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Analysis of ALT and AST levels in HCV infected patients

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Abstract

Background: Hepatitis C infection is spreading worldwide at an alarming rate and Pakistan is the second largest country to be infected with hepatitis C virus (HCV). Abnormal levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) has been reported to be associated with hepatitis C infection and HCV associated pathologies.

Methods: The serum of study participants was isolated and analyzed for confirmed HCV infection and HCV RNA was extracted by QIAamp DSP Virus Kit followed by quantification by real time PCR. For the measurement of ALT and AST analysis, aspartate aminotransferase activity assay kit and alanine aminotransferase activity assay kit of Sigma Aldrich were used.

Results: Gender-wise analysis showed that 58% females while 42% males were HCV infected. Participants' age ranged between ⩾15 to ⩽75 years. A confirmatory test of HCV infection by real time PCR showed 63% positive while 37% participants to be negative for HCV infection. An age-wise comparison of AST and ALT level showed a higher level of ALT in all age groups of HCV patients with significant higher level in 21-60 years of males and 61—80 years of female. The gender-wise comparison of these enzyme level showed higher level of ALT and AST in females as compared to males. The correlation analysis showed a positive association between viral load and AST/ALT level.

Conclusion: Though irregular levels have been reported in HCV patients previously but this gender-based study shows an increased level of both the enzymes in females as compared to males. Moreover, ALT have increased levels in HCV patients as compared to AST indicating ALT to be more specific biomarker of HCV infection and liver damage.

Introduction

Hepatitis C virus (HCV) is a positive sense, single stranded enveloped RNA virus of Family *Flaviviridae,* which has affected approximately 3.3% of world population and is still spreading at an alarming rate. HCV consist of 9.6kb genome with six major genotypes with each genotype having further subtypes. HCV genome encodes three structural (E1, E2, core) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) by a single polyprotein precursor which is processed by the protease catalysis [1]. HCV is a pathogen that transmits into human where it replicates through reverse transcription and grows by utilizing host machinery and become the cause of chronic hepatitis that may lead to liver cirrhosis and hepatocellular carcinoma (HCC) [2]. Use of unsterilized dental and surgical equipment, shaving, blood transfusion methods, tattooing, and body piercing needles are the main risk factors is the transmission of HCV [3]. Interferon was used as a cure for HCV infection with the addition of ribavirin being administered to enhance the treatment response. Interferon plus ribavirin was used as main treatment regimen since 1991 till the development of direct acting antiviral agents (DAAs) which increased the SVR rate up to 90%, providing higher efficacy and improved tolerability [4,5]. Main targeted proteins of HCV by DAAs are NS3/NS4A (Protease), NS5A (Phosphoprotein which regulates virus replication and assembly) and NS5B (RNA-dependent RNA polymerase) [6]. HCV is a globally spread deadly pathogen that has affected almost 3.3% of world population and is still spreading at an alarming rate. Egypt, India, Pakistan, China and Indonesia are the countries where hepatitis C virus is highly prevailed and Pakistani population is the second major population being affected with HCV [7,8]. This infectious disease-causing virus needs to get diagnosed and eradicated from human body. Several biomarkers have been used for screening, diagnosis and to predict the complications linked with hepatitis C infection. Liver Biopsies and liver function tests (LFTs; including ALT and AST) are usually performed to measure the liver damage and to assess the treatment response of body. Alanine transaminase (ALT) also known as serum glutamic pyruvic transaminase (SGPT), and aspartate aminotransferase (AST) are prominent live enzymes. ALT is also found in skeletal muscle and heart cells in trace amounts, and it functions as a catalyst, transferring amino group from L-alanine to α-ketoglutarate to form hepatic metabolite oxaloacetate [9]. 10 IU/L is the standard value of ALT, though, it has been reported that the level of serum ALT elevates during liver injury and damage [10]. Higher level of serum ALT has also been associated with alcohol consumption, intake of some medications (i.e., aspirin, naproxen, sulfonamides etc.) while a lower than standard value of ALT has been seen to be linked with aging [11]. The standard value of AST (also termed as glutamic pyruvic transaminase (SGOT)), in healthy individuals is 5-40 IU/L nevertheless its value is observed to be increased 10-20 times in case of severe liver damage [12]. A higher-than-normal level of ALT and AST has been observed in HCV infection [13]. Pouti and his coworkers [14] reported that some HCV infected patients show normal range of ALT and the possible reason of a normal serum ALT level could be mild liver disease or the absence of fibrosis stage. Moreover, he associated ALT level with liver histopathology with increased level of ALT depicting severity of liver disease. An altered expression of ALT and AST has been observed in HCV related liver disease, which were seen to return to its normal range after the completion of treatment [15].

The aim of this study was to find out the correlation of alanine transaminase and aspartate aminotransferase with HCV RNA level in the serum and to conduct a gender-based comparison of alanine transaminase and aspartate aminotransferase in HCV infected patients.

Methods

Sample collection

This study consisted of 100 participants who had an ELISA positive result for HCV presence. Blood samples of all the study subjects were collected by their consent from Centre of Applied Molecular Biology, Lahore during the time period of August 2017 to July 2018. A written consent that stated to allow the of use their data (age, gender, disease associated and other provided data) for research purpose was taken from study participants. The individuals who refused to give the consent were not included in this study. Moreover, the consent form has been attached with this manuscript (Appendix I). Any individual having HBV and HCV co-infection, HIV and HCV co-infection or liver related pathogenesis other than HCV were excluded from the study. All the demographic features (age, sex etc.) and LFT profiles (ALT, AST) of all the participants was recorded by the consent of all the study participants.

Serum Isolation and HCV RNA extraction

5ml of blood was taken into lavender top EDTA (ethylenediamine tetra-acetic acid) tubes. It was centrifuged for 10 minutes at 6000rpm, the serum was separated and taken into a 1.5 ml Eppendorf and stored at -20˚C. Extraction of HCV RNA was carried by QIAamp DSP Virus Kit (Hilden, Germany) according to the manual that includes four major steps; lysis of viral

particles by lysis buffer and protease, Binding of viral nucleic acids to membrane by Loading of QIAamp MinElute column, membrane washing by wash buffer and elution of extracted HCV RNA by elution buffer. Specific buffers i.e., lysis buffer (AL), wash buffers (AW1, AW2), elution buffer (AVE) and tubes i.e., LT (lysis tube), ET (elution tube), collection tubes, connectors and columns are provided within the kit for the efficient extraction of HCV RNA.

Quantification of HCV RNA

Extracted RNA was amplified by using artus ® HCV RG RT-PCR kit (QIAGEN, GERMANY) in which master mix A and master mix B were mixed followed by vortex and short spin. 15ul of this master mix along with 10ul of quantification standard and 10 ul of extracted RNA sample were added into the PCR tube. Extracted HCV RNA was quantified by using Rotor-Gene Q Real Time PCR System, QIAGEN ®.

Analysis of ALT and AST

The level of ALT and AST was measured using aspartate aminotransferase activity assay kit, Sigma Aldrich and alanine aminotransferase activity assay kit, Sigma Aldrich. Tests for ALT and AST were performed according to the manual provided with the assay kit. For AST analysis, a standard solution was prepared by adding glutamate solution and AST assay buffer. For ALT analysis, pyruvate solution was mixed with ALT assay buffer to make a standard solution. Two separate 96-well plates were prepared for the analysis: one for ALT and one for AST. The respective standard solutions were added in the plates followed by addition of 20ul of the sample and a final volume of 50ul was set in the wells using assay buffer.

Statistical analysis

All the collected data was statistically analyzed and represented. Descriptive statistics of all the given values were calculated by IBM SPSS statistics version 21. Graph pad prism version 6.0.1 was used for comparative analysis of ALT, AST, gender and age groups. For all the statistical tests, the p value less than 0.05 was considered significant.

Results

Characteristics of study subjects

The study subjects were studied and based on their age and gender. Gender wise distribution depicted that 58% were females while 42% were males (figure 1). The age of the participants was ranged between ⩾15 to ⩽75 years.

Detection of HCV by real time PCR

All the HCV ELISA positive patients were tested for the presence of HCV by real time PCR and hepatitis C virus was detected in 63% patients while 37% were reported negative for HCV infection. HCV positive samples were further processed for ALT and AST analysis.

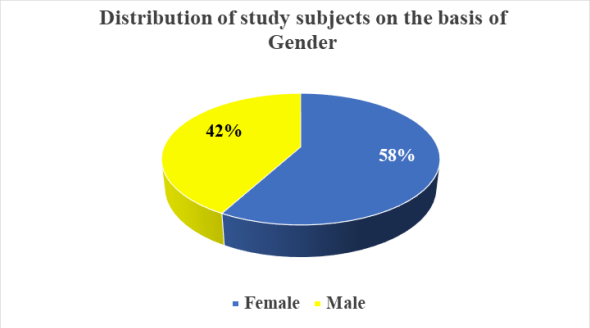

Figure 1: Gender-wise distribution of study participants: The study subjects which were infected with HCV were analyzed based on gender. A higher ratio of females (58%) was found to be infected as compared to males (42%).

Comparative analysis of plasma ALT & AST level in HCV infected patients based on age

HCV patients were divided into 4 groups based on age and were analyzed for their ALT and AST level. Normal value of serum ALT in males is 29-33 IU/L and of AST is 10-40 IU/L. The following graph was plotted by taking age of HCV patients (only males) on x-axis and values of ALT and AST on y-axis. A comparative analysis showed a higher level of ALT in all the age groups of HCV infected patients as compared to AST. A major difference in the values of ALT and AST can be seen in elderly people (61-80 years of age).

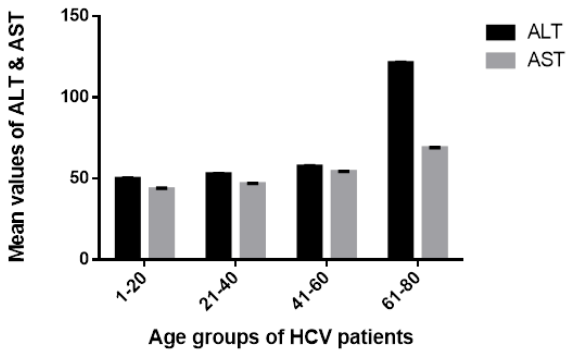

Figure 2: Representation of Comparative analysis of ALT & AST in HCV infected male patients: Infected male patients were grouped based on their age; a higher expression of ALT is observed in the patients of all the age groups. The highest expression of ALT and AST both has been seen in the patients who belong to 61-80 years.

Comparative Analysis of ALT and AST level with different age groups among females HCV patients

ALT and AST levels were assessed separately in males and females. Normal value of ALT in females is 19-25

IU/L and of AST is 10-40 IU/L. No female HCV patient of age group 1-20 was seen in this study. A higher level of ALT expression was seen in 61-80 years of females while a slightly higher level of AST was seen in 41-60 years of females with HCV infection.

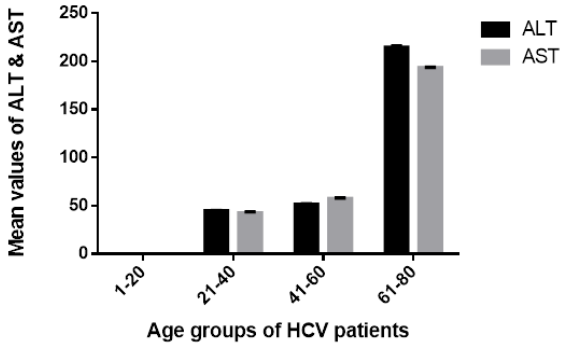

Figure 3: Comparison of ALT & AST in HCV infected female patients: Comparison was performed among female individuals infected with HCV. The highest expression of ALT and AST was observed in 61-80 years age group as *compared* to other groups, quite similar level of expression of AST and ALT was seen in 41-60 years age group, an equal expression of ALT and AST was seen in 21-40 years age group while no female patients of 1-20 years infected with HCV was seen in this study.

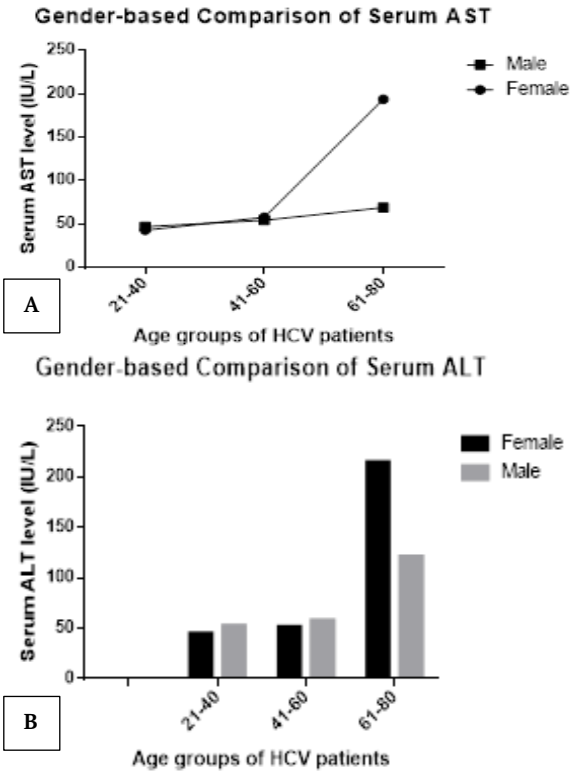

Figure 4: Gender based comparison of serum ALT and AST in HCV patients. A higher expression of ALT and AST both have been observed in females. Serum AST and ALT level was observed to be quite same in both the genders while an increased level of ALT and AST was seen in females of 61-80 years of age.

Gender based comparison of serum ALT & AST in HCV patients

A comparison of alanine transaminase (ALT) and aspartate aminotransferase (AST) was carried out on the basis of gender which showed a quite same level of ALT in males of 21-60 years of age, though, a dramatic increase of serum ALT level was seen in female age group of 61-80 years (Figure 4A). An equal level of AST was observed in both male and female patients of HCV. however, an increased level was observed in females of 61-80 years (Figure 4B).

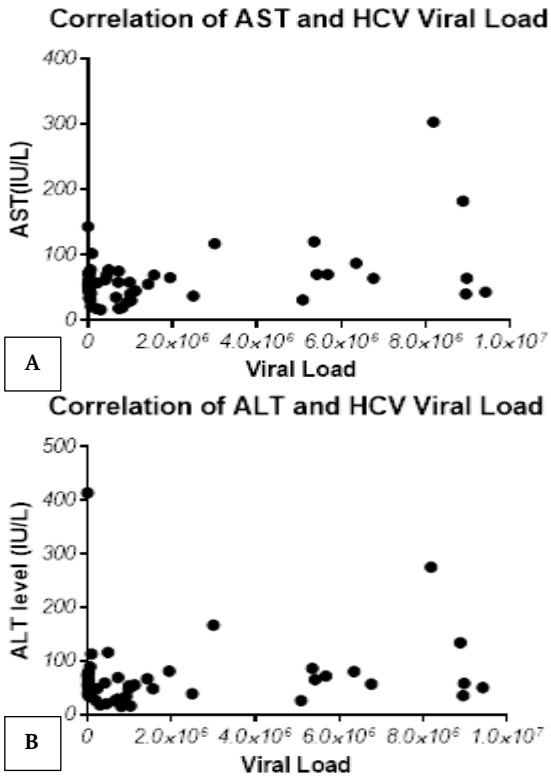

Figure 5: Correlation of HCV viral load with ALT (Pearson correlation coefficient r=0.15, p value=0.28) and AST (Pearson correlation coefficient r=0.42, p value=0.002). A positive association has been observed between the levels of AST, ALT and viral load. It indicates the increase in hepatitis C viral load, the level of ALT and AST also raises.

Correlation of ALT and AST with HCV Viral load

A correlation between HCV viral load, detected by quantitative PCR, was analyzed with ALT and AST. Pearson correlation coefficient indicates a significant positive correlation between AST and HCV viral load (r=0.42) while a positive but weak correlation was observed between ALT and HCV RNA level (0.15).

Discussion

Hepatitis C virus infects hepatic cells which alter the level of liver enzymes [16]. ALT and AST, being the most common liver enzyme [17], also get changed. An

elevated serum level of ALT and AST in chronic liver disease has been shown by many studies [18,19]. This study gives a comparison of altered ALT and AST level in males and females and elucidates the correlation of HCV RNA level with serum ALT and AST level. It has been observed in previous studies that ALT and AST are good biomarkers of liver pathology [20] but in some cases AST alone may have higher than normal level whereas ALT level being in the normal range in HCV infection [15]. A higher-than-normal level of ALT and AST has been observed in all the HCV infected patients in this study. It can be said that in chronic hepatitis C patients ALT shows more nonstandard values than AST, indicating ALT to be a better biomarker of HCV infection. Gender based comparison showed an elevated level of ALT and AST in females than in males who belonged to 61-80 age group. A gender-based comparison showed a comparatively higher values of ALT than AST in males while in female patients ALT and AST levels were seen to be increased equally above normal range in HCV . Though an increased level of ALT than AST was seen in female patients of 61-80 age group. Previous studies have shown the ability of ALT being used as a diagnostic and prognostic marker [21]. Many studies on AST/ALT ratio to be used as a predictive tool of cirrhosis in HCV has been done which showed an increased AST/ALT ratio in cirrhotic patients as compare to non-cirrhotic patients thus to be used as a prognostic and diagnostic biomarker [22,23]. The correlation between HCV viral load and severity of liver disease was assessed by Anand and Velez [24] which showed no significant correlation of HCV viral load with ALT (r=0.06) and AST(r=0.004) level. Likewise, in the present study no correlation has been seen between HCV viral load and serum ALT level (r=0.15) whereas a significant correlation between serum AST level and HCV RNA level (r=0.42) has been seen which is contradictory to Velez and Anand findings. Zechini et al., studied the correlation of AST and HCV viral RNA in HCV patients that support the findings of this study. They found a statistically significant correlation of HCV RNA titres with AST (r=0.24) and ALT (r=0.27) [25]. The histopathological changes in liver were correlated more significantly with AST however no Significant correlation was observed between ALT and pathological status of liver [26].

It can be concluded from the findings of this study that both the liver enzymes showed an elevated expression in HCV infected individuals with ALT being more expressed than AST. ALT and AST depicted an increased expression at ⩾ 60 age in both males and females. The analysis of correlation showed a significant positive correlation of HCV RNA level and serum AST level and a positive but weak correlation with serum ALT level.

Competing Interests

The authors of this study have no financial or commercial conflict of interest with respect to this manuscript.

Author Contributions

Manzoor Hussain and Sadia Amjad supervised the research and helped in troubleshooting, Mirwais khan helped in sample collection and statistical analysis, Ayesha Akram and Iqbal performed the laboratory work and Ayesha Akram drafted the manuscript.

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