Original Article

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Liver Function Profile Anomalies in HIV Seropositive Tuberculosis

Internal Medicine Section

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ABSTRACT

Background: The Human Immunodeficiency Virus (HIV) and the Tuberculosis (TB) co infection are contributory to each other in causing a progressive decline in the cell mediated immunity and a damage to the hepatobiliary system. The aim of our study was to estimate the extent of liver damage which was caused by these infections before the start of the therapy with hepatotoxic drugs like Antiretroviral Therapy (ART) and Antitubercular Drugs (ATD).

Methods: One hundred and ninty three confirmed HIV positive cases were enrolled in this study. The cases were divided into 2 groups; Group 1-100 subjects with TB and *Group* 2-93 subjects without TB.80 age and sex matched controls were also included (Group 0). Some parameters of the serum Liver Function Test (LFT) were estimated biochemically by using an auto analyzer (ERBA XL600,Transasia).

Results: The serum total bilirubin, Alanine Transaminase (ALT), Aspartate Transaminase (AST) and the Alkaline Phosphatase (ALK-P) levels were significantly higher in the cases as compared to those in the controls, more so in the cases with the associated TB co infection, except the AST levels. The Group 1subjects had lower serum total protein and albumin levels and altered albumin/globulin ratios as compared to the controls. A statistically significant difference was absent in the serum total protein levels between the *Group 2* cases and the *Group* 0 controls. No significant differences were observed when the values for serum total protein, albumin and globulin and the albumin: globulin ratios among the two case groups (1 and 2) were compared.

Conclusion: The results have shown the importance of estimating some LFT parameters, prior to the start of ATD and ART in these cases. Hence, a mandatory performance of LFT is recommended, as it is simple and cost effective.

Key Words: Human Immunodeficiency Virus (HIV), Tuberculosis (TB), Liver Function Test (LFT)

INTRODUCTION

The Human Immunodeficiency Virus (HIV) and Tuberculosis (TB) have been closely linked to each other. Since the emergence of the Acquired Immune Deficiency Syndrome (AIDS), the HIV infection has been found to be contributory to a significant increase in the incidence of TB worldwide [1,2]. TB is the most common opportunistic infection which affects HIV seropositive individuals [1] and it is the most common cause of death in the patients with AIDS [3]. By altering the pathogenesis of TB via a progressive decline in the cell-mediated immunity, HIV has given rise to more of extra pulmonary and disseminated TB and atypical presentations [1].

Approximately 10 million people are estimated to be co-infected with Mycobacterium Tuberculosis (MTb) and HIV. 90% of these dually infected individuals reside in the developing countries, where the rate of this co-infection exceeds 1000 per 1,00,000 populations [4].

The HIV infection is known to be an independent risk factor for the reacquisition of the TB infection and a rapid progression to disease [5,6], due to a decrease in the host's ability to contain the new tuberculosis infection [7]. Alternatively, investigations have shown that MTb increases the viral replication in the T lymphocytes and the monocytes [8-10] and that it accelerates the clinical course of the HIV infection [11]. The involvement of the hepato–biliary system is a major concern in the patients with the HIV infection. Approximately one third of the deaths in the patients with the HIV infection are in some way, related to liver disease. Granulomatous hepatitis has been seen as a consequence of the Mycobacterium infection. In late stages of the HIV infection, a primary TB like pattern with or without a diffuse interstitial or a miliary infiltrate is quite common. The physical findings in miliary tuberculosis include hepatosplenomegaly and lymphadenopathy, especially in the abdomen. The elevation of serum Alkaline Phosphatase (ALK-P) and other abnormal values of the liver enzymes in the Liver Function Test (LFT) are detected in patients with a severe hepatic involvement in the HIV illness. Granulomas are also evident in the liver biopsy specimens in many cases [12].

The Antitubercular Drugs (ATD) frequently cause disturbances in the LFTs in 20-25% of the patients and clinical hepatitis in 3% of the cases. Isoniazid and Pyrazinamide raise the serum transaminase levels, while Rifampicin causes hyperbilirubinaemia and centrilobular necrosis of the liver [13].

Similarly, all the Antiretroviral Drugs (ART) like Nevirapine and Efavirenz and almost all the protease inhibitors are potentially hepato-toxic and they cause elevated serum bilirubin and transaminase levels [14].

In this background, a study was conducted to compare the biochemical indices of liver cell damage and hepato-biliary obstruction in the ART naïve cases in the HIV illness with or without TB.

MATERIALS AND METHODS

Between January 2003 and January 2012, a total of 273 subjects were selected for this study. 193 subjects who had been confirmed

to be positive for the HIV antibodies after they had undergone proper laboratory tests in the School of Tropical Medicine/ ICTC clinic, following their referral to the Department of Chest Medicine of a teaching hospital in Kolkata, India, were selected as the cases. The serous samples from all the suspects were screened for the HIV antibody by a rapid immunochromatography test. Those who were found to be reactive were subjected to an Enzyme Linked Immunosorbent Assay (ELISA) or another kit of a different principle was considered as HIV+ve in this study. The cases were further divided into two separate groups, based on the presence or absence of sputum positive pulmonary tuberculosis in them (100 and 93 respectively).There was no case of tuberculosis of the gums or the oropharyngeal region. 80 seronegative (HIV) subjects who had attended the out-patients department or were admitted in the Department of Chest Medicine for non-tubercular pulmonary illness and who were devoid of any hepato-biliary disorder, musculo-skeletal disease or malignancy were selected as the controls. Ethical clearance was obtained for this study. Written consents were obtained from all the cases and the controls. The clinical specimens from all the cases were obtained prior to the commencement of ART or ATD. 5 ml of blood was collected aseptically from all the cases and the controls and the sera which were obtained following the centrifugation of the blood were used to perform different bio chemical tests by using an auto analyzer (ERBA XL600, Transasia). The serum bilirubin was estimated by Jendrassik and Grof's method. Serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST) activities were measured by the modified International Federation for Clinical Chemistry (IFCC) method . The serum ALK-P levels were estimated by the para Nitro Phenyl Phosphate (pNPP) method. The serum total protein and albumin levels were determined by the Biuret and the Bromocresol Green (BCG) methods respectively. The serum globulin levels were calculated by substracting the serum albumin values from those of the total protein.

Statistical software: SPSS, version 16.0 was used to analyze the data and Post hoc ANOVA with Bonferroni corrections was used to compare the multiple parameters.

Parameters	Group 1 (n=100)		Group 2 (n=93)		Group 0 (n=80)	
Gender	Male# (%)	Female# (%)	Male# (%)	Female# (%)	Male# (%)	Female# (%)
# (%)	75 (75)	25 (25)	72 (77.42)	21 (22.58)	60 (75)	20 (25)
Mean age (In years) ± SD	32.56 ± 2.83	31.38 ± 1.02	30.9 ± 0.71	30.23 ± 0.63	39.03 ± 10.6	35.48 ± 9.8

[Table/Fig-1]: Age and sex distribution of cases and controls

NB- Group 0- controls, Group 1-cases with both HIV and TB, Group 2- cases with HIV only. Abbreviations- ± Standard deviation, # Number

Multiple Comparisons Post hoc ANOVA with Bonferroni correction					
Dependent Variable	(I) Grouping	(J) Grouping	Mean Difference (I-J)	Std. Error	Level of significance (p value)
Total protein	0 vs	1	-0.68867*	.16596	.000
	0 vs	2	-0.37333	.16507	.075
	1 vs	2	0.31533	.17249	.208
ALK-P	0 vs	1	-229.40000*	24.61646	.000
	0 vs	2	-102.66078*	24.48447	.000
	1 vs	2	126.73922*	25.58471	.000
Albumin	0 vs	1	0.51067*	.10227	.000
	0 vs	2	0.69843*	.10173	.000
	1 vs	2	0.18776	.10630	.238
Globulin	0 vs	1	-1.24733*	.11524	.000
	0 vs	2	-1.09922*	.11463	.000
	1 vs	2	0.14812	.11978	.654
Albumin: globulin ratio	0 vs	1	0.58365*	.04226	.000
	0 vs	2	0.59484*	.04203	.000
	1 vs	2	0.01119	.04392	1.000
ALT	0 vs	1	-35.70000*	4.24818	.000
	0 vs	2	-18.39608*	4.22540	.000
	1 vs	2	17.30392*	4.41527	.000
AST	0 vs	1	-15.75667*	3.71129	.000
	0 vs	2	-10.39314*	3.69139	.016
	1 vs	2	5.36353	3.85727	.499
Total bilirubin	0 vs	1	-2.88897*	.16986	.000
	0 vs	2	-1.58772*	.16895	.000
	1 vs	2	1.30125*	.17654	.000

[Table/Fig-2]: Comparison of different LFT parameters in three study groups

* The mean difference is significant at the <0.05 level
Control group =0 (I),Cases with both HIV and TB =1(J), Cases with HIV only =2 (J)
(I-J) : Difference between mean values between cases and controls and those between the two case groups. Abbreviation- vs: Versus

RESULTS

All the cases and the controls were distributed in three groups. Group 0 constituted the controls (n=80). Group 1 constituted the cases with both HIV and TB (n=100) and Group 2 included the HIV cases only(n=93).The age and sex distribution among the cases and the controls are shown in [Table/Fig-1].

The results showed [Table/Fig-2] statistically significant higher levels of serum total bilirubin amongthe cases (both Groups 1&2) as compared to those in the controls (Group 0). Similarly, there was a significant increase in the activities of the serum enzymes like ALT,AST and ALK-P in all the cases (both Groups 1 and 2) as compared to those in the controls(Group 0). The Group 1 subjects had lower serum total protein and albumin levels and altered albumin/ globulin ratios as compared to those in the controls (Group 0). The Group 2 cases did not show any statistically significant difference in the serum total protein levels as compared to those in the controls (Group 0).

The Group 1 cases had higher total bilirubin levels and ALT and ALK-P activities as compared to the Group 2 cases. No significant differences were observed when the values for serum total protein, albumin and globulin and the albumin: globulin ratios among the two case groups (1 and 2) were compared with each other [Table/Fig-3].

Parameters	HIV+ve with TB (Group 1) (n= 100)	HIV+ ve only (Group 2) (n = 93)	Control group (Group 0) (n = 80)
Hepatomegaly	64 (64.0)	57 (61.29)	6 (7.5)
Splenomegaly	32 (32.0)	22 (23.65)	0
GB stone	3 (3.0)	3 (3.23)	0

[Table/Fig-3]: USG abdomen (chief findings) in all cases and controls
*Figures in parentheses indicate percentage

The rates of hepatomegaly, splenomegaly and GB stone among the different groups were compared by the Z-test, taking two groups at a time:

Hepatomegaly

Between the HIV+ve with TB cases and the HIV+ve cases only (64.0% vs. 61.29%) ----- z = 0.24 p = 0.810 (not significant)

Between the HIV+ve with TB cases and the controls (64.0% vs. 7.5%) ------ z = 7.57 p = 0.000 (significant)

Between the HIV+ve cases only and the controls (61.29% vs. 7.5%) ------ z = 7.17 p = 0.000 (significant)

Splenomegaly

Between the HIV+ve with TB cases and the HIV+ve cases only (32.0 vs. 23.65) ---- z = 1.13 p = 0.258 (not significant)

Splenomegaly was significant in the HIV+ve cases and in the HIV-TB coinfection, and it was absent in the controls.

DISCUSSION

The results of our study showed altered LFTs in the cases as compared to those in the controls. The concentrations of serum albumin were lower in all the cases as compared to those in the controls. Our findings were similar to those of the study of Geffriend et al., [15] However, Cello [16] and Capell [17] reported an insignificant reduction in albumin concentrations when the HIV positive patients in their study were compared to the controls. The observed reduction in our study suggested a chronic hepatic dysfunction and suppression of the synthetic function of the liver [18]. Reversal of the albumin/globulin ratio was seen among the cases. A similar pattern had been reported by Mohammed [19] who had noticed that the HIV patients had generalized lymphadenopathy and polyclonal hyperglobinopathy which involved IgG, IgA and IgM, which had caused an altered albumin: globulin ratio. A similar pattern of hypergammaglobulinaemia had been confirmed in the HIV patients in northern Nigeria [20]. Except for the serum total protein levels, the cases with both HIV and TB had a more significant alteration of these values than those with HIV alone.This finding suggested that the HIV-related immunosuppresion prevents the containment of TB to a localized portion of a single organ system [21].

Opportunistic infection of the liver by M.tuberculosis is a common complication in HIV. It is implicated as the cause of the liver parenchymal damage [22].

Moreover, previous studies have suggested that the liver is an important site of the HIV replication [23]. HIV attacks the liver cells directly [24], causing cell death and the release of the cellular contents into the surrounding medium, of which the enzymes constitute 20% [25]. This may be responsible for the increase in the serum liver enzymes in the infected patients. As a support to our findings, various studies have shown a significant increase in the serum AST, ALT and the ALK-P activities in the ART naive, HIV positive cases as compared to the control subjects [26-28].

In our study, the serum ALK-P, ALT and the total bilirubin levels were higher in all the HIV cases as compared to the controls. The values were more in the cases with tuberculosis. This may be due to the degeneration of the connective tissue of the liver [29] and it may also be the result of the hepatobiliary obstruction that had occurred in the subjects. The structural abnormalities like papillary stenosis, sclerosing cholangitis, cholecystitis and a thickened gall bladder wall [30] in these HIV patients could be attributed to the TB like opportunistic infection [30, 31].

Our observations were similar to the findings of Dwarkin et al., [32] who found a significant rise in the serum ALK-P levels [33] in the cases with HIV and to those of Cello et al., [16] who found increased levels of ALK-P and cholecystitis in the subjects with AIDS.

Imaging studies which were done by Ultrasonography (USG) in AIDS patients had shown that the HIV infected patients had hepatomegaly, steatosis, cholecystitis and biliary tract abnormalities, which had caused the altered LFTs, which had been seen here also. The granulomatous hepatitis which had occurred in these cases due to Mycobacterium TB, represented the reactivation of the latent disease [34].

This study had limitations that must be considered. The numbers of the patients in the study groups were not large. Many other investigations, though they were desirable, could not be performed because of limitations.

Thus, care must be taken during the extrapolation of the present findings with those of other populations and other racial groups. Despite these limitations, we believe that our study will be helpful, as the assays of the above mentioned LFT parameters are simple and cost effective.

The results of our study clearly indicated the derangement of most

of the LFT parameters (serum bilirubin, total protein, albumin and globulin, the albumin/globulin ratio, ALT,AST and ALK-P) in the HIV infection. This effect on the above mentioned parameters on the LFT was further aggravated by opportunistic infections with Mycobacterium tuberculosis in the TB-HIV co-infection. Nowadays, it is recommended that the LFT must be performed on a routine basis in HIV+ve subjects before the start of the ART. Though it is desirable that the LFT must be done as a routine before giving ATD to the newly diagnosed TB (all forms) patients, this is not adhered to in the routine practice.

Our study demonstrates that LFT must be made mandatory before giving ATD. A similar observation was made by Pukenyte et al., [35]. In such cases or when it is done for other indications, the unexplained high levels of ALK-P should raise the suspicion of a co-existing HIV infection, which was hitherto undetected. Thus, ALK-P can be used as a fruitful marker for identifying more underlying HIV infection.

REFERENCES

[1] AIDS Control and Prevention (AIDSCAP) Project of Family Health Internal, The Francois-Xavier Bagnoud Center for Public Health and Human Rights of the Harvard School of Public Health UNAIDS. *The Status and Trends of the Global HIV/AIDS Pandemic*. Final Report July 5-6, 1996.

[2] Raviglione MC, Narain JP, Kochi A. HIV-associated tuberculosis in developing countries: clinical features, diagnosis, and treatment. *Bull WHO*. 1992; 70:515-26.

[3] Raviglione MD, Snider DE, Kochi A. Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. *JAMA*. 1995; 273:220-26.

[4] Dye C, Scheele S, Dolin P, PathaniaV, Raviglione MC. Global burden of tuberculosis. Estimated incidence, prevalence, and mortality by country. *JAMA*. 1999; 282:677-86.

[5] Alland D, Kakut GE, Moss AR, McAdam RA, Hahn JA, Bosworth W, et al. Transmission of tuberculosis in New York City: An analysis by DNA fingerprinting and conventional epidemiologic method. *N Engl J Med*. 1994; 330:1710-16.

[6] Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, et al. The epidemiology of tuberculosis in San Francisco: a population-based study using conventional and molecular methods. *N Engl J Med*. 1994; 330:1703-09.

[7] Hopewell PC, Bloom BR. Tuberculosis and other mycobacterial diseases. In: Murray JF, Nadel JA, editors. Respiratory Medicine, 3rd ed. Philadelphia, PA:WB Saunders Company; 2000; 1043-50.

[8] Pape JW, Jean SS, Ho JL, Hafner A, Johnson DW. Effect of isoniazid prophylaxis on incidence of active tuberculosis and progression of HIV infection. *Lancet*. 1993; 342:268-72.

[9] Shattock RJ, Friedland JS, Griffin GE. Modulation of HIV transcription in and release from monocytic cells following phagocytosis of Mycobacterium tuberculosis. *Res Virol*. 1993; 144:7-12.

[10] Lederman MM, Georges D, Kusner DJ, Mudido P, Giam CZ, Toossi Z. Mycobacterium tuberculosis and its purified protein derivative activate expression of the human immunodeficiency virus. *J AIDS*. 1994; 7:727-33.

[11] Nakata K, Weiden M, Rom WN. Mycobacterium tuberculosis enhances human immunodeficiency virus-1 replication by transcriptional activation at the long terminal repeat. *J Clin Invest*. 1995; 95:2324-31.

[12] Raviglione MC, O'Brine RJ. Tuberculosis. In:Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J et al, editors. *Harrison's Principles of Internal Medicine*. 18th ed.New York; McGraw Hill 2012; 2: 1340-59.

[13] Ragni MV, Belle SH, Im K, Neff G, Roland M, Stock P, et al.Survival of human immunedeficiency virus-infected livertransplant recipients. *J Infect Dis*. 2003; 188:1412-20.

[14] Spach DH, Barlett JG. Abnormal laboratory values in HIV disease. In: Barlett JG, Cheever LW, Johnson MP, Paauw DS, editors. A guide to primary care of people with HIV/ AIDS.2004 edition. Rockville; U.S. Department of Health and Human Services, *Health Resources and Services Administration HIV/AIDS Bureau* 2004; 10: 77-84.

[15] Geffriaud, Poynard T, Delfraissy JF, Bedossa P, Naveau S, Bourée P, et al. Hepatic involvement inHIV-1 virus infections. *Gastroenterol. Clin Bioc.*1988; 12(5):465-72.

[16] Cello JP.Acquired Immunodeficiency Syndrome Cholangiopathy. Spectrum Disease. *AmJ Med*.1989; 86:539-46.

[17] Cappell MS, Schwartz MS, Biempica L. Clinical utility of liver biopsy in patients with serum antibodies to the HIV. *Am J Med*. 1990; 88: 123-30.

[18] Ogunro PS, Oparinde DP, Okesina AB. Liver function tests in HIV-1 infected asymptomatic patients and HIV-1 AIDS patients without hepatomegaly in Lagos, Nigeria. *Afr J Clin Exper Microb*. 2005; 6(1):40-45.

[19] Mohammed I. AIDS in Nigeria. An immunological perspective. *Nig J Immunol*. 1990; 2:1-3.

[20] Uko GP, Griffiths M, Dawkins RL, Cobain T, Mohammed I, Hedo C, et al. IG2 associated hyperglobinaemia in some NigerianswithHIV infection. *Afr JMed Sci*. 1994;23:385-88.

[21] El-Sadr R, Abrams. Diagnosis and Management of HIV-related Tuberculosis. *The Columbia Clinical Manual*. 2004; 5.5:1-11.

[22] Housset C, Boucer O, Girard PM, Leibowitch J, Saimot AJ, Brechot C, et al. Immunohistochemical evidence for human immunodeficiency virus-1 infection of Liver Kupfer cells. *HumanPathology*. 1990; 21(4):404-08.

[23] Richardson I, Melester T, Gold D.Liver disease and HIV. *Gay Men's Health Crisis* "*Treatment Issues*" 1994;8(5).

[24] Oluwafemi O, Oguntibeju B, Olatubosan B. A Study on the Activities of Liver Enzymes in HIV/AIDS Patients. *J Med Sci.* 2003; 3(1):106-09.

[25] Wild-Up M, Fortuin F, Whittle DHC, Hall AJ, Wolf CR, Montesonai R. Liver and hepatitis Bvirusin Gambian children. *Cancer Epidemiol Biomarkers*. *Prev*.1990; 2(6):555-61.

[26] Bowen DC, Lane HC, Fauci AS. Immunopathogenesis of the acquired immune deficiency syndrome. *Ann Intern Med*. 1993; 103:704-09.

[27] Wit FW, Weverling GJ, Weel J, Jurrian S, Lange JM. Incidence of and risk factors for severe hepatotoxity associated with antiretroviral combination therapy. *J Infect Dis*. 2002; 186(1):23-31.

[28] Schneidermen DJ, Cello JP, Caing FC. Papillary stenosis and sclerosing cholangitis in the acquired immunodeficiency syndrome. *Ann Intern Med*. 1987; 106:546-49.

[29] Margulis SJ, Honig CL, Soave R, Govoni AF, Mouradian JA, Jacobson IM.Biliary tract Obstruction in the AIDS. *Ann Intern Med*.1986; 105:207-10.

[30] Prufer-Kramer L, Kramer A, Weigel R, Rogler G, Fleige B, Krause PH, et al. Hepatic involvement in patients with human immunodeficiency virus infection:discrepancies between AIDS patients and those with earlier stages of infection. *J Infect Dis*. 1991; 163(4):866-69.

[31] Forbes A, Blanshard C, Gazzard B. Natural history of AIDS related sclerosing cholangitis: a study of 20 cases. *Gut*. 1993; 34:116-21.

[32] Dworkin, et al. The liver in acquired immune deficiency syndrome: Emphasis on patients with intravenous drug abuse. *Am J Gastroenterol*.1987; 2(3):231-36.

[33] Astageau, et al. Hepatic involvement in AIDS.A retrospective clinical study in 71 patients. *Ann Med Intern Paris*.1990; 141(5):459:63.

[34] Reeders JWAJ, Bartesman JFWM, Huibregste K. AIDS-related manifestations of the bile duct system: A common finding? *Abdominal Imaging*. 1994; 19: 423:24.

[35] Pukenyte E, et al., Incidence of and risk factors for severe liver toxicity in HIV-infected patients on anti-tuberculosis treatment. *The International Journal of Tuberculosis and Lung Disease.* January 2007; 11(1): 78-84(7).

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