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RELATIONSHIP BETWEEN HEPATITIS C VIRUS (HCV) GENOTYPES AND ALANINE AMINOTRANSFERASE LEVELS IN PATIENTS OF TERTIARY HEALTH CARE CENTRE, KANPUR

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ABSTRACT High frequency of HCV chronicity, leading to hepatic failure, cirrhosis and hepatocellular carcinoma. The Aim and objective of this study was to assess Relationship between Hepatitis c virus genotypes and serum Alanine aminotransferase (ALT). Hepatitis C virus (HCV) serum titers have been examined with Genotype.

A total of 58 RNA Confirmed samples were tested for serum alanine aminotransferase (ALT) with HCV genotypes in the department of Microbiology Rama Medical College-Hospital & Research Center, Rama Medical University Kanpur from June 2016 till June 2017.

Serum alanine aminotransferase was determined by UV (international Federation of Clinical Chemistry recommended) Kinetic Method by using kit. mean±SD age of 58, (44.81±15.43) male were 47.56 (±16.98) years, female were 42.05 (±33.16) with ALT Label male 71.60 and female 71.41 ($p<0.05$), indicate that in severity of liver disease role of ALT is independent with hepatitis c virus genotypes.

KEYWORDS : HCV, Polymerase Chain Reaction, Genotyping, Alanine aminotransferase.

INTRODUCTION

Hepatitis C virus (HCV) is a blood borne pathogen that is endemic in most parts of the world, with an estimated overall prevalence of nearly 3% [1]. Hepatitis C is an emerging infection in India and an important pathogen can cause chronic liver disease, approximately 80% patients with hepatitis C virus develop chronic blood-borne infection, and progression to cirrhosis occurs in nearly 20% of these subjects within 20 years of infection, and those with cirrhosis are at risk of clinical decompensation and developing hepatocellular carcinoma [2, 3]. Moreover, patients with HCV-related cirrhosis are at an increased risk of developing hepatocellular carcinoma, which is estimated to occur at the rate of 1.5% to 4% per year [4]. In most individuals, liver disease progresses slowly over several decades, but the rate of progression is highly variable [3,5-6]. Whereas some patients develop cirrhosis and end-stage liver disease within one to two years of exposure, others may die of old age or an entirely unrelated cause[7]. Although it is mostly unclear why some patients progress more rapidly than others, several factors have been identified as having a role in disease severity.

ALT/SGPT is a liver enzyme. When Hepatitis C infects the liver, the hepatocytes (liver cells) produce higher-than-normal enzymes such as ALT, indicating inflammation of the liver. When initially infected, ALT may skyrocket to 10 times the normal level. When hepatitis C becomes chronic, ALT usually drops to a lower level, but remains persistently elevated. About two-thirds of people with chronic hepatitis C have continuously elevated ALT levels, reflecting ongoing damage to liver cells. The other third have normal ALT levels, even though they have a detectable HCV viral load. Although most people with HCV and normal ALT will live without any liver-related problems, roughly one-quarter of these people may have progression of liver disease. Elevations of serum alanine amontransferase (ALT) and aspartate aminotransferases (AST) activity serve as important markers for liver injury.

Distribution of HCV Genotypes in India:

The genotypic distribution of HCV in India has been found according to following table;

Table-1 Prevalence of HCV genotypes among patients

HCV1	HCV2	HCV3	HCV1&3	Author
21	25	54		Amarapurkar et al.1992[8]
88		13		Valliammai et al.1995 [9]
10		90		Chowdhury et al.1995 [10]
36		64		Panigrahi et al.1996[11]
19	1.1	62		Raghuraman et al.2003 [12]
17	10	71		Hazari et al.2004 [13]
13.8	5.5	66.6		Sompal Singh et.al.,2004 [14]
13.1	2.5	80.2		Hissar et al.2006 [15]
31	0.05-4.5	62		Narahari S et al.2009[16]
21		59		Rehan HS et al.2011 [17]
30.98	5.63	63		Anita Chakravarti et.al.,201[18]
15	7	20		Medhi S et al.2012 [19]
25.72%	0.002%	63.85%		J Christdas et.al.,2013[20]
24.13%	1.72%	72.41%	1.72%	Present study

The knowledge of HCV genotype is the strongest predictive factor for sustained virological response (SVR), since patients with different HCV genotypes react differently to α-interferon therapy [21]. The reported rates of SVR to interferon plus ribavirin combination therapy are 65% and 30%, in patients infected with HCV-2 or and HCV-1genotypes respectively [22]. The patient genotype has a vital role in treatment outcome therefore it is necessary to check genotype of isolated before the starting treatment.

RESEARCH AIM AND OBJECTIVES

The aim of this study was to find out serum alanine aminotransferase (ALT) with HCV genotypes in the department of Microbiology Rama Medical College-Hospital & Research Center, Rama Medical University Kanpur from June 2016 till June 2017.

INCLUSION CRITERIAAND EXCLUSION CRITERIA

Inclusion criteria: Patients who were reactive for anti-HCV antibodies.

Exclusion criteria:

Studies in non-representative populations, e.g.,

1. Epilepsy
2. Other malignancy
3. Pregnancy and lactation period
4. Therapy involving cytotoxic and bone marrow depressant.
5. HIV positive patients

MATERIALS AND METHODS

A total of 58 RNA samples were tested for Relationship between serum alanine aminotransferase (ALT) and HCV genotype, in the department of Microbiology Rama Medical College-Hospital & Research Center, Rama Medical University Kanpur from June 2016 till June 2017. Out of these 58 patients 29 (50%) were females and 29 (50%) were males. The overall mean age of all the patients was 44.81 ±15.43years ranging from 16 to 75 years. In females the mean age was 42.05 years (±33.16), ranging from 18 to 67 years and in males the mean age was 47.56 years (±16.98), ranging from 16 to 75 years.

Serum alanine aminotransferase was determined by UV (international Federation of Clinical Chemistry recommended) Kinetic Method by using kit (ERBA diagnostics kit) [23], The upper limit of normal ALT at 37°C is generally between 40 and 50 U/L for males and slightly lower for females (Normal Range: UP to 40U/L)[24].

Molecular diagonostic tests:

Total RNA extraction: Total viral RNA was extracted from Chromous Blood Total RNA Minispin kit according to manufacturer's instruction.

First Strand Synthesis KIT (cDNA Preparation)

PCR of Products: Polymerase chain reaction was performed on PCR machine (T-100, Bio-Rad).Primers were got synthesized from the GCC Biotech, Kolkata. The primers sequence was as follows; forward primer: 5'-GTCTAGCCATGGCGTTAGTA-3' and reverse primer; 5'-GTACTCAACGGTTCCGC-3'. Primers were dissolved with sterile double distilled water and TAE buffer based on the manufacturer's instruction. The PCR conditions were 95°C for 3min (initial denaturation), 35 cycles of 95°C for 30sec, 48°C for 30sec, 72°C for 1 min and final extension was at 72°C for 5min.Then PCR product was run with 1% agarose gel containing ethidium bromide. Genotype was determined by fragment size under UV light in gel documentation system (Bio-Rad). The PCR product (unpurified) was directly submitted for sequencing to Chromous Biotech Pvt. Ltd (Bengaluru).

STATISTICALANALYSIS

Automated data analysis and HCV genotype viral loads determination were performed using a Microsoft Excel based HCV genotyping invader data analysis worksheet (IDAW) developed and produced by TWT. The data was entered and analyzed through the SPSS version 10.0 (SPSS Inc, Chicago, US). Descriptive statistics was used to summarize the continuous and categorical data. Results were expressed as mean ± standard deviation (SD), frequencies and percentages.

RESULT

A total of 58 samples were tested for serum Alanine aminotransferase (ALT) with HCV-RNA levels in HCV genotypes in the department of Microbiology Rama Medical College-Hospital & Research Center, Rama Medical University Kanpur from June 2016 till June 2017. Serum alanine aminotransferase (ALT) was performed on those patients who had earlier tested positive for HCV-RNA and Genotype. Out of these 58 patients 29 (50%) were females and 29 (50%) were males. The overall mean age of all the patients was 44.81(±15.43) years ranging from 16 to 75 years. In females the mean age was 42.05 years (±33.16), ranging from 18 to 67 years and in males the mean age was 47.56 years (±16.98), ranging from 16 to 75 years. With ALT Label male 71.60 and female 71.41 ($p<0.05$), indicate that in severity of liver disease role of ALT is independent with hepatitis c virus genotypes.

Table 2- Association of Liver enzymes

Sr.No.	Enzymes	Mean±Std	p-value
1	Total Serum Bilirubin (Normal Range: 0.3-1.10 Mg / DL)	(0.84±0.91)	0.07
2	AST(SGOT) Levels (Normal Range: UP to 40U/L)	(81.77±51.48)	0.04*
3	ALT(SGPT) Levels (Normal Range: UP to 40U/L)	(72.81±49.98)	0.03*
4	SAP (Serum Alkaline Phosphates) Levels (Normal Range: 40-130 U/L)	(131.77±98.99)	0.01*
5	Serum Protein Levels (Normal Range: 6.4-8.3 Gm/DL)	(6.75±0.91)	0.01*
6	Serum Albumin Levels (Normal Range: 3.5-5 Unit Gm /DL)	(3.15±0.62)	0.02*

Table 2 shows different enzymes levels in HCV confirmed patients. Among all enzymes except Total Serum Bilirubin all were found to be statistically significant ($p<0.05$). This leads to the inference that enzymes have a significant level in both males and females.

In HCV infected patients, the liver function tests especially the predominant biomarkers viz. total bilirubin was determined by Malloy and Evelyn method, 1937 [25],Serum glutamine oxalo transaminase (SGOT) and Serum glutamate pyruvic transaminase (SGPT) was determined by Reitman and Frankel (1975) (Malloy HT,et. al., 1937) [23]. Alkaline phosphatase and albumin were determined by Kind and King (1971) [26].

Table 3- Prevalence of Hepatic diseases in different HCV genotypes in confirmed patients (N=58)

Hepatic Disease	HCV	HCV1	HCV2	HCV3	HCV1&3	p-value
Chronic hepatitis	40 (68.96%)	11 (27.5%)	-	28 (70%)	1 (2.5%)	0.01*
Liver Cirrhosis	15 (25.86%)	2 (13.33%)	1 (6.66%)	12 (80%)	-	
HCC	1 (1.72%)	-	-	1 (100%)	-	
Acute liver failure	2 (3.44%)	1 (50%)	-	1 (50%)	-	
Total	58	14 (24.13%)	1 (1.72%)	42 (72.41%)	1 (1.72%)	

***p-value <0.05 is considered statistically significant.**Table 3 shows proportion of different hepatic diseases in patients with confirmed HCV. The most common hepatic disease was found to be chronic hepatitis (68.96%), followed by liver cirrhosis (25.86%). Among the HCV genotypes, HCV 3 was the most common among all showing 72.41% confirmatory results in patients. To see the strength of association was found to be statistically significant which shows that HCV 3 is the main causation for different hepatic diseases. *(p=0.01)*

DISCUSSION

Correlation between HCV RNA confirmed cases, and serum ALT values produced conflicting results, but majority of studies found no correlation between HCV RNA confirmed cases, and serum ALT values [27-31]. On the other hand, Kato et al. observed significantly higher HCV RNA titers in patients with chronic active hepatitis and cirrhosis compared to those with milder histological abnormalities such as chronic persistent hepatitis [32]. Similarly, Fanning *et al*. in a study on Irish women who acquired their HCV infection through the administration of contaminated anti-D immunoglobulin obtained a significant correlation between serum HCV viral loads and the degree of hepatic inflammation in liver biopsy specimens[33].

CONCLUSION

The present study was carried out to detect the Hepatitis c virus genotypes and Alanine aminotransferase levels in Patients of tertiary health care centre, Kanpur. A total 58 confirmed RNA Patients were analyzed with screened samples with anti HCV antibodies. The patients admitted for the diagnosis of HCV from June 2016 to June 2017 at Rama Medical College Hospital and Research Centre, Kanpur which is a tertiary care centre for health and diagnosis. Total 58 confirmed RNA isolates were selected for the study in the age of between 16 to 80 years.

Following conclusions were drawn from the study:

- In this study it was seen that distribution of RNA confirmed patients (N=58) HCV genotype 3 and genotype 1 were found to be the predominant genotypes in the Indian sub-continent.
- Out of 58 confirmed patients,ALT Label male 71.60 and female 71.41 ($p<0.05$), indicate that in severity of liver disease role of ALT is independent with hepatitis c virus genotypes.
- Among all enzymes except Total Serum Bilirubin all were found to

be statistically significant ($p<0.05$). This leads to the inference that enzymes have a significant level in both males and females in most common hepatic disease was found to be chronic hepatitis (68.96%), followed by liver cirrhosis (25.86%). Among the HCV genotypes, HCV 3 was the most common among all showing 72.41% confirmatory results in patients. To see the strength of association was found to be statistically significant which shows that HCV 3 is the main causation for different hepatic diseases. *(p=0.01)*

- Our results indicate that the severity of liver disease is independent of serum levels of hepatitis C virus. The precise mechanism by which hepatitis C virus damages the liver remains poorly understood.

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