trimoxazole (ANTINORI *et al.*, 1995; JONES *et al.*, 1996; KAPLAN *et al.*, 1996). It may also be beneficial for the prevention of other opportunistic or common infections, like *Pneumocystis carinii* pneumonia (HARDY *et al.*, 1992; SCHNEIDER *et al.*, 1992), bacterial pneumonia (HIRSCHTICK *et al.*, 1995), and *Isospora belli* diarrhoea (DEHOVITZ *et al.*, 1986). However, its use may be limited by side effects (SCHNEIDER *et al.*, 1995), and would require careful evaluation in an Ethiopian population.

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