

*Full Length Research Paper*

# **Seroprevalence of HTLV1/2, HSV1/2 and *Toxoplasma gondii* among chronic HIV-1 infected individuals in rural northeastern South Africa**

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**Infection with human immunodeficiency virus (HIV) leads to immunosuppression and subsequent onset of AIDS. The management of HIV/AIDS takes into account co-morbidities due to viral, bacterial and protozoal infections. The aim of this study was to determine the seroprevalence of human T-cell lymphotropic virus types 1/2 (HTLV1/2), herpes simplex virus types 1/2 (HSV1/2) and *Toxoplasma gondii* in a cross-sectional population of HIV infected individuals in rural Northeastern South Africa. Antibodies to HTLV1/2, HSV1/2 and *T. gondii* were assessed by commercial ELISA systems. Statistical differences with p-values less than 0.05 were taken as significant. One hundred and seventy individuals were tested for HTLV1/2, total IgG; while 160 were tested for HSV1/2 total IgG and *T. gondii* IgG antibodies. Seroprevalence of 24.0, 83.0 and 18.1% were observed for HTLV1/2, HSV1/2 and *T. gondii* respectively. For *T. gondii*, 82% of infections were found in females between the ages of 21 and 35 years and this observation was significant ( $p < 0.05$ ). Generally, infection with either of the pathogens tested was more common in individuals in the age group of 21 - 35 years and this was significant irrespective of gender ( $p < 0.05$ ). The results suggest a relatively high seroprevalence of HTLV1/2, HSV1/2 and *T. gondii* infections in the studied population. In addition, data on HSV1/2 and *T. gondii* seroprevalence among HIV infected individuals is presented for the first time from rural Northeastern South Africa.**

**Key words:** HTLV1/2, HSV1/2, HIV, co-infection, Northeastern South Africa.

## **INTRODUCTION**

Human T-cell lymphotropic virus type 1 (HTLV-1) causes tropical spastic paraparesis (TSP)/HTLV-1 associated myelopathy (HAM) and adult T cell Leukaemia/Lymphoma (ATLL) (Proietti et al., 2005; Verdonck et al., 2007) and possibly depression (Stumpf et al., 2008). On the other hand, HTLV-2 has not been associated with haematological malignant disease (Feuer et al., 2005) and only a few reports have described lymphomas in HTLV-2 carriers (Poiesz et al., 2000). However, HTLV-2 is linked with the development of neurological symptoms and with susceptibility to bacterial and fungal infections (Murphy et al., 1999).

Genital ulceration caused by herpes simplex virus (HSV) is a risk factor for HIV infection because of the disruption of the epithelial barrier and infiltration by CD4+lymphocytes which are targets of HIV. Genital herpes is frequently reactivated in HIV infected individuals and viral particles are often detected in herpes lesions, with titres higher than seen in plasma (Gupta et al., 2007). The transmission routes of HTLV and HSV are similar to that of HIV which includes contaminated blood and blood products, mother to child through breast feeding, and through sexual intercourse (Bittencourt, 1998; Menna-Barreto, 2006; Pimenta et al., 2008). On the other hand, mother to child transmission of *Toxoplasma* is well established with higher chances of transmission by HIV infected mothers (Minkoff et al., 1997; Asgari et al., 2006; Bachmeyer et al., 2006; Cruz et al., 2007; Lago et al., 2009). Overall, a close association

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of HTLV (Casoli et al., 2007; Brites et al., 2009), HSV (Bhargava et al., 2009; Chohan et al., 2009; Tobian and Quinn, 2009) and *Toxoplasma* infections (Linstrom et al., 2006; Alfonso et al., 2009; Lago et al., 2009) with HIV infection is well documented.

Human immunodeficiency virus infection is endemic in South Africa, with about 5 million individuals infected as at the end of 2009 (UNAIDS, 2009). The Limpopo Province (Northeastern South Africa) has an HIV prevalence of about 20% based on seroprevalence data from first time antenatal pregnant women attendees (DOH, 2009). Although sexually transmitted infections are strongly associated with HIV infection, data on co-infection with HTLV, HSV and *Toxoplasma* among the HIV population in Limpopo province has received very little attention. Very few studies about HTLV have been undertaken in South Africa and only a single study has been conducted in the Northeastern region of the country on the co-infection of HIV and HTLV (Taylor et al., 1992).

It is important to understand the burden of co-infections in HIV infected individuals with other significant etiologies because of implications in pathogen screening policy and subsequent patient management (Regis et al., 2009).

The main objective of this study was to determine the seroprevalence of HTLV, HSV and *Toxoplasma gondii* infections in a cross-sectional population of HIV infected individuals in Northeastern South Africa.

## MATERIALS AND METHODS

### Study design and study population

This study was a cross-sectional retrospective investigation on archived HIV positive plasma samples collected between 2007 and 2008 within an HIV diversity investigation. A total of 170 samples were available for analysis. Samples were obtained from chronically infected patients attending health establishments in Musina, Madimbo and Bela Bela in Northeastern South Africa. The patients were not on antiretroviral therapy. The study protocol was approved by the University of Venda Health, Safety and Research Ethics Committee. Demographic data such as age, sex, probably year and place of infection and history of antiretroviral therapy were obtained by questionnaire administration.

### Serological assays and statistical analysis

Specific anti-HTLV immunoglobulin gamma (IgG) antibodies were detected using Detect HTLV ELISA kits (Adaltis, Italy) according to the manufacturer's instructions. The assay has a sensitivity and specificity of 100 and 99.5% respectively. The enzyme captured on the solid phase, acting on the substrate chromogen mixture generates an optical signal that is proportional to the quantity of HTLV1/2 antibodies present in the sample. Similarly, the detection of total IgG antibodies to HSV1/2 was performed with RIDASCREEN ELISA kits (Mikrogen Diagnostic, Germany) (sensitivity 97.8%, specificity 100%). Immunoglobulin G antibodies to *T. gondii* were also detected by ELISA (Adaltis) according to the manufacturer's instructions, with a sensitivity and specificity of 64 and 84% respectively. Absorbance values were obtained using the ELx808 ELISA reader (BioTek, USA). Statistical differences and significance were determined with the SPSS software.

## RESULTS

### Study group demographics

Of the 170 HIV infected individuals, 117 (69%) were females and 53 (31%) were males. The age range was 11 - 65 years. All the patients were in WHO stage 1 of disease. Estimated period of infection with HIV was between 2 - 5 years. Sequencing and phylogenetic analysis of partial polymerase genes showed that all the subjects were infected with HIV-1 subtype C variants (data not shown).

### HTLV serology

Of the 170 plasma samples tested 41 (24.1%) were reactive to HTLV1/2 IgG antibodies. Of these, 26 (63.4%) were females, and 15 (36.6%) were males. Prevalence distribution among the age groups was 2.4% (11 - 20 years), 8.8% (21 - 35 years) and 12.9% for those above 35 years of age. The p-value was 0.279 for gender and 0.352 for age groups.

### HSV serology

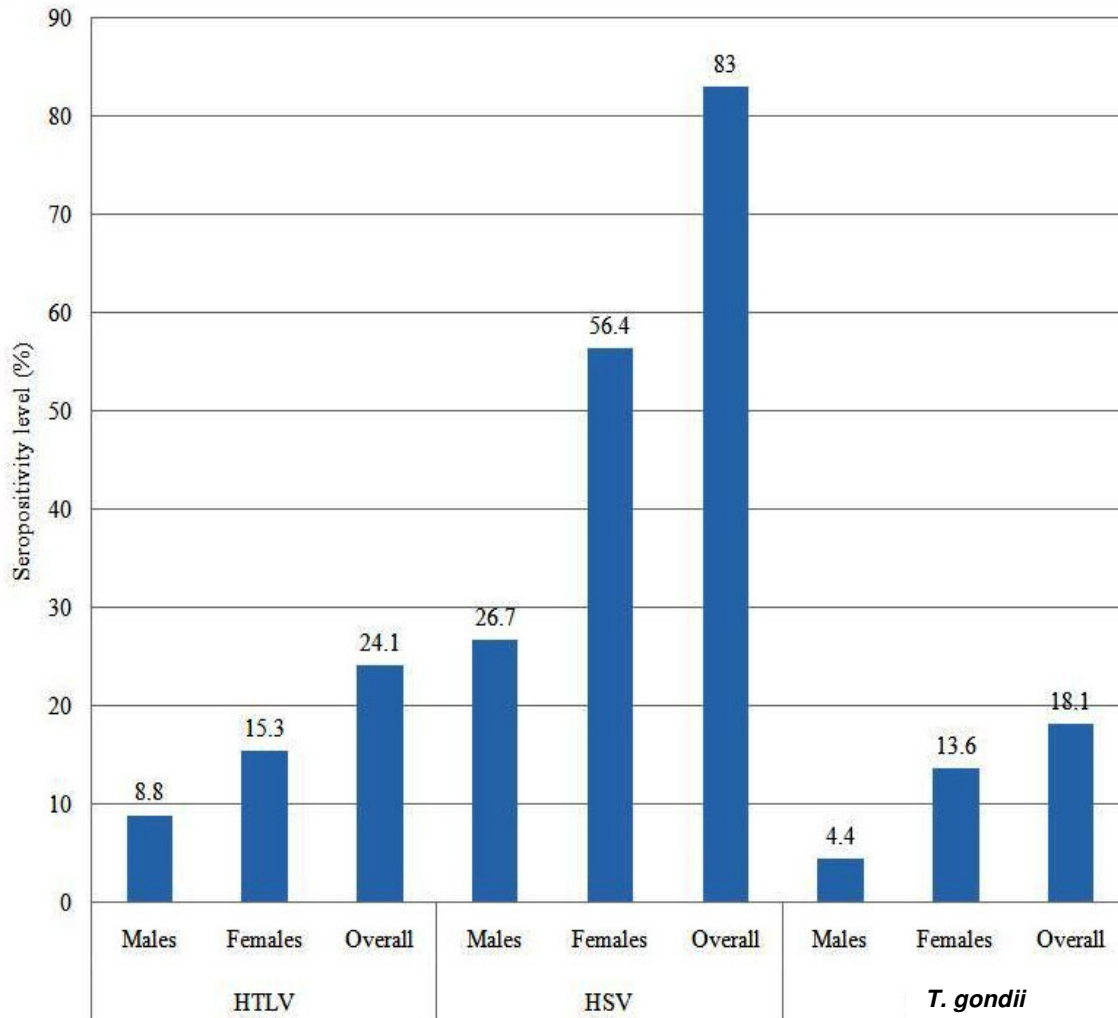
One hundred and sixty five samples (5 samples had insufficient quantities) were screened for HSV1/2 IgG antibodies. Of these 51 were males and 114 were females. Overall seroprevalence was 137/165 (83%). Seroprevalence in males was 44/51(86.2%) and 93/114 (81.6%) in females. Seropositivity stratified according to age was 6.7% (11/165) amongst those between 11 to 20 years, 40% (66/165) for those between 21 to 35 years, and 37% (61/165) for those above 36 years old. The calculated p-value was 0.407 for gender and 0.518 for age groups.

### *Toxoplasma gondii* serology

One hundred and sixty samples were available for *T. gondii* serology comprising 42 from males and 118 from females. The overall seroprevalence was 29/160 (18.1%). Seroprevalence in males and females were 7/42 (16.7%) and 22/118 (18.6%) respectively and the difference was not statistically significant ( $p > 0.05$ ). The age distribution was 0.63% (1/160) for individuals 20 years and below, 10.6% (17/160) for those between 21 and 35 years old and 6.9% (11/160) for individuals who were 36 years old and above. Within the 21 - 35 years age group 83% of those infected were females. Figure 1 shows the relative seroprevalence of HTLV1/2, HSV1/2 and *T. gondii* in the studied population.

## DISCUSSION AND CONCLUSION

Infection with HIV leads to immunosuppression with



**Figure 1.** Seroprevalence of HTLV, HSV, and *Toxoplasma gondii*, among antiretroviral drug naïve HIV positive patients in rural Northeastern South Africa. Generally, infection levels were higher in females than in males.

subsequent bacterial, fungal, protozoal and viral infections. The present study looked at the frequency of detection of HTLV1/2, HSV1/2 and *T. gondii* in a population of HIV positive individuals in rural Northeastern South Africa. In the present study, 24% of HIV infected subjects were reactive to HTLV1/2 antibodies. This is higher than reported in Spain (1%) among different groups at risk for HIV (Soriano et al., 1990), and in an urban setting in Brazil (6.4%) (Morimoto et al., 2005). Although the current study did not look at the correlation of HIV and HTLV infection, a previous study in the Free State Province of South Africa found a correlation between HIV seropositivity and HTLV-1 infection (Van der Ryst et al., 1996). However, no correlation was found in another study in Gauteng Province of South Africa (Taylor et al., 1996).

Infection with HSV is common worldwide and prevalence appears to vary with age, race, geographic location and life style (Stock et al., 2001; Clemens and Farhat, 2010; Stowe et al., 2010). Very high infection

levels have been documented in developing societies. For example, Cory and Handsfield (2000) reported rates of 60 - 90% among gold miners and commercial sex workers respectively in South Africa. A recent longitudinal study shows that HSV-2 was responsible for approximately 25% of incident HIV infections among men in a farm settlement in South Africa (Sobngwi-Tambekou et al., 2009). In the present study, an HSV1/2 seroprevalence of 83% in HIV infected individuals was observed (86.2% in males and 81.6% in females). This is relatively higher compared to a study in India in which about 17% of HIV infected females were found to be infected with HSV (Devi et al., 2008). Nevertheless, the results seem to be in line with reports on HIV/HSV co-infections worldwide (Ramaswamy and Geretti, 2007).

Toxoplasmosis is an important opportunistic infection in HIV/AIDS (Nissapatorn, 2009). Prevalence of *T. gondii* infection in HIV infected individuals varies probably because acquisition and reactivation of infection depends on several factors. For example seroprevalences of 32, 6,

54, 4 and 32%, were found among Mexican, Nigerian, South African (Kwazulu-Natal Province), and Burkinabe HIV populations respectively (Ouermi et al., 2009; Akanmu et al., 2010; Alvarado-Esquivel et al., 2010; Murphy et al., 2010). A seroprevalence of 18% was observed in the current study suggesting variability of spread of infection in HIV patients. Furthermore, the observed high significant level of *Toxoplasma* in women of child bearing age suggests that newborn babies may be at risk.

Overall, this study has updated the seroprevalence of HTLV1/2 antibodies in HIV infected patients in rural Northeastern South Africa and for the first time presents data on the seroprevalence of HSV1/2 and *T. gondii* in the HIV population of the study area. Subsequent studies with antigen determination parameters and discrimination between HSV-1/HSV-2 and HTLV-1/HTLV-2 may shed more light on the epidemiology of these infections in Northeastern South Africa.

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