ORIGINAL ARTICLE

LEVELS OF SERUM HIV-1 RNA VIRAL LOAD IN TUBERCULOSIS PATIENTS WITH OR WITHOUT INTESTINAL PARASITES DURING TREATMENT OF TUBERCULOSIS IN GONDAR, ETHIOPIA

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ABSTRACT

Background: *HIV-1 RNA viral load is a powerful predictor of risk for disease progression in subjects infected with HIV. However, studies assessing VL in co-infected patients are very scarce. This study was, therefore, aimed at determining VL in tuberculosis (TB) and HIV-1 co-infected patients with or without intestinal parasites and also to assess its variation with treatment.*
Methods: *TB was diagnosed following standard clinical, bacteriological, radiological and histological procedures. HIV serostatus was checked by enzyme linked immunosorbent assay. One hundred nineteen TB/HIV-1 co-infected patients were includedas a baseline and 22 were re-examined at the end of intensive phase of anti-TB chemotherapy. Stool samples were examined for intestinal parasites by conventional microscopy and serum viral load was determined using an Amplicor HIV-1 Monitor RT-PCR assay.*
Results: *Forty-five (37.8%) patients were found infected with one or more species of intestinal parasites. Ascaris lumbricoides and Strongyloides stercoralis were the most frequently detected species. The mean (±SD) serum viral load (log_{10} RNA copies/ml) of patients at baseline was 4.82 (±0.66) without a significant difference by status of intestinal parasitoses. In patients with follow up treatment the viral load declined from 4.84 (±0.45) to 4.52 (±0.66) at the end of the intensive phase of anti-TB chemotherapy (P=0.07). In five patients who were also treated for intestinal parasites, viral load declined from 5.02 (±0.38) to 4.47 (±0.66) (P<0.05). A mean increase of 0.58 (±0.33) was seen in seven patients (P<0.01).*
Conclusion: *The lack of significant decline in viral load at the end of the intensive phase of anti-TB treatment may indicate increased morbidity in the patients. Intervention measures such as provision of anti-retroviral and anti-parasite therapy may help to reduce morbidity.*
Key words: *HIV-1 viral load, tuberculosis, intestinal parasites, anti-TB treatment*

INTRODUCTION

Mycobacterium tuberculosis and HIV-1 are the two leading infectious causes of death worldwide (1). Increasing morbidity and mortality among *M. tuberculosis* infected individuals in areas where HIV-1 is hyper-endemic are ascribed to *M. tuberculosis*/HIV-1 co-infection (2). In Ethiopia, an estimated 1.5 million people were infected and live with HIV/AIDS as of the end of 2004 (3). Tuberculosis (TB), on the other hand, is amongst the leading causes of outpatient morbidity, hospital admission, and death in the country (4). According to a recent report, Ethiopia is classified as one of the 22 high-burden countries ranking 7th based on the number of estimated TB cases (1).

In addition, infection with intestinal parasites is widely distributed in Ethiopia due to the low level of living standards coupled with poor environmental sanitation and personal hygiene practices (5). Although the prevalence rates of individual parasites vary considerably in different parts of the country, several studies show that *Ascaris lumbricoides* is the most prevalent intestinal parasite followed by *Trichuris trichiura*, hookworm and *Strongyloides stercoralis* (5).

Chronic immune activation caused by infection with intestinal parasites has been suggested as the main cause for elevated HIV plasma viral load (VL) reported in patients of sub-Saharan Africa (6,7).

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Furthermore, active TB leads to the activation of T-cells and macrophages resulting in the production of cytokines (8). The cytokines in turn promote expression of latent HIV in monocytes and lymphocytes through interaction with the nuclear factor-kB binding sites in the HIV-1 genome leading to elevated viral replication (9). As a result, VL which is a marker for HIV disease progression, increases and leads to increased morbidity in TB and HIV co-infected patients. Measurement of the VL has been used routinely in clinical practice and has proved to be a powerful predictor of risk for disease progression in subjects infected with HIV (10). Serial measurements of the VL help patients and physicians decide when to begin antiretroviral drug therapy, assist in establishing the effectiveness or failure of therapy, and help ascertain when the beneficial effect of treatment is being lost and therapies must be changed (11).

Even though studies from developed countries (12,13) have reported significant reductions in HIV-1 plasma VL in patients after a successful treatment of TB, those conducted in a few sub-Saharan African countries did not find a significant decrease in HIV-1 VL months after anti-TB therapy (14-17). This study was, therefore, aimed at assessing the effect of treatment of active TB on plasma VL in TB and HIV-1 co-infected patients in Gondar, Ethiopia.

METHODS

Study subjects, diagnosis and treatment: The study was conducted at the University of Gondar Teaching Hospital, in Gondar, Northwest Ethiopia. Subjects included were 119 consecutive patients who were diagnosed for tuberculosis and HIV-1 infection at the hospital in 2003. A specialist medical doctor working in the TB clinic performed the necessary clinical and diagnostic work-up. Diagnosis and treatment of TB were made following the protocol of the National Tuberculosis and Leprosy Prevention and Control Programme (4).

In brief, TB was diagnosed by combining clinical, radiological, histopathological, and laboratory features of the patients. All relevant x-ray films were seen for the radiological features of TB and interpreted by a consultant radiologist. Three consecutive sputum samples (spot, morning, morning) were collected following the standard procedure from eligible patients and microscopically examined for acid fast bacilli on direct smears using Ziehl Neelsen stains. Fine needle aspiration specimens collected from extra-pulmonary TB (EPTB) cases were examined cytologically by a pathologist following the standard procedures. To be considered as a case of TB, an individual had to have two or more of the following: 1) positive sputum smear, 2) histopathological evidence, 3) radiographic examination consistent with TB, and 4) clinical response to anti-TB chemotherapy. After TB diagnosis, all patients were classified into treatment categories based on the WHO criteria and received appropriate anti-TB chemotherapy (4). Socio-demographic and clinical features of the patients were collected by questionnaire. None of the participants received any antiretroviral treatment before or during the study as the treatment was not in practice during the time of the study.

Stool microscopy: Stool specimens were collected from all patients on three consecutive days at the time of TB diagnosis and two months after anti-TB therapy in a sub-sample of the patients. The stools were examined microscopically for ova, larva, cysts or trophozoites of intestinal parasites following direct and formalin-ether sedimentation concentration methods (18).

Serum collection and HIV serology: Venous blood was collected from all TB patients prior to the initiation of anti-TB treatment and examined for the presence of HIV antibodies in serum by an enzyme linked immunosorbent assay following the manufacturer's instructions (Vironostica HIV Uni-Form II plus O, Organon Teknika, Boxtel, the Netherlands). Blood samples were also collected at the end of an intensive phase of anti-TB chemotherapy from sub-samples of the patients for further studies. The sera were separated and stored at -20^{o}C until used for VL determination.

Determination of serum HIV-1 viral load: The frozen serum samples were kept on dry ice and air freighted to Japan. Viral load (serum HIV-1 RNA copy number) was measured using an Amplicor HIV-1 Monitor RT-PCR assay version 1.5 (Roche Diagnostic Systems, Inc., Tokyo) according to the manufacturer's instructions, at AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan. The lower detection limit of the assay was 400 copies/ml (2.6 log10 RNA copies/ml). Samples with RNA concentrations below the detection limit were not included in the analyses.

Statistical analyses: Data were analyzed by SPSS Version 10 statistical package. VL levels were log-transformed for analysis. Baseline characteristics and VL of groups with and without intestinal parasitic

infections were compared by Student's *t* test. Changes in VL levels at baseline and after two months of anti-TB chemotherapy were compared by paired *t* test. Two-tailed *P* values were determined and considered significant when found <0.05.

Ethical considerations: Written informed consent was obtained from all study participants, and the study was approved by the Research Ethics Committee of the University of Gondar, Ethiopia. Subjects found positive for intestinal parasites were given appropriate treatment.

RESULTS

One hundred-nineteen TB and HIV co-infected patients (48 males and 71 females) were included in the study. Their mean age was 31.3 years (range 16-60 years). A substantial majority (83.2%) of the patients were young adults less than 40 years of age . Table 1 shows socio-demographic and clinical characteristics of the patients.

Table 1. Socio-demographic and clinical characteristics of the study subjects

Characteristics	Frequency (%)
Sex	
Male	48 (40.3)
Female	71 (59.7)
Age	
<20	1 (0.8)
20-29	54 (45.4)
30-39	44 (37.0)
40-49	15 (12.6)
50+	5 (4.2)
Religion	
Christian	110 (92.4)
Muslim	9 (7.6)
Residence	
Urban	98 (82.4)
Rural	21 (17.6)
Clinical features	
Cough	99 (83.2)
Fever	112 (94.1)
Weight loss	111 (93.3)
Night sweats	106 (89.1)
Loss of appetite	21 (17.6)
Chest pain	41 (34.5)
Tuberculosis diagnosis	
Smear positive pulmonary	31 (26.1)
tuberculosis	18 (15.1)
Smear negative pulmonary	70 (58.8)
tuberculosis	
Extrapulmonary tuberculosis	

At baseline, 45 (37.8%) of the patients were found to be infected with one or more species of intestinal parasites. *Ascaris lumbricoides* and *Strongyloides stercoralis* were the most prevalent species with a detection rate of 16.8% and 13.4%, respectively. Single, double (*Ascaris lumbricoides* and *Schistosoma mansoni*, *Ascaris lumbricoides* and hookworm, *Giardia lamblia* and *Entamoeba histolytica*, or hookworm and *Entamoeba histolytica*) and triple (hookworm, *Strongyloides stercoralis* and *Schistosoma mansoni*) parasitic infections were detected in 39, 4 and 2 patients, respectively (Table 2).

Table 2. Type and frequency of intestinal parasites and serum viral load (mean±SD) in TB/HIV co-infected patients visiting the University of Gondar Hospital, Northwest Ethiopia

Parasite species	Frequency (%) (N=119)	Viralload (log_{10}RNA copies/ml)
S. stercoralis	16 (13.4)	5.21±0.90 (n=11)
A. lumbricoides	20 (16.8)	4.70±0.78 (n=14)
Hookworm	8 (6.7)	4.98±0.73 (n=6)
S. mansoni	8 (6.7)	4.76±0.69 (n=4)
E. histolytica	4 (3.4)	5.29±0.58 (n=4)
G. lamblia	5 (4.2)	4.98±0.59 (n=5)
T. saginata	1 (0.8)	4.85±0.00 (n=1)
All protozoa	9 (7.6)	4.81±0.76 (n=9)
All helminths	53 (44.5)	4.63±0.76 (n=36)
Total no. of patients infected	45 (37.8)	4.67±0.73 (n=45)
Infection with single species	39 (32.8)	4.62±0.75 (n=33)
Infection with two species	4 (3.4)	5.11±0.68 (n=4)
Infection with three species	2 (1.7)	4.54±0.88 (n=2)

Twelve patients had VL below 400 RNA copies/ml and hence were not included in the analysis. Figure 1 shows the profile of serum VL in the study population.

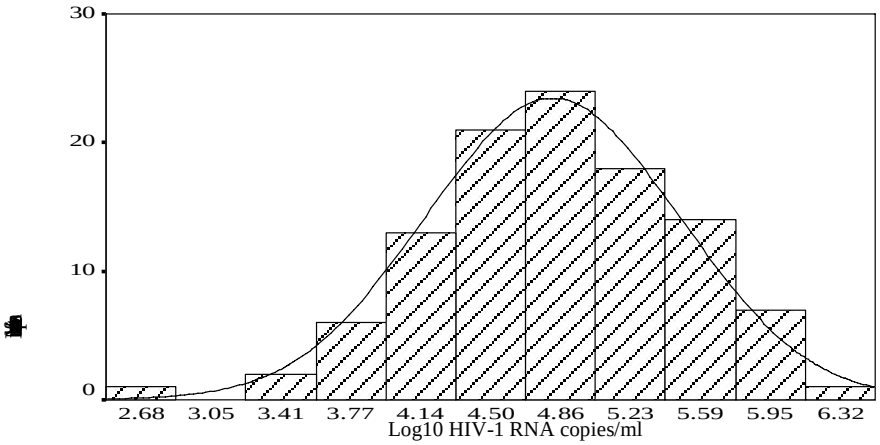

Figure 1. Profile of serum HIV-1 RNA viral load in tuberculosis patients, Gondar, Ethiopia (n=107)

The mean±SD serum VL of patients at baseline (n=107) was 4.82±0.66 $\log_{10}$ RNA copies/ml. It was 4.67±0.74 $\log_{10}$ RNA copies/ml in patients co-infected with intestinal parasites (n=38) and 4.91±0.60 $\log_{10}$ RNA copies/ml in those without intestinal parasites co-infection (n=69), with a mean difference of 0.24 $\log_{10}$ RNA copies/ml and having no significant difference between the two groups. No significant association was found between the presence of individual intestinal parasites and VL.

However, relatively higher VL was observed in patients infected with *Strongyloides stercoralis* and *Entamoeba histolytica* (Table 2). Also, individuals infected with multiple intestinal parasite species (n=6) had higher mean VL (4.92±0.72 $\log_{10}$ RNA copies/ml) than those infected with single parasites (4.62±0.75 $\log_{10}$ RNA copies/ml, n=33) although the difference was not statistically significant. In addition, serum VL was relatively higher in patients with EPTB (4.89±0.72 $\log_{10}$ RNA copies/ml, n=63) than those diagnosed with PTB (4.71±0.53 $\log_{10}$ RNA copies/ml, n=44, P>0.05).

HIV-1 VL data were available for 22 patients both at baseline and two months after anti-TB treatment. This was due to the referral of patients to health service delivery setups closer to their residence for TB treatment after TB diagnosis was made in the hospital.

The group with follow-up treatment did not differ from the whole group at baseline in age and sex distribution, clinical features of TB, or serum HIV-1 RNA VL. Serum HIV-1 RNA VL of the 22 patients declined to 4.52±0.66 $\log_{10}$ RNA copies/ml from the pretreatment reading of 4.84±0.45 $\log_{10}$ RNA copies/ml, although the difference was not statistically significant. In 15 patients, HIV-1 RNA VL declined by a mean of 0.75 log10 RNA copies/ml from a baseline reading of 5.03+0.37 log10 RNA copies/ml to 4.28+0.58 log10 RNA copies/ml, P<0.001. Five of the 15 patients were those who were also treated for intestinal parasites and their HIV-1 RNA VL significantly declined at the end of the intensive phase of anti-TB chemotherapy (5.02±0.38 versus 4.47±0.66 $\log_{10}$ RNA copies/ml, P<0.05) (Table 3).

In the other 10 patients, the HIV-1 RNA VL significantly declined to 4.18±0.54 $\log_{10}$ RNA copies/ml at the end of the intensive phase chemotherapy from 5.03±0.37 $\log_{10}$ RNA copies/ml at baseline (P<0.01), (Table 3). In the other seven patients, there was a significant increase in the mean serum HIV-1 RNA VL from 4.43±0.36 $\log_{10}$ RNA copies/ml at baseline to 5.02±0.57 $\log_{10}$ RNA copies/ml after treatment (P<0.01) (Table 3). The mean changes in HIV-1 RNA serum VL of each group are presented in Table 3.

Table 3: Serum viral load (mean±SD) at baseline and at the end of intensive phase of anti-TB chemotherapy by type of TB and status of intestinal parasitoses in TB/HIV co-infected patients visiting the University of Gondar Hospital, Gondar, Ethiopia.

Patient code	Sex	Age	TB type	Baseline		After two months	
				Parasite	VL	Parasite	VL
109	Male	45	EPTB	Ascaris	4.85	Negative	4.62
117	Female	20	EPTB	Ascaris	5.25	Negative	4.37
79	Female	33	EPTB	Ascaris	4.72	Negative	3.89
23	Female	31	EPTB	Giardia	5.57	Negative	5.52
1	Male	30	PTB	Ascaris	4.69	Negative	3.94
D Mean (±SD) HIV-1 RNA load -0.54 (±0.37)							
126	Male	26	PTB	Negative	5.03	Negative	4.51
135	Female	30	PTB	Negative	4.69	Negative	4.34
114	Male	30	EPTB	Negative	5.41	Negative	4.31
116	Male	25	PTB	Negative	5.39	Negative	3.97
83	Male	28	EPTB	Negative	4.56	Negative	4.44
86	Male	42	PTB	Negative	5.13	Negative	3.08
87	Female	28	PTB	Negative	5.53	Negative	5.05
74	Male	23	EPTB	Negative	5.21	Negative	4.51
53	Female	22	PTB	Negative	4.90	Negative	3.71
21	Male	29	PTB	Negative	4.42	Negative	3.89
D Mean (±SD) HIV-1 RNA load -0.84 (±0.58)							
69	Female	22	PTB	Negative	4.71	Negative	5.52
46	Male	25	PTB	Negative	4.76	Negative	5.52
60	Female	25	EPTB	Negative	4.50	Negative	5.26
28	Female	35	PTB	Negative	4.32	Negative	5.26
30	Female	24	PTB	Negative	3.85	Negative	3.90
10	Female	30	PTB	Negative	4.09	Negative	4.70
77	Female	35	PTB	Negative	4.81	Negative	5.01
D Mean (±SD) HIV-1 RNA load +0.58 (±0.33)							

DISCUSSIONS

HIV/AIDS, TB and intestinal parasites are amongst the various health problems people of the poor nations are facing. The pandemic of HIV is causing a resurgence of TB, with TB incidence increasing rapidly in countries with a high burden of HIV infection (1). On the other hand, due to poor environmental sanitation and personal hygienic practices, the burden of intestinal parasitic infections in underdeveloped nations of the world, especially in sub-Saharan Africa, is very high. The exposure of hosts to such a multitude of environmental antigens has been suggested to cause chronic immune activation which would make the host more susceptible to infections and less able to cope once infected (6,19,20).

We observed a high prevalence of intestinal parasites in TB and HIV co-infected patients in the present study. A few previous studies had also demonstrated the high burden of intestinal parasites in AIDS patients from the country (21-24). Our finding of a 0.24 $\log_{10}$ RNA copies/ml difference in baseline VL between patients negative for intestinal parasites and those positive for intestinal parasites is in line with a recent study from Zambia which showed a median pretreatment plasma concentration of HIV-1 RNA higher by 0.33 $\log_{10}$ copies/ml in the helminth-uninfected group compared to helminth infected group (25). To the contrary, however, a report by Brown *et al.* (6) from a cohort study in Uganda showed that helminth infected patients had a significantly higher plasma VL than those negative for helminthes (4.92+0.85 vs 4.74+0.91 $\log_{10}$ HIV-1 RNA copies/ml, P=0.03).

Our observation of a lack of a significant association between HIV-1 RNA VL and the presence of a specific parasite species is also in good agreement with a study from Brazil where no association between the level of CD4 count or plasma VL and infection with a specific parasite was reported (26). The significant decline in the HIV plasma VL of the five patients after deworming and anti-TB treatment in this study is in line with that shown by Wolday *et al.* (7) where a successful treatment of intestinal helminthes resulted in a significant decline in VL. On the other hand, Lawn *et al.* (27) did not observe a reduction of HIV-1 plasma VL after treatment of schistosomiasis in dually infected patients in Kenya.

It is interesting to note, in the present study, that HIV-1 RNA VL declined by a mean of 0.32 log10 copies/ml two months after treatment of TB in the twenty two patients although the result was statistically significant (P=0.07). Among twenty HIV-infected patients in Ghana, Lawn *et al.* (15) did not observe a significant decrease in plasma VL three months after treatment of TB. In that study, five patients had significant increases (>0.5 log10 copies/ml) in VL, four had significant decreases, and ten had no significant changes in VL. This is in agreement with our observation of a significant decline by a mean difference of 0.75 log10 HIV-1 RNA copies/ml in fifteen patients, and a significant increase by a mean of 0.58 log10 HIV-1 RNA copies/ml in seven.

Our observation is also partly in agreement with a report by Wolday *et al.* (17) which showed a significant increase in plasma VL (>0.5 log10 HIV-1 RNA copies/ml) in 48% of TB patients, where 20% had no change but 24% showed a significant decline during the initial two months of anti-TB treatment in Addis Ababa, Ethiopia.

On the other hand, Morris *et al.* (16) reported a significant increase in VL level one month after treatment of TB in South African patients from a baseline median VL of 5.58 log10 copies/ml to 5.71 log10 copies/ml. Further, in a study in Abidjan, Cote d'Ivore, Kalou *et al.* (14) demonstrated a significant increase by a median of 0.64 log10 HIV-1 RNA copies/ml in forty four patients from a baseline of 4.2 log10 HIV-1 RNA copies/ml to a 4.9 log10 HIV-1 RNA copies/ml 12 months after successful treatment of TB.

Our findings that HIV VL increases significantly despite the initial intensive treatment of TB among the seven HIV-infected TB patients have important clinical implications as plasma VL is a strong predictor of disease progression and mortality. This may explain the persistent elevation in VL and mortality among HIV-infected persons even after they were cured of their TB in a previous study in South Africa (16). However, the decline in VL after anti-TB treatment in the fifteen patients corroborates with the significant decrease in plasma VL that have been observed after treatment of other co-infections (such as sexually transmitted infections, opportunistic infections, and falciparum malaria) in HIV-1 infected patients (28-33). On the other hand, studies conducted in the developed world showed a significant decline in VL after treatment of TB (12,13).

In a study in the United States, a significant decrease in VL in four TB patients after one month of treatment of TB was observed (12). Dean *et al.* reported a significant decrease in VL (from 4.9 log10 copies/ml at baseline to 3.8 log10 copies/ml at twelve months) after treatment of TB in HIV-1 infected patients in England (13). The discrepancy in changes in HIV-1 viral load observed among HIV-infected TB patients treated in developed countries and those treated in Africa could be due to the general chronic activation in African patients whereby dysregulation of cytokines production occurs despite the resolution of TB in these patients (15).

Taken together, the few human studies which tried to address the role of anti-TB (12-17) or anti-helminthes (6,7,25,27) chemotherapy on VL have ended up with inconsistent findings which may be due to limitations such as small sample size or absence of comparison/control groups. The present study also suffers from limitations such as the small size of the study population, lack of complete follow-up of the patients due to the clinical arrangements, and inability to include CD4 count due to lack of such service.

It is, therefore, not possible to reach a definite conclusion at this point, despite the role intestinal parasites and TB play in immune dysregulation and HIV disease progression (6,19,20). Future studies should take into consideration the above mentioned missing issues, and others such as sputum and culture conversion, drug resistance profile of mycobacterial isolates, radiographic and clinical improvements of TB, infection with blood or tissue parasites, etc.

In conclusion, the mean serum HIV-1 RNA VL did not decline significantly two months after anti-TB treatment except in those who were simultaneously treated for intestinal parasites. Intervention measures such as the provision of anti-retroviral and anti-

parasite therapy may help to reduce morbidity in TB patients with co-infections. Further longitudinal studies with larger sample sizes and follow-up of the patients during the entire course of the treatment are required to substantiate the present findings.

ACKNOWLEDGMENTS

The study was financially supported by a grant from the National Institute of Biomedical Innovation, Japan and partly by the University of Gondar, Ethiopia. We would like to thank the study participants without whom this study could not have been completed.

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