Quantitative Analysis of Serum Level Alanine and Aspartate Aminotransferases, γ-Glutamyl Transferase and Alkaline Phosphatase as Predictor of Liver Diseases
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Abstract: The diagnostic evaluation of different enzymes associated to liver such as Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Gamma Glutamyl Transferase (GGT) can give an easy follow through for detection of liver homeostasis. The hepatic diseases can be better evaluated by analyzing the serum levels of alanine and aspartate amino transferase and Alkaline Phosphatase (ALP). The study includes serum level of enzymes of hepatic disorders such as alcoholic liver disease, hepatitis, and liver cirrhosis. The serum level of ALT, AST, GGT and ALP were analysed by their standardized methods. There was a significant relationship between ALT and GGT. Comparative elevation in the above discussed enzyme indicates the nature and degree of the hepatic damage.

Keywords: Hepatic damage, serum level, Liver cirrhosis, viral hepatitis, Alcoholic liver damage, Alanine amino Transferase, Aspartate amino Transferase, Gamma-Glutamyl Transferase, Alkaline Phosphatase.

I. Introduction
Liver is the major organ that performs many vital functions of our body. It helps primarily in metabolism, digestion, detoxification and immunity [1]-[3]. Various forms of liver associated diseases are alcohol cirrhosis [1], viral hepatitis [4], non-alcoholic fatty liver disease (NAFLD) [5]. Alanine amino transferase (ALT) also known as serum glutamic pyruvic transaminase (SGPT) normally found largely in liver. It is not fact that it is only present in liver but the concentration of this enzyme is more in liver and generally are released to bloodstream as a result of liver injury. Its level in serum of blood is frequently used to determine these type of hepatic diseases and functionality of liver. Elevated serum level of ALT determines the liver damage such as hepatitis and jaundice [6]. The normal level of ALT in blood ranges between 7-56 U/L and it increases more than 50 times or greater than 500 U/L during hepatitis, liver cirrhosis, ischemic liver injury, toxin induced liver damage [7]. Despite the association between greatly elevated ALT level and its specificity to hepatocellular diseases, the absolute peak of the ALT elevation does not correlate with the extent of liver cell damage. Aspartate amino transferase (AST) also known as serum glutamic oxaloacetic transaminase (SGOT) is found in diverse tissue system such as liver, heart, muscles, kidney and brain. The normal range of AST is 5-40 U/L. It is released into the bloodstream when the above tissues are damaged. Its serum level increases with heart attacks and muscle disorders. So AST can’t be used as specific indicator for liver damage. AST and ALT are sensitive indicators for liver damage as the interpretation of elevated serum level of two enzymes, but depends solely on the whole clinical picture and the physician experience in evaluating the liver disease. The precise level of these enzyme does not correlate the extent of liver damage or the prognosis. The ratio of AST/ALT ratio can be more clinically valid than assessing individual concentration. Alkaline Phosphatase (ALP) is present in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta. It helps in lipid transportation in intestine and calcification of bone. The serum ALP activity is mainly from liver with half that is contributed by bone. The normal ALP level is 41-133 U/L [8]. Elevated serum level of ALP found in infiltrate liver disease, granulomatous liver disease and amyloidosis, hepatitis etc. [9]. Gamma-glutamyl transferase, GGT, GGTP, gamma-GT (E.C. 2.3.2.2) is found in many tissue system including kidney, duct, pancreas, gall bladder, spleen, heart, brain and seminal vesicles the most notable one being the liver. It catalyse the transfer of gamma-glutamyl moiety of glutathione to a acceptor may be amino acid, a peptide or water. It also involve the transfer of amino acid across cellular membrane and leukotriene synthesis. Elevated serum GGT can be found in diseases related to liver, biliary system and pancreas. Indeed unlike ALP, it is also related to detecting liver diseases. The two markers i.e ALP and GGT indeed correlate well though there is a conflicting data about whether GGT has better sensitivity. The main value of GGT over ALP is in verifying that ALP elevations are, in fact, due to biliary diseases, ALP also has increased serum level in certain bone diseases but GGT has no elevated level in this case. [9]
II. Material and Methodology

In the present investigation, a total of 40 male subjects aged between 30 to 50 years having history and suffering from viral hepatitis, alcoholic liver disease, and liver cirrhosis. The inclusion criteria were for liver cirrhosis were jaundice, ascites, edema, enlarged veins over abdomen and palpable spleen whereas for hepatitis pyrexia, jaundice tendency and repeated vomiting. For alcoholic liver disease the inclusion criteria were fever, repeated vomiting, pain in liver area, hepatic tenderness, cyst, vegetative forms seen in stool. The exclusion criteria were cases of liver carcinoma, cardiovascular associated liver disease and other infectious diseases. Controls were selected on the basis that they do not have a history or are not suffering from any chronic diseases so as they have a controlled serum level of ALT, AST, GGT and ALP. Each group i.e. controls, cases suffering from viral hepatitis, alcoholic liver disease and liver cirrhosis containing 10 individuals. The estimation for the ALT, AST, GGT and ALP was carried out for three consecutive weeks.

Estimation of Alanine amino Transaminase (ALT) and Aspartate amino Transaminase (AST) by Reitman and Frankel method [10]

The estimation is based on the quantification of pyruvate produced by Alanine amino Transferase(ALP). In this assay pyruvate and NADH are converted to lactate and NAD by enzyme lactate dehydrogenase (LDH). The decrease in NADH absorbance at 340 nm is proportional to ALT activity. This method is very simple and a convenient assay. For the assay, two quartz cuvettes, one for the blank and one for the sample were taken. To each cuvette, 1000 µL of assay buffer, 25 µL of co-substrate, 5 µL of enzyme mix and 20 µL of double distill water. The cuvettes were equilibrated to 37°C. The sample serum was prewarmed to 37°C and 100 µL of serum was added to the sample cuvette. To the blank, 100 µL of double distill water was added to the blank cuvette. The content of the assay were mixed thoroughly by mixing. The absorbance was measured at 340 nm in a double-beam spectrophotometer at 5th and 10th minutes of the reaction.

For quantification of Aspartate amino Transferase (AST) is done by the quantification of oxaloacetate hydrazone formed with 2,4 dinitrophenyl hydrazine. The oxaloacetate was formed by the action of AST on L-aspartate and α-keto-glutarate. This oxaloacetate formed then reacts with 2,4 dinitrophenyl hydrazine to oxaloacetate hydrazone.

Estimation of Gamma-Glutamyl Transferase (GGT) by SZASZ method

The Estimation of Gamma-Glutamyl transferase was done by the method as proposed by Szasz G. [11]. The test utilizes substrate as L-γ-glutamyl-3-carboxy -4-nitro-anilide in Tris Glycylglycin buffer pH-8.25. The enzyme Gammaglutamyl transferase transfers the gamma glutamyl group of L-γ-glutamyl-3-carboxy -4-nitro-anilide to glycylglycine. The amount of 5-amino-2- nitrobenzoate liberated is proportional to amount of GGT activity and is determined spectrophotometrically at 420nm.

Estimation of Alkaline Phosphatase (ALP)

The serum Alkaline Phosphatase in blood was assayed by colometric and point method as described in Moss and Henderson, 1999 [12]. Two clean and dry test tubes were taken and marked as Standard and Test. To both the test tubes 0.5 mL of Alkaline phosphatase substrate(di-sodium phenyl phosphohate in carbonate-bicarbonate buffer at pH 10) was taken and incubated at 37°C for 10 minutes. The standard and sample enzymatic solution were also equilibrated at 37°C. 0.05 mL of the standard and sample were then added respectively to the Standard and Test test tubes respectively. Then they were mixed gently and incubated at 37°C exactly up to 10 minutes. Sodium arsenate was added to stop the enzymatic activity. The absorbance was taken using a double beam spectrophotometer at 590nm.

III. Results

The serum enzyme level of different enzymes such as ALT, AST, GGT, ALP were analysed after 1st week, 2nd week and 3rd week. (Table :1, Figure :1, Figure :2, Figure :3, Figure :4).

Table 1: Estimation of serum level ALT, AST, GGT and ALP for three consecutive weeks.

<table>
<thead>
<tr>
<th>TIME OF DIAGNOSIS</th>
<th>Control (n=10) (mean±SD)</th>
<th>Viral Hepatitis (n=10) (mean±SD)</th>
<th>Alcoholic Liver disease (n=10) (mean±SD)</th>
<th>Liver Cirrhosis (n=10) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alanine amino transferase (ALT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEEK 1</td>
<td>7.73±3.50</td>
<td>251.13±80.65</td>
<td>72.18±30.12</td>
<td>45.70±6.43</td>
</tr>
<tr>
<td>WEEK 2</td>
<td>9.14±2.50</td>
<td>255.64±83.12</td>
<td>76.62±34.33</td>
<td>48.97±7.98</td>
</tr>
<tr>
<td>WEEK 3</td>
<td>11.29±3.98</td>
<td>269.32±79.23</td>
<td>82.43±29.32</td>
<td>56.09±5.76</td>
</tr>
<tr>
<td><strong>Aspartate amino transferase (AST)</strong></td>
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<td></td>
</tr>
<tr>
<td>WEEK 1</td>
<td>4.10±0.65</td>
<td>154.80±54.23</td>
<td>157.83±45.43</td>
<td>54.14±6.56</td>
</tr>
<tr>
<td>WEEK 2</td>
<td>6.11±0.49</td>
<td>157.3±34.22</td>
<td>162.56±39.76</td>
<td>59.07±6.27</td>
</tr>
<tr>
<td>WEEK 3</td>
<td>13.06±3.43</td>
<td>161.66±59.87</td>
<td>165.47±50.12</td>
<td>65.77±12.17</td>
</tr>
<tr>
<td><strong>Gamma-glutamyl transferase (GGT)</strong></td>
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<tr>
<td>WEEK 1</td>
<td>17.65±2.03</td>
<td>107.45±10.12</td>
<td>171.07±40.26</td>
<td>236.74±32.08</td>
</tr>
<tr>
<td>WEEK 2</td>
<td>19.21±2.30</td>
<td>110.49±10.10</td>
<td>176.98±39.87</td>
<td>243.43±35.69</td>
</tr>
<tr>
<td>WEEK 3</td>
<td>27.64±4.32</td>
<td>117.23±19.65</td>
<td>183.35±54.67</td>
<td>255.29±43.52</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase (ALP)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>WEEK 1</td>
<td>26.20±3.65</td>
<td>197.78±49.45</td>
<td>172.22±23.30</td>
<td>54.13±6.70</td>
</tr>
<tr>
<td>WEEK 2</td>
<td>28.21±2.63</td>
<td>203.66±37.56</td>
<td>178.23±13.10</td>
<td>58.23±8.76</td>
</tr>
<tr>
<td>WEEK 3</td>
<td>37.88±6.23</td>
<td>211.03±54.60</td>
<td>183.88±33.30</td>
<td>64.23±12.77</td>
</tr>
</tbody>
</table>
Figure 1: Comparative analysis of Alanine aminoTransferase (ALT) for different liver diseases.

![Image of Figure 1](image1)

Figure 2: Comparative analysis of Aspartate aminoTransferase (AST) for different liver diseases.

![Image of Figure 2](image2)

Figure 3: Comparative analysis of Gamma-Glutamyl Transferase (GGT) for different liver diseases.

![Image of Figure 3](image3)

Figure 4: Comparative analysis of Alkaline Phosphatase (ALP) for different liver diseases.

![Image of Figure 4](image4)
The analysis of the level of serum liver enzymes such as ALT, AST, GGT and ALP was done as they are proposed to be the main biomarkers for the liver diseases and these can tested routinely for the clinical analysis. The elevation in the concentration of ALT and AST may indicate predominantly hepatocellular diseases while the elevation in ALP and GGT may indicate cholestatic diseases of the liver. The concentration of ALT, AST and GGT are considered to be the measures for liver homeostasis. The two Transferases such as serum ALT and AST are the sensitive indicators as those hepatic enzymes have leaked into blood stream due to some hepatocellular injury such as hepatitis. The pattern of the elevation of serum aminotransferase elevation can be useful to treat liver diseases diagnostically. In most of the cases of acute hepatocellular disorders, the ALT concentration is higher than or equal to AST. The ratio of AST:ALT also determines the diseases status and their ratio greater than 3:1 highly suggest alcoholic liver disease.

In the present study from Figure 5, mostly the concentration of AST:ALT is almost equals to 1 for normal individuals whereas it is less than 1 in case for viral hepatitis, greater than 2 for alcoholic liver disease and within 1-2 for liver cirrhosis. The present study has suggested that there is a huge increase in the level of ALT, AST, GGT and ALP as compared to the controls in the diseased conditions. The concentration of serum ALT, AST and ALP are significantly higher in viral hepatitis as compared to alcoholic liver disease and liver cirrhosis. The alcoholic liver diseased person have higher serum level of ALT, AST and ALP as compared to liver cirrhosis. Increased ALT to AST ratio may be due to also fatty liver diseases (non-alcoholic hepatosteatosis), viral infections (Hepatitis A, B, C), haemochromatosis, adverse medication effect, auto-immune diseases and inherent factors whereas it is reversed in case of alcoholic liver disease. The level of ALP increase upto 200-300 U/L in viral hepatitis and upto 300 U/L in alcoholic liver disease. In liver cirrhosis ALP level is normal or slightly elevated. Increased ALP level is observed in intra or extra hepatic bile duct obstructions due to regurgitation of enzyme into circulating blood. The level of GGT is significantly low in case of viral hepatitis and high in case of alcoholic liver disease and cirrhosis. GGT level is high in case of cirrhosis than alcoholic liver disease. The GGT present in hepatobiliary system is extremely sensitive to identify cholestatic disease. In viral hepatitis, it increases upto 5 times in absence of cholestatis and 10 times in presence of cholestatis. There is a 8-10 folds increase in upper limit of GGT and the persistence increase in the GGT is an indicator of liver cirrhosis. If medication and alcohol are suspected cause, then serum level of aminotransferase level must be checked upto 6-8 weeks. High ALT also found in obesity induced fatty liver and diabetes. Commonly encountered serum liver enzyme levels in clinical practices are hepatocellular predominance with elevated ALP and AST and cholestatic predominance with elevated ALP and GGT. The joint analysis of GGT with other liver associated enzyme may yield additional information regarding disease risks and diagnosis. Elevated GGT combined with elevated ALP suggest hepatobiliary injury that distinguishes form elevated level for ALP that suggest bone related diseases.

V. Conclusion

The abnormal elevation in the level of ALT, AST, GGT and ALP than normal values indicative of different liver diseases. In case of the serum ALT level it was found out that the mean value was 23 folds in case of viral hepatitis, 7.13 folds in case alcoholic liver disease and 4.6 folds in case of liver cirrhosis elevated concentration than normal mean value whereas in case of AST, the serum level was 12 folds in case of viral hepatitis, 12.7 folds in case of alcoholic liver disease and 4.7 folds in case of liver cirrhosis elevation than normal value. The serum GGT showed 4 folds elevations in case viral hepatitis whereas it was 6 folds and 9 folds elevation in case of liver diseases and cirrhosis respectively than normal value. In case of ALP, the serum level were 5.7 folds, 5 folds and 3.2 folds elevated in case viral hepatitis, alcoholic liver disease and liver cirrhosis respectively. So these study can a possibility in the early and correct diagnosis of the different liver diseases. These can helpful in detecting the severity of the diseases that in future considered for the right medication with right dose at right time.
References


