Bioresearch Communications
Volume 05, Issue 01, January 2019
Journal Homepage: www.bioresearchcommunications.com

Original Article

Correlation of serum alanine aminotransferase and hepatitis C viral RNA levels in Bangladeshi hepatitis patients

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BACKGROUND: Hepatitis C virus (HCV) is the second largest causative agent for liver infection (0.2-1% general population) in Bangladesh. In hepatitis, bothserum alanine aminotransferase (ALT)and aspartate aminotransferase are elevated without showing correlation of disease severity.However, serum ALT is the commonest and reliable biochemical parameter forliver function test. Hence, the correlation study of ALT and HCV RNA levels is warranted to observeprospective treatment outcomes through biochemical assay.**OBJECTIVE:**The investigation of serum ALT and HCV RNAlevels inacute and chronic hepatitis patients.**METHODS:**Whole blood was collected from 112 patients.Serum ALT levels were measured biochemically, serum antibody by EIA and HCV-RNA was confirmedby NAT.**RESULTS:**Among the enrolled hepatitis patients, there werecomparable demographic characteristics irrespective of their normal or elevated ALT levels. Although 59% patients were HCV RNA undetectable, the higher ALT levelswere significantly correlated with HCV RNA positive patients (*p=0.0015*). The latter patients group was mostly infected with genotype 3 (67%) than genotype 1 (22%) and other genotypes (11%).**Conclusion:** The confirmatory test and genotyping are essential to determine the optimal duration of therapy.

Keywords: Hepatitis C virus, Alanine aminotransferase (ALT), Enzyme immunoassay (EIA), Nucleic acid amplification test (NAT).

Article History
Received: 16 September 2018
Accepted: 17 December 2018

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Citation : Mohammed Mohasin , Tasnin Akter Nila ,Tahfima Sultana Tushy , Kamrun Nahar Keya , Md . Enamul Haque ; Correlation of serum alanine aminotransferase Biores Comm. V5-(1) 678-683.

INTRODUCTION

The hepatitis C virus (HCV) is ahepatotropic, small, enveloped, single-stranded and positive-sense RNA virus.It is a member of the genus *Hepacivirus* in the family *Flaviviridae*.The prevalence of HCVinfection is approximately 0.2-1% of general population in Bangladesh[1] and 180 million people (around 3%) are infected globally[2]. Like hepatitis B virus, HCV is primarily transmitted throughparentral route, which includes unscreened blood transfusion, injection drug use, infected body fluids, unsafe shaving and haircut in barber shops[1]. The highest transmission way is the intravenous drug abusers (24.8%)[3]. Acute HCV infection is usually asymptomatic and is very rarely associated with life-threatening disease. In 2014, WHO reported that around 15–45% of HCV infected person spontaneously clear viral particle within 6 months of infection. The rest of them usually develop chronic HCV infection. Around 20-30% of the latter group may have risk ofliver cirrhosis within 20 years[4]. In patients with clinical or biological signs of chronic liver disease, chronic hepatitis C is certain when both anti-HCV antibodies and HCV RNA (50 IU/ml or less) are present[5].Detectable HCV replication in the absence of anti-HCV antibodies is exceptional with the current third-generation Enzyme Immunoassays (EIAs), which ismostly observed in immune depressed

patients[6]. Moreover, an elevated alanine transaminase (ALT) level is indicative of hepatocellular necrosis and has been used as a surrogate marker of liver injury. Another aminotransferase enzyme is aspartate aminotransferase (AST),which is also elevated at liver injury as like asALT. AST is expressed in various tissues, which is released into serum when any one of these tissues is damaged, for example its level in serum rises with heart attacks and with muscle disorders. It is therefore not a highly specific indicator of liver injury. In contrast, ALT normally found largely in the liver, where it is most concentrated and releasedinto the bloodstream due to liver injury. Hence, ALT is mostly considered as a biomarker to assess liver function. The reference values of serumALT are 7 to 56 units per liter. Patients with chronic HCV have elevated levels of serum ALT and these patients commonly exhibit histological evidence of active inflammation and fibrosis. However, approximately 25-46% of patients with HCV have persistently normal ALT[7]. The presence of elevated ALT levels in HCV patients, which is frequently used as a guideline for commencing treatment, except for genotypes 2 and 3[8]. Patients with normal ALT were found to demonstrate an elevation of ALT in up to 27% of cases when monitored for 5 years[9]. We hypothesized that elevated serum ALT level and hepatitis viral load could be correlated during acute or chronic hepatitis. With a view to find out the associative features of HCV infection the particular objective of the present study includes: (i) To find out the correlation of the aminotransferase level with the acuities of the infection in order to develop a well specific treatment option, (ii) To find out the detection rate of HCV RNA in the patients who have shown anti-HCV antibody as it is an effective method to detect the stage of infection, and (iii) To identify the most common HCV genotype among Bangladeshi population.

MATERIALS & METHODS

In this study, blood samples were collected from 112 clinically tested patients (69 male and 43 female) who were infected with HCV. The patients were of different age and socio-economic groups. The specimens of this study were collected in a tertiary hospital in Dhaka,most of the patients were inhabitant of urban area. The whole study took place under the supervision of Department of Biochemistry, University of Dhaka and all the laboratory work was carried out in IbnSina Hospital, Dhanmondi, and Dhaka, Bangladesh after getting their approval.

Blood collection and Serum separation: Whole blood from 112 patients was drawn in a Lavender top tube. Then Serum from the blood samples were separated through centrifugation and preserved in CryoTube™ vials (Thermo scientific).Allow the blood to clot by leaving it undisturbed at room temperature (RT). This usually takes 15-30 minutes.Remove the clot by centrifuging at 10,000 rpm for 10 minute in a centrifuge machine. The resulting supernatant is designated serum.Immediately transfer the liquid component (serum) into a clean1.5 mL Thermo scientific CryoTube™ vials.

Detection of antibodies against HCV in serum: Anti-HCV has been detected through the Enzyme Immunoassay (EIA) method. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated surface to bind complementary analytes[10].

Determination of ALT level: The LX20 method was carried out to determine the level of ALT[11].This method basically replicates the cellular transaminases reaction, for example ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The absorbance was recorded at 340 nm over a fixed-time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample.

Isolation of HCV RNA and determination of viral load: RTA Viral Nucleic Acid Isolation Kit (Thermo Fisher) was used for viral RNA extraction from clinical samples. HCV RNA load was determined by RT-PCR assay. Real-time PCR assay is a one-step real time assay where RNA templates are first reverse-transcribed to generate cDNA strands then DNA polymerase-mediated cDNA amplification takes place. The viral load was determined using Stratagene Mx3000p/Mx3005p instrument or LightCycler 1.5/2.0 or INCEPTRA Cycler.

HCV genotyping: There are 6 genotypes and more than 90 subtypes of HCV.Most patients with HCV are found to have only one principal genotype, rather than multiple genotypes [12].Each area of the world has its own distribution of genotypes. Genotype 6 is the most common in Southeast Asia. Detection of HCV genotypes 1a, 1b, 2, 3a, 4, 5a, and 6 were carried out by polymerase chain reaction (PCR) usingHCV-genotype-FRT PCR kit (AmpliSens Biotechnologies). Briefly, total RNA was extracted from blood plasma, cDNA produced by reverse transcriptase carried out, and finally HCV cDNA copy was amplified using PCR technique.

Statistical analysis

Variables are presented as counts and percentages.Data were analyzed by Graphpad Prism 7.0 and the statistical test was performed using two-tailed Chi-Square (and Fisher exact) test, a $p<0.05$ was considered as statistically significant.

RESULTS

HCV infected patients mostly display symptomatic hepatitis

Hepatocytes are the main reservoir of HCV virus. It primarily attacks liver and can stay without showing any symptoms for as long as several years. At the terminal stage, it causes liver carcinomathough it's a rare scenario.We found chronic symptomatic infection in more than 55% (n=62) of infected individuals (Table-1). Moreover, 14% (n=16) eventually developed liver cirrhosis, which is clinically defined at the end-stage of liver carcinoma (Table-2).

Table 1. Demographic features of the study participants

Demographic Features		No. of Patients (%)
Sex	Male	69 (62%)
	Female	43 (28%)
Inhabitants	Rural	67 (60%)
	Urban	45(40%)
Age (Years)	<20	4 (3%)
	21-35	11 (10%)
	36-50	48 (43%)
	>50	49 (44%)
	Average	45.47
	Median	49

Table 2. Common clinical features of HCV patients

Clinical Features	No. of Patients (%)
Asymptomatic patients	23 (20%)
Weight Loss	67 (60%)
Jaundice	78 (70%)
Peripheral edema	33 (29%)
Ascites	21 (19%)
Thyroid dysfunction	13 (11%)
Painful joint and skin	19 (17%)

Alanine Aminotransferase level

The alanine amino-transferase (ALT) level is mostly determined to assess liver function. We found 6-fold increased ALT levels (Mean = 91 IU/L, range 13-378 IU/L) in two-thirds (66%) hepatic patients compared to standard reference level (15-56 IU/L). However, we found ALT level less than the reference value in 7% patients, which implies that this biochemical assay is not hundred percent accurate (Figure 1).

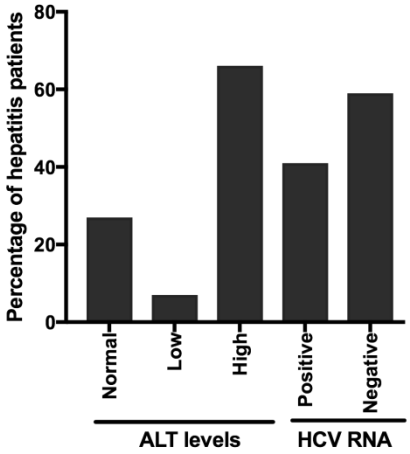

Figure 1. Determination of antibody to hepatitis C viral and ALT levelsfrom hepatitis-infected patients. Antibody specific to HCV was detected by enzyme immunoassays (EIAs) method and ALT levels were measured by a colorimetric biochemical assay described in method section. Percentage of normal, below and higher ALT levels containing patients were evaluated through comparing with standard ALT level. The bar-chart shows the percentages of hepatitis patients whoseserum ALT levels was measured biochemically and anti-HCV was detected by EIAs.

HCV RNA Detection:

The range of viral copy number or viral load usually varied among the hepatitis patients. We carried out RT-PCR to quantify HCV viral load in hepatitis patients whose ALT level was either normal or higher than the baseline level. Moreover, we found aundetectable HCV RNA level among 28.85% hepatitis patients. Whereas, 71.15%patients had a detectable RNA level. Interestingly, among the HCV RNA detectable patients, only 62.16% patients (46 out of 74 patients) had elevated ALT level (Table-3). This implies that elevated ALT levels were not 100% associated with acute viral infection.

Table 3. Comparison of ALT levels and detection of HCV RNA in hepatitis-infected patients

Data analyzed	*Normal ALT level	Higher ALT level	Total	Chi-Square (and Fisher exact) test
HCV RNA positive	28	46	74	*p=0.0015* (two-sided)
HCV RNA negative	2	28	30	
Grand Total	30	74	104	

Subtypes of HCV:

All the participants were tested to find the genotypes by which they were infected. There are 6 subtypes of HCV. But our limited kits only can identify 2 genotypes (e.g. genotype 1 & 3). All other subtypes were not found in these settings.In our setting, we found mostly genotype 3 (71.5%)compared to genotype 1 (22%) in HCV infected patients (Figure 2).

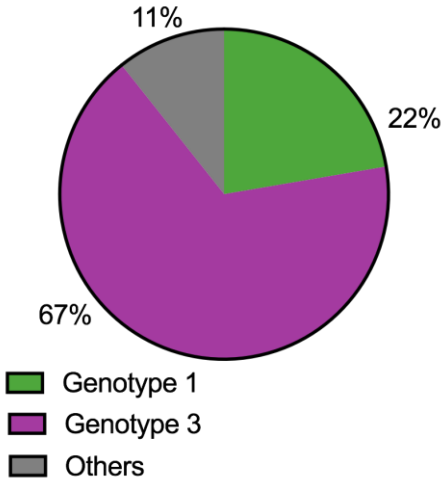

Figure 2. Subtypes of hepatitis C virus. HCV RNA was subtype by qPCR technique as described in the methods. Figure shows the percentages ofgenotypes, which are mostly predominate in HCV patients.

DISCUSSION

Hepatitis C virus (HCV) is a major cause of chronic liver disease, frequently progressing to liver cirrhosis and increased risk of hepatocellular carcinoma[13,14,15].HCV is ahepatotropic RNA virus, transmitted primarily through the parental route.It is mainly diagnosed through initially screening of high-risk groups for antibodies to HCV (anti-HCV). Moreover, prior to initiating treatment, another supplemental assay called nucleic acid amplification tests (NAT), which is mostly used as confirmatory tools as well as to determine viral load. In addition, genotyping is an important tool in clinical management to predict the likelihood of response and determine the optimal duration of therapy.

This study investigated the association of serum ALT and HCV RNA levels in acute and chronic hepatitis patients in Bangladesh. High ALT levels were independently associated with the presence of HCV viral load in serum particularly in acute hepatitis patients, which could be associated with up-regulation of inflammatory cytokines and chemokines as described[16]. The current demographic study also demonstrated that different subtypes of HCV prevalent among the hepatitis patients in Bangladesh. It has been reported that genotype 3 is mostly predominating over other genotypes, which arecorroborated with anotherstudy conductedon Bangladeshi hepatitis patients[17].

Aminotransferases are a group of enzymes, which transfer amino group from a donor molecule to a recipient molecule. The two major enzymes are aspartate aminotransferase (AST) is also known as serum glutamic oxaloacetic transaminase (SGOT) and alanine aminotransferase (ALT) is also known as serum glutamic pyruvic transaminase (SGPT). AST is normally found in various tissues including liver, heart, muscle, kidney, and brain. It is released into serum when any one of these tissues is damaged, for example its level in serum rises with heart attacks and with muscle disorders. It is therefore not a highly specific indicator of liver injury. In contrast, ALT (SGPT) is largely produced in hepatocytes though it can produce a few amounts from other tissues as well. It is released into the bloodstream as a result of liver injury.Moreover, an elevated alanine ALTlevel is indicative of hepatocellular necrosis and has been used as a surrogate marker of liver injury. Recently, several studies have shown that elevated ALT is an important predictor of the development and progression of liver fibrosis in chronic HCV infection irrespective of their HCV RNA levels[18,19]. These observations underpin a hypothesis that intra-hepatic inflammation is mostly responsible for development and progression of cirrhosis instead of cytotoxic effects of HCV infection. Another longitudinal study over 30 years has demonstrated that acute HCV infection over the years causes rapid liver disease progression, which was correlated with persistent elevation of ALT levels[20]. In contrast, some studies suggested that up to 25% of patients with chronic hepatitis C virus infection have persistently normal ALT levels[14,21,22]. In the literature, it has shown that the reference value for ALT level was set in the 1950s and has changed little since then. In this circumstance, it has been suggested to revise the normal ALT levels[23,24,25].

On the other hand, HCV has a remarkable degree of genomic diversity. It has six major genotypes and numerous subtypes in different geographic distribution. Moreover, HCV genotype has emerged as an important factor both in prognosis and to determine

the duration of antiviral therapy. Although nucleotide sequencing of a phylogenetically informative region remains the gold standard method for genotyping[26], whereas applied qPCR technique for genotyping and we found genotype 3 is more prevalence than genotype 1. However, Infection with any genotype can lead to cirrhosis, end-stage liver disease, and hepatocellular carcinoma.Neumann AU et al, has demonstrated that interferon therapy decline HCV viral load for patients with genotype 2 and 3 than genotype 1 [27]. For example, genotypes 2 and 3 istreated with a lower dose ribavirin (800mg) for 24 weeks rather than 48 weeks[28].

Overall, this study demonstrated the demographic and genotypic distributions of HCV as well as levels of ALT, as a critical biomarker, which is elevated as a host response along with the viral load irrespective of acute or chronic hepatitis in Bangladesh. However, it is warranted to observe the intra-hepatic or plasma inflammatory cytokines levelsin chronic hepatitis patients to explore disease prognosis as well as to monitor the outcomes of anti-viral therapy.

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