

Clinical Significance of Elevated Serum Aminotransferases Levels in Asymptomatic Individuals with Hepatitis C Infection

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Background: Hepatitis C is a common and important cause of chronic liver disease that often remains asymptomatic and most of the times discovered incidentally by routine serologic or biochemical testing. Aminotransferases (AST and ALT) reflect alterations in liver function/inflammation in liver diseases. The current study was conducted to examine the utilization and limitations of these biochemical markers in subjects with asymptomatic HCV infection.

Aims and Objectives: To find out how many subjects with asymptomatic HCV infection have normal or elevated serum AST and ALT levels.

Subjects and Methods:

Study Design: Cross sectional.

Study Duration: Seven months from November 2008 to July 2009.

Study Universe: Services Hospital, Lahore.

Study Population: This study included 413 subjects attending the outpatient departments of hospital with minor complaints. The random population of subjects was referred to the clinical laboratory of Services Hospital, Lahore for LFTs, HBsAg and anti-HCV screening from OPD department of the hospital. A total of 413 persons of all ages were analyzed during this study. There were 263 subjects who were sero – positive for anti-HCV (141 females, 122 males; median age 35 ± 11.34 years) and 150 in the control group (80 of them were females and 70 males with median age 32 ± 12.97 years) were sero-negative for both HBsAg and anti – HCV.

Subjects Selection Criteria: In this study, only anti – HCV sero – positive subjects were included who was sero – negative for HBsAg or dual infection (both HBsAg and anti – HCV) and not on anti – viral therapy. Control group was sero – negative for both – HBsAg and anti – HCV antibodies.

Data Collection: We assayed levels of serum aminotransferases (AST and ALT) and screened blood for HBsAg and anti-HCV. ELISA technique was used for viral hepatitis markers.

Results: Out of 263 subjects tested positive for anti-HCV antibodies in their blood, 90.76 % and 87.45 % had elevated AST and ALT levels ($ALT \geq 40$ U/L) respectively. Similarly, 9.23% and 12.54% had serum AST and ALT levels < 40 U/L. Frequency of elevated AST and ALT levels in individuals who were sero – negative for both infections (HBV or HCV) were 10.66% and 13.33%, respectively. 56.95% of anti – HCV antibody positive subjects had serum ALT elevation of less than two times the upper level of normal ($ALT \leq 80$ U/L).

Conclusion: The current study revealed that 90.76% and 87.45% had elevated AST and ALT levels ($ALT \geq 40$ U/L) respectively. We concluded from this study that biochemical markers (AST, ALT and AST: ALT ratios) are useful, dependable and highly specific parameters for monitoring HCV infected patients (particularly asymptomatic) and frequent retesting is recommended to assess progression or regression of chronic liver disease.

Key words: HCV infection, AST and ALT, clinical significance, prognosis.

Introduction

Hepatitis C is a common and important cause of chronic liver disease that often remains asymptomatic and most of the times discovered incidentally by routine serologic or biochemical testing. This infection is becoming an increasing challenge to health professionals and is now a global public health issue.¹ In spite of the fact that about 170 million people worldwide are currently infected with HCV, its clinical course is still not well understood.² Frequently, pati-

ents are discovered incidentally when anti – HCV antibodies are detected during general medical checkup or at the time of blood donation.^{2,3} A positive test prompts consideration of liver function tests (LFTs) to assess the severity of liver damage.³⁻⁶

Liver enzyme studies usually reflect hepatocellular damage with often strikingly elevated serum aminotransferases levels.^{3,5} Both, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are biochemically related to

each other and are the two most useful liver enzymes. They are highly concentrated in the liver. AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain and red blood cells, and ALT has low concentrations in these body tissues.⁵⁻⁷ Therefore, estimation of both enzymes (ALT and AST) has been advocated as a means of improving the specificity of enzymic tests for liver disease. Differentiation of acute and chronic forms of hepatocellular injury is aided by examining the ratio of ALT to AST, called the De Ritis ratio. In acute hepatitis, serum ALT predominates whereas in alcoholic liver disease, chronic hepatitis, and cirrhosis, the serum AST predominates.⁷⁻¹⁰ Therefore, the level of AST compared to the level of ALT or the ratio of AST to ALT, provides many important clues to what is going on inside.¹⁰

In clinical practice, serum ALT level is commonly used (due to its liver specificity) to assess progression, evaluate the need for therapy and establish a prognosis because elevated ALT levels may reflect severity of ongoing damage to liver particularly in apparently healthy people with chronic hepatitis C.^{4-6,9-11} However, clinical significance of elevated serum ALT levels in prediction of severity of liver injury and progression rate in patients with chronic hepatitis C is still a matter of debate. The current study was conducted to examine the utilization and limitations of these biochemical markers in persons with asymptomatic HCV infection.

Aims and Objectives

Keeping in mind the importance of biochemical markers in asymptomatic individuals with 'Hepatitis C infection', a study was performed to find out how many subjects with asymptomatic HCV infection has normal or elevated serum ALT and AST levels.

Subjects and Methods

Study Design: This was a cross sectional, hospital based study.

Study Duration: Seven months from November 2008 to July 2009.

Study Universe: Services Hospital, Lahore. It is a tertiary care hospital, associated with the Services Institute of Medical Sciences, Lahore.

Subjects Selection Criteria

In this study, only anti – HCV sero – positive subjects were included who was sero – negative for HBsAg or dual infection (both HBsAg and anti-HCV) and not on anti-viral therapy. Control group was sero – negative for both – HBsAg and anti – HCV.

Study Population

The study population included 413 subjects attending the outpatient department of the hospital with minor complaints (e.g. generalized weakness and tiredness, lethargy, body

aches, darkening of color, lack of interest in the daily routine etc). This random population of subjects was referred to the clinical laboratory of Services Hospital, Lahore for laboratory investigations including LFTs, HBsAg and anti-HCV screening.

We collected a cross – sectional sample of 413 persons. 263 subjects who were sero – positive for anti-HCV (141 females, 122 males; median age 35 ± 11.34 years) and 150 in the control group were included in the study. They were divided into seven groups according to age (Table 1 and 2).

Reference Population

For reference and comparison of results, age and sex matched control population was included in this study. Their ALT and AST levels were examined. An abnormal ALT or AST level was defined as ≥ 40 U/L.¹² The control group represented non-repeat blood samples that were anti-HCV and HBsAg – negative. The reference laboratory controls included 150 individuals who had ALT and AST measurements performed as part of a full chemistry panel in the same laboratory as study individuals. 80 (52.98%) of them were females and 70 (47.01 %) males with median age 32 ± 12.97 years.

Data Collection and Laboratory Evaluation

Blood samples for the evaluation of aminotransferases (AST and ALT) and other biochemical parameters were obtained. Routine biochemical tests were carried out using Hitachi system 902, Japan. HBsAg and anti-HCV 3rd generation ELISA kits were used to screen all blood specimens. Following instrument and kits were used. Multiskan – EX (Thermo Electron Corporation, Finland), Ortho HCV 3.0 – ELISA Test System Enhanced SAVE (sample addition verification) and MONOLISA HBsAg Ultra (Bio – Rad, France).

AST and ALT Upper Reference Limit (URL)

The concept of normal serum aminotransferase levels remains highly arbitrary and the precise meaning of URL has not been defined particularly in chronic hepatitis C.^{2,6} The cut-off value for diagnosis of elevated AST and ALT levels was set at 40 U/L and abnormal AST and ALT levels are defined as ≥ 40 U/L. ALT to AST ratio is normally less than 1.0.^{10,11}

Statistical Analysis

SPSS version 12.0 was applied for statistical analysis. Results are expressed as median \pm standard deviation. Percentages were calculated directly. The Chi – square test with Yates correction was used for qualitative variables – to check the relationship of AST and ALT. Statistical significance was accepted at a level of $P < 0.05$.

Results

Incidence of elevated AST and ALT levels in the control population was 10.66% and 13.33% respectively, showing that ALT elevations were slightly more common than serum

AST levels in persons tested sero – negative for both –HBsAg and anti – HCV antibodies [Table 2].

Table 1: Distribution of anti – HCV positive subjects according to age and sex.

Age – Years	Number Tested	Male	Female
≤ 10	00	00	00
11 – 20	14	08	06
21 – 30	74	36	38
31 – 40	97	43	54
41 – 50	47	19	28
51 – 60	25	11	14
≥ 61	06	05	01
Total	263	122 (44.86%)	141 (55.13%)

Out of 263 subjects tested positive for anti – HCV antibodies in their blood, 122 (46.38%) were male and 141 (53.61%) were females (Table 1). Prevalence of raised AST and ALT levels in these people was 90.76% and 87.45% respectively; demonstrating that elevated serum AST levels were slightly more frequent than ALT elevations (Table 2). Simultaneous increase in the activity of both enzymes (AST and ALT) in serum was observed in 83.46% (217 / 260) of HCV positive persons. AST / ALT ratio was calculated and the median value was 1.07 ± 0.34 . It was found less than 1.0 in 50.38% of subjects and 0.88% (2 / 227) had ratio above 2.0.

Frequency of abnormal AST levels in people who were non-reactive for HBV or dual infections but reactive only for anti – HCV were 10.66% and 90.76% respectively

(Table 2). Similarly, ALT elevations in subjects who were negative for HBV or both infections and positive for anti-HCV alone were 13.33% and 87.45% respectively (Table 2). By comparison, AST and ALT elevations were far more common in the anti – HCV positive subjects than the control population. The difference was statistically highly significant ($P < 0.001$).

In HCV positive subjects, raised serum AST levels were encountered much more frequently than normal serum AST levels. Among them, 90.76% had elevated serum AST levels ($AST \geq 40$ U/L), and 9.23% had serum AST levels < 40 U/L (Table 2). 47.88% of anti – HCV antibody positive subjects had serum AST elevation of less than two times the URL ($AST \leq 80$ U/L). Only 2.69% had an AST elevation of more than 5 times the URL (Table 3).

Elevated serum ALT levels ($ALT \geq 40$ U/L) were observed in 87.45% of HCV positive subjects and only 12.54% had serum ALT levels < 40 U/L (Table 2). Whereas 56.95% had serum ALT elevation of < 2 times the URL ($ALT \leq 80$ U/L) and only 3.04% had an ALT elevation of > 5 times the URL (Table 3).

A significant number of HCV positive subjects showed that elevation of serum AST and ALT levels fell in the range of mild to moderate elevation (81 – 200 U/L) (Table 3).

Serum ALT levels were elevated in 87.45% (230 of 263) of anti – HCV – positive subjects compared with 13.33% (20 of 150) anti – HCV – negative subjects. Statistically, the difference was highly significant ($P < .001$) (Table 2).

Discussion

In our study, serum AST and ALT levels were elevated in 90.76 % and 87.45 % of subjects analyzed, respectively. The findings of our study in asymptomatic persons with hepatitis C are higher from those many studies, which reported that 60- 80 % of HCV infected people have elevated serum ALT levels.^{2,3} However, the frequency of elevated AST and ALT levels varies greatly depending on

Table 2: Prevalence of serum AST and ALT levels in HCV positive and negative subjects.

Population	Number Tested	AST < 40 U/L	AST ≥ 40 U/L	ALT < 40 U/L	ALT ≥ 40 U/L
Anti – HCV Positive	263	24 (9.23%)	236 (90.76%)	33 (12.54%)	230 (87.45%)
Anti – HCV Negative	150	134 (89.33%)	16 (10.66%)	130 (86.66%)	20 (13.33%)

Table 3: Prevalence of AST and ALT elevated levels in anti- HCV positive subjects.

Enzyme	Number Tested	≥ 40 U/L	40-80 U/L	81- 200 U/L	> 200 U/L
AST	260	236 (90.76%)	113 (47.88%)	116 (49.15%)	7 (2.69%)
ALT	263	230 (87.45%)	131 (56.95%)	91 (39.56%)	8 (3.04%)

the population studied and is slightly higher in our study population. In the present study only 9.23% and 12.54% of subjects had normal serum AST and ALT levels (< 40 U/L). Persons with chronic hepatitis C infection typically have only a little elevation in their ALT and AST levels.¹² In the studied population, more than half (56.95%) of anti-HCV antibody positive subjects had an ALT elevation of less than two times the URL (ALT ≤ 80 U/L). AST levels were also raised in 90.76% and both (ALT and AST) were raised in 83.46% persons. By comparison, AST and ALT elevations were far more common in the anti – HCV positive subjects than the control population. Elevated levels of serum AST were observed slightly more frequently than ALT in HCV positive subjects. AST / ALT ratio was calculated 1.07 ± 0.34 and was less than 1 in 50.38% of subjects.

Increases in both aminotransferases are a common finding in liver diseases. Activities of AST and ALT in serum are increased when liver cells are damaged and the extent of the increase being roughly proportional to the amount of liver cells damaged. However, moderately raised serum AST and ALT levels are typically found in chronic hepatitis C and cirrhosis- in proportion to the degree of active cell damage.⁹⁻¹¹ The ratio of AST to ALT has some clinical utility. Progressive liver functional impairment is reflected by an increase in the AST/ALT ratio (reversal of the ALT / AST ratio).⁷⁻¹¹ In many forms of acute and chronic liver diseases, the ratio is often ≤ 1 . This is particularly true in persons with hepatitis C where serum ALT levels are usually higher than AST and the ratio is generally less than 1.0. In other words, a ratio of 1.0 or more may indicate underlying cirrhosis while a ratio below 1.0 is found in patients with and without cirrhosis.⁷⁻¹¹ Park et al. found that an AST / ALT ratio ≥ 1.0 had 96% specificity and 74% positive predictive value in distinguishing cirrhotic from non-cirrhotic patients, with 47% sensitivity and 88% negative predictive value.⁸ An AST : ALT ratio of > 2.0 is very suggestive of alcoholic liver disease.⁷⁻¹¹

Thomas et al¹² showed that among individuals with four or more visits, ALT values were persistently normal in 42%, persistently elevated in 15%, and intermittently elevated in 43%. Hayashi et al. reported from Japan that all of their HCV positive patients were categorized into three groups: in group A, 121 patients (39.5%) had normal ALT levels on all occasions; in group B, 127 patients (41.5%) showed intermittently abnormal ALT levels, and 58 patients in group C (19.0%) had consistently abnormal ALT levels.¹³

Most of the available studies revolve around estimation of serum ALT levels and liver biopsy to establish relationship between the two most commonly used parameters to assess progression of underlying liver disease in chronic hepatitis C. These studies showed a strong correlation between persistently elevated serum ALT levels and severity of liver disease proved by liver biopsy. These studies further provided evidences that individuals with normal ALT and AST levels may have significant liver disease ("silent" liver damage).¹⁴⁻¹⁸

A prospective study showed a strong correlation between liver function test results elevated to greater than twice the upper limit of normal for at least six months and underlying liver disease proved by liver biopsy.¹⁶ In the study by Pasquale et al¹⁷ in patients with persistently normal ALT levels, none had cirrhosis and only 3.1% had bridging fibrosis. In another study, only 4% of patients with persistently normal ALT values had chronic hepatitis and only 1% had cirrhosis.¹⁸ The data of above studies clearly indicate that HCV infection with persistently normal ALT levels is associated with less severe histological liver disease and a lower fibrosis progression rate.¹⁶⁻¹⁸

Most of the reported studies demonstrated that serum aminotransferases is the best test- simple, cost effective and non – invasive – for monitoring HCV infection and the efficacy of therapy but a single normal value does not rule out active infection, progressive liver disease, or even cirrhosis.^{5,10,11,18,19} Despite of some limitations, monitoring of these enzymes over time generally provides a reasonable assessment of the severity of liver disease and may reflect the current activity of the disease process. Thus, evaluation of aminotransferases particularly ALT activity remains the most sensitive enzymatic indicator of hepatocellular damage available at present in chronic hepatitis C.^{9-11,19}

The real advantage of measuring AST and ALT levels lies in its cost and simplicity. The AST/ALT ratio further provides useful additional information at no cost which is readily accessible as part of the noninvasive and non-histological assessment of patients with chronic hepatitis C infection. However, the clinical significance of elevated serum ALT and AST levels in prediction of severity of liver injury and progression rate in persons with chronic hepatitis C is still a matter of debate. More prospective studies and a more targeted work – up are required to determine the potential utility of these enzymes as biochemical markers in following disease progression and guiding treatment decisions. Anyhow, these simple tests might prove useful in following such patients if prognostic information can be derived from changes in the aminotransferase levels and ratio over time.

Conclusion

We conclude that biochemical markers (AST, ALT and AST: ALT ratios) are useful, dependable and highly specific parameters for monitoring HCV infected patients (particularly asymptomatic). It is further concluded that aminotransferases estimation is a simple, non – invasive, non-histological test that is easy to perform and cost effective and a useful prognostic tool in monitoring HCV positive individuals. AST : ALT ratio further provides additional useful assessment at no cost and is a specific indicator of presence of cirrhosis. Furthermore, frequent retesting of asymptomatic individuals with elevated AST and ALT levels is recommended to assess progression or regression.

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