

Assessment of Liver Enzymes in Asymptomatic HIV-Seropositive Patients

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Abstract: Elevations in the liver enzymes signal injury to liver cells and in some cases, to other cells in the body. The activity of liver enzymes in 100 patients, aged 20-50 years, with asymptomatic HIV seropositive infection was assessed and 50 age-matched, apparently healthy subjects who tested negative for antibodies for HIV 1 and 2 served as control. The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) observed in HIV infected asymptomatic patients were significantly higher ($p < 0.05$), than those in the reference group. Non-significant difference was observed in serum Alkaline Phosphatases (ALP) of HIV infected asymptomatic patients ($p > 0.05$), when compared to the control subjects. Even in the absence of hepatomegaly there is evidence of greater hepatic damage in HIV infected asymptomatic patients as suggested by the results. Increase in ALT and AST is most likely due to impairment or involvement of the liver in asymptomatic HIV infection.

Key words: Assessment, HIV-seropositive, liver enzymes, asymptomatic

INTRODUCTION

Enzymes are proteins with catalytic properties due to their powers of specific activation of their substrates. They are also known markers of cellular damage (Reichling and Kaplan, 1988; Burtis *et al.*, 1996).

Serum levels of numerous cytosolic, mitochondrial and membrane-associated enzymes are increased in individuals with various forms of liver disease. The clinically important liver enzymes include Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) (Reichling and Kaplan, 1988). Elevations in the liver enzymes signal injury to liver cells and in some cases, to other cells in the body (Burtis *et al.*, 1996).

AIDS (Acquired Immune-deficiency Syndrome) is one of the most severe infections ever known to have attacked the human population (Watson, 2006).

The first well-documented case of AIDS was in an African man I, 1959 (Morgan *et al.*, 2002). AIDS has been reported in every country and parts of Africa and Asia are

especially devastated by it. Estimates of the number of individuals currently infected with the virus range from 35-40 million with a large number of them not showing any symptoms because they are in the latent phase of the disease (Bonnet *et al.*, 2004). HIV is an enveloped retrovirus containing single stranded RNA (ssRNA), which is the etiologic agent of AIDS and an infection is initiated by binding of the virion envelope gp 120 to the CD4 receptor on the host cell (Feldman, 2005). Persistent HIV infection with depletion of CD4 T-helper cells is central to the pathogenesis of HIV disease, as manifested by immune-deficiency, susceptibility to opportunistic infection and other AIDS-defining illness (Palefsky, 2007). The clinical manifestations of HIV/AIDS are many and diverse. These manifestations can be seen as three phases: the initial acute phase, the latent phase and the advanced stage (Watson, 2006).

In asymptomatic seropositive HIV patients, there are moderate, significant elevations in ALT, AST and ALP (Morgan *et al.*, 2002; Schneider *et al.*, 2005). It is possible nonetheless that other disease conditions such as

hepatitis, cirrhosis, hepatic cholestasis, hepatobiliary disease with increased production of enzymes, enzyme induction and proliferation of enzyme-producing cells as in cancer patients could be secondary to HIV infection and thus, contribute to the increase in the activities of the liver enzymes at different degrees (Lawn, 2004; Bonnet *et al.*, 2004).

MATERIALS AND METHODS

Subjects: One hundred asymptomatic patients (aged between 20 and 50 years), confirmed to be HIV positive were recruited for this study, while the control group consisted of 50 apparently healthy age-matched volunteers, who were confirmed HIV negative. All the subjects were negative for hepatitis B surface antigen and were not known to suffer from any major liver disease or bone disease. Pregnant women were also excluded from the study. Informed consent was obtained from all the subjects verbally and ethical covering was granted by the institution before the commencement of the study.

Sample collection and processing: Blood samples were collected by clean venepuncture from the antecubital fossa into already labeled plain test tubes, without undue pressure on either the arm or the plunger of the syringe. (Cheesbrough, 2002).

The samples were allowed to clot and were then centrifuged to obtain the sera. The separated clear sera were transferred into sterile bottles and were used for the enzyme assay. When not used immediately, they were stored at -20°C and later used within 5 days.

Analytical methods

Estimation of the transaminases (AST and ALT): Aspartate transaminase (AST) and Alanine transaminase (ALT) activities were assayed by the Reitman and Frankel method (Burtis *et al.*, 1996) using Beckman Spectrophotometer.

Alkaline phosphatase estimation: The method of King and Armstrong (Reichling and Kaplan, 1988) was employed for the estimation of Alkaline Phosphatase (ALP) activity.

HIV screening test: HIV screening was done by ELISA (enzyme linked immunosorbent assay) technique (Cheesbrough, 2002).

The kit reagents were manufactured by Biosystems S.A. Barcelona, Spain.

Principle: The reactant, a known antigen is adsorbed to the surface of a well and the serum is added. After

incubation, the well is washed and an enzyme-antibody reagent that can react with the test antibody is placed in the well. The substrate to the enzyme is then added and the wells are scanned for colour changes. Colour development indicates the presence of the antibody in the patient's serum.

HIV confirmatory test: The confirmatory test was done by the western blot method (Cheesbrough, 2002). The kit reagents were manufactured by Biosystems S.A, Barcelona, Spain.

Principle: The test material (Serum sample) is electrophoresed in a gel to separate out particular bands. The gel is transferred to a special blotter that binds the reactant in place. The blot is developed by incubating it with a solution of antibody or antigen labeled with radioactive, fluorescent or luminescent labels. Sites of specific binding will appear as pattern of bands that can be compared with known positive and negative samples. The specific sites allow for the identification of specific antibodies.

The technique detects more antibody types and is less subject to misinterpretation than other antibody tests.

Statistical analysis: Data was analyzed separately using paired t-test and results were expressed as Mean±Standard Deviation (±SD).

RESULTS

The results show a general increase in the liver enzymes ALT, AST and ALP in the asymptomatic HIV seropositive subjects.

Table 1 shows the different enzyme activities of the test and control subjects. There was a significant increase ($p < 0.05$), in the mean levels of AST and ALT for the test subjects (14.56 ± 7.13 ; $10.30 \pm 5.10 \mu\text{L}^{-1}$, respectively) when compared to the control (9.53 ± 2.62 ; $5.87 \pm 2.66 \mu\text{L}^{-1}$).

The Table 1 also shows a non-significant increase ($p > 0.05$), in the mean level of ALP for the test subjects ($51.39 \pm 14.92 \mu\text{L}^{-1}$) when compared to the control subjects ($48.47 \pm 11.99 \mu\text{L}^{-1}$).

Table 1: Liver enzyme activities of the test and control subjects

	Test subjects	Control subjects	p-value
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No. subjects	n = 100	n = 50	
AST (μL^{-1})	14.56±7.10	9.53±2.600	<0.05*
ALT (μL^{-1})	10.30±5.10	5.87±2.660	<0.05*
ALP (μL^{-1})	51.39±14.9	48.47±11.9	>0.05

* Statistically significant

DISCUSSION

The results of the study show a statistically significant increase ($p < 0.05$) in the activities of the liver enzymes AST and ALT in asymptomatic HIV seropositive subjects when compared to the controls. The International Federation of Clinical Chemistry (IFCC) estimated the reference ranges for AST, ALT and ALP as 8-20, 10-40 and 38-94 μL^{-1} , respectively (Burtis *et al.*, 1996).

From Table 1, the activities of the 2 enzymes (ALT and AST) agree with the finding of Morgan *et al.* (2002), in which there was significant increase in the activities of AST and ALT.

The serum ALP level was however, not statistically significant ($p > 0.05$) compared to the control group. This finding is in contrast to the previous findings of Morgan *et al.* (2002), who recorded significant increases in the activities of the 3 enzymes investigated.

The increase in the liver enzymes may be due to the release of cellular contents of dead or injured cells into the surrounding medium, of which enzymes constitute 20%. An event that takes place in HIV infection (Schneider *et al.*, 2005).

CONCLUSION

The study indicates the presence of significant elevation in AST and ALT and non-significant elevation in ALP activity in asymptomatic HIV infection.

Since, wrong management in sequel to misdiagnosis, there is need to monitor prognosis and the progressive involvement of the liver cells in the pathology of HIV infection, via the estimation of serum levels of AST, ALT and ALP. This in turn will help prevent the progressive destruction of liver cells and ensure better management of HIV patients.

REFERENCES

Bonnet, F., C. Lewden and T. May *et al.*, 2004. Malignancy-related causes of death in human immunodeficiency virus-infected patients in the era of highly active antiretroviral therapy. *Cancer*, 101 (2): 317-324. PMID: 15241829. DOI: 10.1002/ncr.20354.

Burtis, C., E. Ashwood and B. Border, 1996. Liver Enzymes: In Tietz Fundamentals of Clinical Chemistry. 5th Edn. In: Aldrich, J.E. (Ed.). Saunders Company. Pennsylvania USA., pp: 353-355. ISBN: 0-7216-3763-9.

Cheesbrough, M., 2002. Clinical Chemistry Tests. District Laboratory Practice in Tropical Countries, Part 1. In: Cheesbrough, M. (Ed.). Low Price Edn. Cambridge University Press, Cambridge UK., pp: 358-362. ISBN: 0-521-66548-5.

Feldman, C., 2005. Pneumonia associated with HIV infection. *Curr. Opin. Infect. Dis.*, 18 (2): 165-170. PMID: 15735422. DOI: 10.1097/01.qco.0000160907.79437.5a.

Lawn, S.D., 2004. AIDS in Africa: The impact of coinfections on the pathogenesis of HIV-1 infection. *J. Infect. Dis.*, 48 (1): 1-12. PMID: 14667787. DOI: 10.1016/j.jinf.2003.09.001.

Morgan, D., Mahe, B. Mayanja and J.A. Whitworth, 2002. Progression to symptomatic disease in people infected with HIV-1 in rural Uganda; prospective cohort study. *BMJ.*, 324 (7331): 193-196. PMID: 64788. DOI: 10.1136/bmj.324.7331.193.

Palefsky, J., 2007. Human papilloma virus infection in HIV-infected persons. *Top. HIV. Med.*, 15 (4): 130-133. PMID: 17720998.

Reichling, J.J. and M.M. Kaplan, 1988. Clinical use of serum enzymes in liver disease. *Dig. Dis. Sci.*, 33 (12): 1601-1614. PMID: 2904353. DOI: 10.1007/BFO1535953.

Schneider, M.F., S.J. Gange, C.M. Williams, K. Anastos, R.M. Greenblatt, L. Kingsley, R. Detels and A. Munoz, 2005. Patterns of the hazards of death after AIDS through the evolution of antiretroviral therapy; 1984-2004. *AIDS.*, 19 (17): 2009-2018. PMID: 16260908. DOI: 10.1097/01.aids.0000189864.90053.22.

Watson, J., 2006. Scientists, activists sue South Africa's AIDS' denialists. *Nat. Med.*, 12(1): 6. PMID: 1639537. DOI: 10.1038/nm0106-6a.