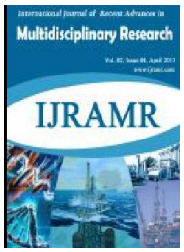

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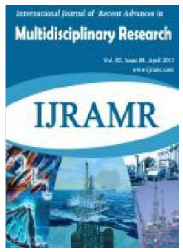

Research Article

A STUDY OF HEPATIC ENZYMES IN HIV SEROPOSITIVE PATIENTS ATTENDING GOVERNMENT GENERAL HOPITAL, KURNOOL

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ABSTRACT

Abnormalities of Liver Enzymes are common in Human immunodeficiency virus (HIV). Infected patients. Hepatic injury due to HIV leading to an increase in the Hepatic Enzymes (ALT, AST, ALP). Elevations in the liver enzymes signals injury to the 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 HIV Seropositive infection was assessed and 50 age matched apparently healthy subjects tested negative for antibodies for HIV 1 and 2 sereved as control. The activities of Serum alanine amino transferase (ALT) and Serum aspartate aminotransferse (AST) were observed significantly higher in HIV infected patients than those to the reference group. There is no significant difference observed in serum alkaline phosphatase (ALP) of HIV infected when compared to control subjects. Increase in ALT and AST is most likely due to impairment or involvement of the liver in HIV infection.

INTRODUCTION

AIDS (Acquired Immuno-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 in 1959 (M organ *et al*, 2002). AIDS has been reported in every country and parts of Africa and Asia are especially devastsed by it. Estimates of the large number of individuals currently infected with virus range from 35-40 million people. HIV is an enveloped retrovirus containing single stranded RNA (ssRNA), which is the etiological 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 byimmunodeficiency, susceptibility to opportunistic infection and other AIDS defining illness (Palefsky, 2007). The clinical manifestations of HIV/AIDS are amny and diverse. These manifestations can be as three phases: the initial phase, the latent phase and the advanced stage (Watson, 2006). 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 bassed enzymes are increased in individuals with various forms of liver disease.

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The clinically important liver enzymes includes Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST) and Alkaline phosphatase (ALP) (Reichling and Kaplan, 1988). Elevations in liver enzymes signals injury to liver cells and in some cases, to other cells in the body (Burtis *et al.,* 1996). In 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 the 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 incrase in the activities of the liver enzymes (Lawn, 2004; Bonnet *et al*, 2004).

MATERIALS AND METHODS

Subjects

One hundred patients (aged between 20-50 years) confirmed HIV positive were recruited for this study, while the control group consisted of 50 apparently healthy age matched volunteers who were confirmed as HIV negative. All the subjects were not known to suffer from any major liver disease of bone disease. Pregnant women were also excluded from the study. Informed consent wass 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 venepunture from the antecubital fossa into already labled 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 in to sterile bottles and were used for the enzyme assay. When not used immediately, they were stored at -20^{o} c and later used within 5 days.

Methods

Analysis was conducted for measuring enzyme activity for ALT, AST, and ALP in Government General Hospital, Kurnool.

Descrition of Methods

- AST: Reitman- Franken Method.
- ALT: Reitman- Franken method.
- ALP: Kind and Kings method.

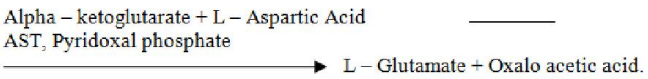
Estimation of Transminases Activity

Procedure: Adopted is Reitman and Frankel method (Burtis *et al,* 1996).The kit reagents (ALT and AST) were manufactured by AGAPPE Diagnostics, Ernakulam, Kerala.

Aspartate Amino Transferase

This serum enzyme preferably called as Aspartate transaminase (AST).

This enzyme catalyses the following reaction:



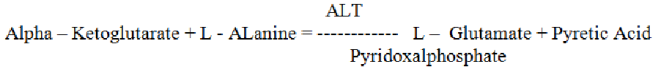
This reaction has been used to estimate the concentration of transaminases in the serum.

Principle

The transamianse present in serum act on Aspartate, when they are incubated together at 37^{0}C in a buffer of around neutral pH, to form respective ketoacid. The ketoacids are made to react with 2 – 4 Dinitrophenyl Hydrazine (DNPH) in Alkaline medium to form reddish – brown complex of Hydrazones. The intenstiy of colour is proportional to the amount of ketoacid present which inturn is proportional to the amount of serum transaminase present. Thus a measure of the optical density of the coloured solution indicates the concentration of the serum transaminase levels.

Alanine Amino Transferase

Serum glutamatge pyruvic transaminase also preferably called Alanine transaminase (ALT) catalyzing following reaction.



This reaction is used for estimating the ALT levels in serum.

Principle

The transaminase ALT present in serum act on Alanine. When they are incubated together at 37^{0}C in a buffer of around neutral pH, to form the respective Ketoacid. The Ketoacid made to react with 2-4 dinitro phenyl hydrazine (DNPH in Alkaline medium to form reddish-brown complex of hydrazones. The intensity of colour is proportional to the amount of Ketoacid present which inturn is proportional to the amount of serum transaminase present. Thus measure of the optical density of the coloured solution indicates the concentration of serum transaminase levels.

Estimation of Serum Alkaline Phosphatase (ALP)

Method: Method of kind and king using the substrate phenyl phosphate. The reagent kits manufactured by RECKON Diagnostics, Baroda.

Principle: Serum alkaline phosphatase acts on disodium phenyl phosphate at pH around 10 to form phenol and phosphate radical. The phenol formed reacts with 4-aminoantipyrine in the present of Alkaline oxidizing agent ferriccyanide to give purple colour. The amount of phenol formed as indicated by the intensity of the colour developed is a measure of enzyme activity.

HIV Screening test: HIV screening test was done by ELISA (enzyme linked immunosorbent assay) technique (Cheesbrough, 2002). The kit reagents were supplied manufactured by SD – Biostandard Diagnostics, Gurgoan, Haryana.

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 antbody 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 SD – Biostandard Diagnostics, Gurgoan, Haryana.

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 HIV seropositive subjects. Table-1 shows the different enzyme activities of the test and control subjects. There was a significant increase ($p<0.0510.63 \pm 3.76$), in the mean levels of AST and ALT for the test subjects (15.76 ±

8.23; 11.35 ± 6.11 iu/l, respectively) when compared to controls (10.63 ± 3.76; 6.88 ± 3.77 iu/l). Table -1 also shows a non significant increase ($p>0.05$), in the mean level of ALP for the subjects (61.40 ± 25.72 iu/l) when compared to the control subjects (58.57 ± 21.88 iu/l)

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

No of subjects	Test subjects (n=100)	Control subjects (n=50)	p-value
AST (iu/l)	15.76 ± 8.23	10.63 ± 3.76	< 0.05*
ALT (iu/l)	11.35 ± 6.11	6.88 ± 3.77	< 0.05*
ALP (iu/l)	61.40 ± 25.72	58.57 ± 21.88	> 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 HIV seropositive subjects when compared to the controls. The International Federation of the clinical chemistry (IFCC) estimated the reference ranges for AST, ALT and ALP as 8-20, 10-40 and 38-94 iu/l, respectively (Burtis *et al*, 1996). From the 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 of 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 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 to prevent the progressive ddestruction of liver cells and ensure better management of HIV patients.

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