Correlation of Liver Function Tests with HIV/AIDS in North Indian Population

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Abstract—Liver function tests (LFTs) are a major indicator for assessing the hepatic condition. HIV/AIDS affect the whole body’s system in a progressive manner which deteriorates various metabolic functions. This preliminary work is aimed to assess the effect of HIV/AIDS on liver function parameters including aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), serum albumin (ALB), bilirubin total (Bil-T) and bilirubin direct (Bil-D) in North India. A total of 317 human blood samples of normal and HIV +ve subjects (age 18-49 years) from two locations in North India (Punjab and Rajasthan) were obtained during September, 2010 to June, 2011. All samples were subjected to biochemical analysis. The serum concentration of hepatic enzymes AST, ALT and ALP increases significantly (p<0.001) in HIV subjects as compared to normal subjects in both the states. The activity of these enzymes is further elevated in AIDS subjects in both the states. The serum concentration of ALB protein decreases significantly (p<0.001) in HIV and AIDS subjects as compared to normal subjects from both the states. Similar results were obtained in all age groups formed in the present study. The serum concentration of Bil-T and Bil-D reveals a gradual increase in HIV subjects as compared to normal subjects in all the three age groups. However this trend reverses in AIDS subjects where Bil-T and Bil-D shows marginal decrease in their concentration. The present investigation reveals that the deviations in AST, ALT, ALP and ALB are significantly correlated with the HIV +ve infection and its progression to AIDS in the blood samples from different subjects considered in the analysis. Further, no significant differences were observed in the various biochemical parameters, when HIV +ve subjects from the two states are compared in the present study. These results can be of significant importance in future studies on LFTs, particularly in resource limited settings.

Keywords—Liver function tests; AST; ALT; ALP; ALB; HIV; AIDS; North India.

I. INTRODUCTION

Biochemical markers can act as an important tool to increase efficiency in drug development. An optimal combination of biochemical markers together with clinical risk factors and imaging will most likely provide a useful toolkit to identify patients at high risk [1]. Anti-retroviral (ARV)-related liver enzyme elevation may be related in part to immune reconstitution, as measured by changes in CD4+ T-cell counts [2]. Abnormalities of liver enzymes are common in patients with human immunodeficiency virus / acquired immune deficiency syndrome (HIV/AIDS) in developed countries [3]. A correlation between HIV viral load and amino transferases as markers of hepatic damage in HIV infected naïve patients emphasized on improved recognition, diagnosis and potential therapy of hepatic damage in HIV infected patients [4]. CD4+ cell count, lipid profile and liver enzymes can be a good index of disease progression in HIV infection and AIDS patients. The serum concentration of hepatic enzymes aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) increases significantly during HIV infection and its progression to AIDS [5, 6]. Further, the above mentioned biochemical variables were found to be affected more significantly in AIDS patients when compared with HIV infected subjects. Increases in non-alcoholic fatty liver disease and drug-induced hepatotoxicity, together with development of hepatocellular carcinoma, also potentiate the burden of liver disease in individuals with HIV infection [7]. A mandatory follow-up for hepatotoxicity, when ART is initiated in patients with <200 CD4+ T cells/mm3 suggested that anti-hepatitis pre- or co-medication could be an effective preventive or curative measure [8]. The serum albumin [ALB] level may be a useful additional marker of HIV-1 disease progression, particularly among asymptomatic women with little or no evidence of immune-suppression [9]. The potential risk of hepatotoxicity for HIV seropositive patients on ARV drugs calls for continuous monitoring of ARV administration so as to prevent fatal effects of hepatotoxicity [10]. Highly active anti-retroviral therapy (HAART) is associated with low level hepatotoxicity at therapy initiation, regardless of drug class or combination [11]. Effect of acute administration of antiretroviral drugs in albino rats is found to be associated with hematologic and hepatic enzyme alterations [12]. The burden of HIV/AIDS in India is reported higher in south and north-east region of India [13]. The North Indian region is still considered to be far less affected by this epidemic.
The aim of this study was to assess the effect of HIV/AIDS on the liver function tests; viz. AST, ALT, ALP, ALB, bilirubin total (Bil-T) and bilirubin direct (Bil-D) in North India.

II. MATERIAL AND METHODS

A. Blood samples

A total of 317 human blood samples (age 18-49 years) of normal and HIV +ve subjects from two locations in North India were obtained between September, 2010 to June, 2011 with due permission from the concerned authorities. Out of these 46 HIV +ve blood samples were collected from Ludhiana (Punjab: State-1) and 102 HIV +ve, 53 AIDS affected and 118 age and sex matched normal blood samples were obtained from Jodhpur (Rajasthan; State-2). The inclusion criteria included male and female human subjects of 18-49 years age, native of Punjab or Rajasthan state, newly diagnosed HIV +ve persons (Untreated for HIV/AIDS through drugs) or human subjects affected with AIDS (may or may not have undergone drug treatment for HIV/AIDS. The exclusion criteria included HIV +ve persons (not affected with AIDS) who have undergone drug treatment for HIV or normal human individuals affected by diabetes or any of major heart, kidney or liver disease (and have consumed drug for any major aliment in past one month).

Serum was separated by centrifugation within 2 hr of collection and stored frozen at -20°C until transport to the Meerut Institute of Engineering and Technology (MIET), Meerut, where they were stored for long term at -80°C till further analysis.

B. Chemicals

All reagent kits used in biochemical tests were of the AUTOPAK make from SIEMENS (Siemens Healthcare Diagnostics Ltd., Gujarat, India). Rest all chemicals used in the study was of analytical grade.

C. Methods

The tests for AST, ALT, ALP, ALB, Bil-T and Bil-D were conducted at YPL Diagnostics, Meerut on biochemistry analyser (RA-50 Bayer). All the analysis was performed in triplicate by using the pre-set software programme.

The study was approved by Institutional Research Board (IRB) and Institutional Ethical Committee (IEC) of MIET.

III. RESULTS

The results of the various biochemical tests were compared using the student’s t-test for comparison between different groups of Normal, HIV and AIDS subjects in both the states. Table 1 and 2 shows the mean concentration and t-test analysis of Liver Function Tests (LFTs) in Normal and HIV subjects of 18-49 years age group from state-1 (Punjab) and state-2 (Rajasthan) respectively.

A. State-1 (Punjab) Liver Function Analysis (18-49 years)

The mean concentration of enzyme AST, ALT and ALP for HIV subjects of age 18-49 years in state-1 were much higher as compared to the normal subjects. The t-tests for all the three enzymes show highly significant t-value of -7.94, -8.36 and -5.55 (p<0.001) with in the same state. The mean concentration of serum protein albumin in HIV subjects was lower as compared to the normal subjects in state-1. The t test of ALB shows highly significant t-value of 10.43 (p<0.001) between normal and HIV subjects within the same state. The mean concentration values for Bil-T and Bil-D in HIV subjects in state-1 were significantly higher as compared to the normal subjects within the same state. The increase in Bil-T and Bil-D shows significant t-value of -2.74 each between normal and HIV subjects in state-1.

The mean concentration for enzymes AST, ALT and ALP in HIV subjects of 18-49 years age in state-2 was higher as compared to the concentration in normal subjects. The t-test for enzyme AST, ALT and ALP shows highly significant t-values of -11.28, -10.80 and -8.40 (p<0.001) respectively between normal and HIV subjects of the same state. The mean concentration of serum ALB for HIV subjects was significantly lower as compared to the normal subjects in state 2 with a highly significant t-value of 13.04. The mean concentration of Bil-T in HIV subjects was higher as compared to the Bil-T in normal subjects in state-2. The t-test for Bil-T shows significant t-value of -2.23 between normal and HIV subjects within the same state.

Table 1. Mean concentration and t-test analysis of Liver Function in Normal and HIV subjects from State-1 (Punjab)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Tests</th>
<th>Mean ± SD</th>
<th>Normal (n=118)</th>
<th>HIV (n=46)</th>
<th>t-value</th>
<th>Significant/ In-significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspartate aminotransferase</td>
<td>23.03 ± 8.37 IU/L</td>
<td>33.50 ± 7.27 IU/L</td>
<td>-7.94</td>
<td>Significant***</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Alanine aminotransferase</td>
<td>19.58 ± 8.25 IU/L</td>
<td>28.83 ± 5.45 IU/L</td>
<td>-8.36</td>
<td>Significant***</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Alkaline phosphatase</td>
<td>143.06 ± 30.48 IU/L</td>
<td>197.11 ± 63.28 IU/L</td>
<td>-5.55</td>
<td>Significant***</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Serum albumin</td>
<td>3.63 ± 0.56 g/dl</td>
<td>2.81 ± 0.40 g/dl</td>
<td>10.43</td>
<td>Significant***</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Bilirubin total</td>
<td>0.46 ± 0.13 mg/dl</td>
<td>0.60 ± 0.33 mg/dl</td>
<td>-2.74</td>
<td>Significant***</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Bilirubin direct</td>
<td>0.24 ± 0.05 mg/dl</td>
<td>0.34 ± 0.25 mg/dl</td>
<td>-2.74</td>
<td>Significant***</td>
<td></td>
</tr>
</tbody>
</table>

**p < 0.01, ***p < 0.001
The mean concentration of Bil-D in HIV subjects was slightly higher as compared to the Bil-D in normal subjects from state-2. The increase in Bil-D was in-significant with a t-value of -1.70 for normal and HIV subjects within the same state.

C. Liver Function Comparison between HIV Groups of State-1 and State-2

The t-value comparison of normal Vs HIV subjects for all parameters in LFT’s (AST, ALT, ALP, ALB, Bil-T and Bil-D) shows significant result in state-1. State-2 also shows similar significant results in terms of t-values except for Bil-D, which were in-significant, while comparing normal and HIV subjects. The result of t-values in AST, ALT, ALP, and ALB shows more significant and hence higher t-values in state-2 as compared to state-1. While comparing HIV subjects in state-1 and state-2, all LFTs (AST, ALT, ALP, ALB, Bil-T and Bil-D) show in-significant results in terms of t-values with values of -0.65, -0.74, -0.70, 0.20, 1.14 and 1.66 respectively. This shows that there is no difference or very in-significant difference between the various concentrations of LFT’s in HIV subjects of the two states.

To analyze the overall age based liver function, the data from the two states was combined and the total samples were divided into different age groups of normal [(N_{30-39}), (N_{40-49})], HIV [(H_{30-39}), (H_{40-49}), (H_{18-49})] and AIDS [(A_{18-49})] subjects.

D. Liver Function Analysis in 18-29 yrs age group (Table 3)

The mean concentration of enzymes AST, ALT and ALP in HIV subjects was much higher as compared to the normal subjects of 18-29 years age group. The t-test for the same shows highly significant t-value of -12.68, -8.27 and -8.82 (p<0.001) respectively in the 18-29 years age group between normal (control) and HIV subjects. The mean concentration of ALB for HIV subjects was lower as compared to the normal subjects in the 18-29 years age group. The t-test of ALB shows highly significant t-value of 7.67 (p<0.001) between normal and HIV subjects within the same age group. The mean concentration values for LFT’s; Bil-T and Bil-D in HIV subjects (Group H_{30-39}) were slightly higher as compared to the concentration values in normal subjects (control group) in the 18-29 years age group. The t-test for Bil-T and Bil-D did not show significant differences between normal and HIV subjects as seen by t-values of -1.47 and -1.19 respectively within the same age group.

E. Liver Function Analysis in 30-39 yrs age group (Table 3)

The mean concentration of enzymes AST, ALT and ALP in HIV subjects was much higher as compared to the normal subjects of 30-39 years age group. The t-test for the same shows highly significant t-value of -4.32, -4.69 and -5.60 (p<0.001) respectively in the 30-39 years age group between normal (control) and HIV subjects. The mean concentration of ALB in HIV subjects was also significantly lower as compared to the normal subjects in the 30-39 years age group. The t-test of ALB shows highly significant t-value of 11.55 (p<0.001) within the same age group between normal (control) and HIV subjects. The mean concentration values of Bil-T and Bil-D in HIV subjects (Group H_{30-39}) of 30-39 years age were slightly higher as compared to concentration values in normal subjects. This increase in concentration of Bil-T and Bil-D was not significant with t-values of -1.63 and -1.70 respectively between normal (control) and HIV subjects.

F. Liver Function Analysis in 40-49 yrs age group (Table 3)

The mean concentration of enzymes AST, ALT and ALP in HIV subjects was much higher as compared to the normal subjects of 40-49 years age group. The t-test for the same shows highly significant t-value of -7.25, -8.30 and -4.05 (p<0.001) respectively in the 40-49 years age group between normal (control) and HIV subjects. The mean concentration of ALB in HIV subjects was significantly lower as compared to the normal (control) subjects in the 40-49 years age group with a significant t-value of 5.5 (p<0.001) within the same age group. The mean concentration values of LFT’s; Bil-T and Bil-D in HIV subjects (Group H_{40-49}), of 40-49 years age were significantly higher as compared to the concentration values in normal (control) subjects. The t-tests of Bil-T and Bil-D were significant with t-values of -2.67 and -2.44 respectively between normal and HIV subjects.

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**Table 2. Mean concentration and t-test analysis of Liver Function in Normal and HIV subjects from State-2 (Rajasthan)**

<table>
<thead>
<tr>
<th>Liver Function Tests [State-2 (Rajasthan)]</th>
<th>[Age Group: 18-49 years]</th>
<th>Mean ± SD</th>
<th>t-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. No.</td>
<td>Tests</td>
<td>Normal (n=118)</td>
<td>HIV (n=102)</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Aspartate aminotransferase</td>
<td>23.03 ± 8.37 IU/L</td>
<td>34.31 ± 6.45 IU/L</td>
<td>-11.28</td>
</tr>
<tr>
<td>2.</td>
<td>Alanine aminotransferase</td>
<td>19.58 ± 8.25 IU/L</td>
<td>29.54 ± 5.28 IU/L</td>
<td>-10.80</td>
</tr>
<tr>
<td>3.</td>
<td>Alkaline phosphatase</td>
<td>143.06 ± 30.48 IU/L</td>
<td>189.80 ± 48.51 IU/L</td>
<td>-8.40</td>
</tr>
<tr>
<td>4.</td>
<td>Serum albumin</td>
<td>3.63 ± 0.56 g/dl</td>
<td>2.80 ± 0.38 g/dl</td>
<td>13.04</td>
</tr>
<tr>
<td>5.</td>
<td>Bilirubin total</td>
<td>0.46 ± 0.13 mg/dl</td>
<td>0.53 ± 0.31 mg/dl</td>
<td>-2.23</td>
</tr>
<tr>
<td>6.</td>
<td>Bilirubin direct</td>
<td>0.24 ± 0.05 mg/dl</td>
<td>0.27 ± 0.19 mg/dl</td>
<td>-1.70</td>
</tr>
</tbody>
</table>

* p < 0.05, *** p < 0.001
Table 3. Mean concentration and t-test analysis of LFTs in Normal and HIV Subjects in three Age Groups

<table>
<thead>
<tr>
<th>Liver Function Tests [Age Group: 18-29 years]</th>
<th>Mean ± SD</th>
<th>Significant t/ Insignificant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n=37)</td>
<td>HIV (n=48)</td>
</tr>
<tr>
<td>1. Aspartate aminotransferase</td>
<td>18.97 ± 4.48 IU/L</td>
<td>33.88 ± 6.35 IU/L</td>
</tr>
<tr>
<td>2. Alanine aminotransferase</td>
<td>17.65 ± 6.55 IU/L</td>
<td>28.81 ± 5.63 IU/L</td>
</tr>
<tr>
<td>3. Alkaline phosphatase</td>
<td>147.59 ± 23.37 IU/L</td>
<td>211.25 ± 42.31 IU/L</td>
</tr>
<tr>
<td>4. Serum albumin</td>
<td>3.51 ± 0.52 g/dl</td>
<td>2.73 ± 0.39 g/dl</td>
</tr>
<tr>
<td>5. Bilirubin total</td>
<td>0.41 ± 0.15 mg/dl</td>
<td>0.49 ± 0.36 mg/dl</td>
</tr>
<tr>
<td>6. Bilirubin direct</td>
<td>0.23 ± 0.06 mg/dl</td>
<td>0.27 ± 0.25 mg/dl</td>
</tr>
</tbody>
</table>

Table 4. Mean concentration and t-test analysis of Liver Function in HIV and AIDS subjects [Age Group: 18-49 years]

<table>
<thead>
<tr>
<th>Liver Function Tests [Age Group: 18-49 years]</th>
<th>Mean ± SD</th>
<th>Significant t/ Insignificant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n=45)</td>
<td>HIV (n=55)</td>
</tr>
<tr>
<td>1. Aspartate aminotransferase</td>
<td>26.56 ± 10.38 IU/L</td>
<td>34.29 ± 6.70 IU/L</td>
</tr>
<tr>
<td>2. Alanine aminotransferase</td>
<td>21.58 ± 10.38 IU/L</td>
<td>29.64 ± 5.56 IU/L</td>
</tr>
<tr>
<td>3. Alkaline phosphatase</td>
<td>140.36 ± 26.56 IU/L</td>
<td>173.69 ± 32.93 IU/L</td>
</tr>
<tr>
<td>4. Serum albumin</td>
<td>3.89 ± 0.50 g/dl</td>
<td>3.85 ± 0.38 g/dl</td>
</tr>
<tr>
<td>5. Bilirubin total</td>
<td>0.48 ± 0.12 mg/dl</td>
<td>0.54 ± 0.23 mg/dl</td>
</tr>
<tr>
<td>6. Bilirubin direct</td>
<td>0.24 ± 0.05 mg/dl</td>
<td>0.28 ± 0.16 mg/dl</td>
</tr>
</tbody>
</table>

Table 4 shows that the mean concentration of enzymes; AST, ALT and ALP in AIDS subjects (Group A18-49), were significantly higher as compared to the concentration values in HIV subjects (Group H18-49) of 18-49 years age group. The t-test for enzymes; AST, ALT and ALP show significant t-values of -5.78, -4.61 and -6.95 (p<0.001) respectively between HIV and AIDS subjects within the same age group. These results show that the activity of liver enzymes increases significantly with the progression of HIV infection into AIDS expression.

The mean concentration of ALB in AIDS subjects was significantly lower as compared to the HIV in the 18-49 years age group. The t-test for ALB shows significant t-value of 7.13 (p<0.001) within the same age group. The Bil-T and Bil-D levels show in-significant results in terms of differences in mean concentration and t-value in the 18-49 years age group between HIV (Group H18-49) and AIDS (Group A18-49) subjects.

The graphical representation of LFT enzymes; AST, ALT and ALP is shown in figures 1, 2 and 3 respectively. The mean concentration values of normal, HIV and AIDS subjects under different groups for all the parameters under study are shown in the form of a graphical representation. Each figure shows the corresponding changes in the concentration values of respective parameter in the form of a bar graph. The mean concentration of AST shows increasing trends in all the age groups of HIV subjects (H18-29, H30-39 and H49-49) as compared to all the age groups of normal subjects (N18-29, N30-39 and N40-49).

Table 4. Mean concentration and t-test analysis of Liver Function in HIV and AIDS subjects [Age Group: 18-49 years]

G. Liver Function Comparison between HIV and AIDS in 18-49 yrs age group (Table 4)
Similar trend of increase in mean concentration of AST was also observed while comparing of AIDS (A<sub>18-49</sub>) and HIV (H<sub>18-49</sub>) subjects. Likewise, results were shown for enzymes ALT and ALP. The mean concentration values of ALT and ALP shows increasing trends in all the three age groups of HIV subjects (H<sub>18-29</sub>, H<sub>30-39</sub> and H<sub>40-49</sub>) as compared to normal subjects (N<sub>18-29</sub>, N<sub>30-39</sub> and N<sub>40-49</sub>). Similar trend of increase in mean concentration values for ALT and ALP was also observed in AIDS subjects (A<sub>18-49</sub>) as compared to HIV subjects (H<sub>18-49</sub>). These results show that there was a significant increase in the activity of liver enzymes with the progress of HIV infection to AIDS expression. This indicates that the increased activity of liver enzymes was induced by HIV. The graphical representation of serum protein albumin (ALB) is shown in figure 4. The mean concentration of ALB shows decreasing trends in all the age groups of HIV subjects (H<sub>18-29</sub>, H<sub>30-39</sub> and H<sub>40-49</sub>) as compared to all the age groups of normal subjects (N<sub>18-29</sub>, N<sub>30-39</sub> and N<sub>40-49</sub>). Similar trend of decreasing mean concentration for ALB was also observed in AIDS subjects (A<sub>18-49</sub>) as compared to HIV subjects (H<sub>18-49</sub>). It can be seen from the above table and figures, that the increase in the activity of liver enzymes with a decrease in serum albumin is a function of HIV.

The graphical representation of LFT parameters Bil-T and Bil-D is shown in figures 5 and 6 respectively. The mean concentration values of Bil-T and Bil-D shows insignificant increasing trends in the lower age groups of HIV subjects (H<sub>18-29</sub> and H<sub>30-39</sub>) as compared to the lower age groups of normal subjects (N<sub>18-29</sub> and N<sub>30-39</sub>). Whereas the higher age group of HIV subjects (H<sub>40-49</sub>) show significant increase in mean concentration values of Bil-T and Bil-D as compared to the normal subjects (N<sub>40-49</sub>). This trend of increasing mean concentration was found to be reversed in AIDS subjects (A<sub>18-49</sub>) where mean concentration values for Bil-T and Bil-D decreases (insignificantly) as compared to HIV subjects (H<sub>18-49</sub>). The results of Bil-T and Bil-D indicate that their concentration values are affected marginally and in an insignificant manner in AIDS and HIV subjects as compared to normal subjects.
their concentration. Earlier, Emejulu et al. [10] reported an increase in bilirubin (Bil-T and Bil-D) concentration in HIV subjects as compared to non-HIV subjects with a significance level of p < 0.05 in Nigerian population. Our results on bilirubin do not agree with the above report, but finds similarity with reports of Ebuehi et al. [5] on Nigerian population to the fact that there is no significant variation in bilirubin levels in HIV subjects as compared to normal subjects.

V. CONCLUSION

A comparison of enzyme activities among normal and HIV +ve subjects revealed that activities of AST, ALT and ALP enzymes were significantly higher in HIV +ve subjects as compared to the normal subjects from both the North Indian states (Punjab and Rajasthan). Similarly, the ALB concentration in HIV +ve subjects from both the states was significantly lower as compared to the normal subjects. The increase in the activities of three enzymes (AST, ALT and ALP) was highly significant irrespective of distant regional locations. The lowering of concentration of ALB is also highly significant in the two states. The results of Bil-T and Bil-D in higher age group (40-49 years) are significant as compared to results from the lower age groups (18-29 and 30-39 years). These results could be because of higher age of the subjects, which might result in higher formation of the bilirubin pigment at age greater than 40 years.

These results reveal that the deviations in the various liver function parameters (AST, ALT, ALP and ALB) are significantly correlated with the HIV +ve infection and its progression to AIDS in the blood samples from different human subjects considered in the present investigation. Further, no significant differences were observed in the various biochemical parameters, when HIV +ve subjects from the two states are compared in the present study. The results can be of significant importance in future studies on LFTs, particularly in resource limited settings.

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REFERENCES


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