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CHRONIC HEPATITIS;

HCV GENOTYPES IN CORRELATION WITH ALANINE AMINOTRANSFERASE LEVEL IN PATIENTS IN INTERIOR OF SINDH

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ABSTRACT… Objective: To determine the correlation between HCV genotypes and serum ALT levels in various patients of chronic hepatitis induced by hepatitis C virus, in our setup. **Design:** A prospective study carried out from August 2006 to December 2009. **Setting:** Research Medical Center LUMHS Jamshoro, Pathology Department Peoples University of Medical & Health Sciences Nawabshah and Biotechnology Department University of Karachi. **Patients:** A total of 344 HCV-PCR positive patients with different genotypes were evaluated 239 men and 105 women with ages between 18–55 years of age years were included in the study. **Methodology:** All the patients went for ELISA test for the presence of HCV antibodies by ELISA kit of Biokit Spain, then a 10.0 ml sample of blood was collected in a tube with separating gel, to obtain serum, which was stored at -80^{O}C, for the determination of HCV RNA by RT- PCR (Real time cephid smart cycler) and for determination of HCV-RNA genotypes by comparison in sizes of the products amplified by RT-PCR using HCV genotype- specific primers, then subjecting the product to electrophoresis by Anagen kit. 5.0 ml of blood was also collected in a tube with separating gel, to obtain serum, which was stored at room temperature for the determination alanine aminotransferase by using UV enzymatic kinetic method. **Results:** when HCV genotypes were correlated with serum ALT levels, 09 cases were found < 50 mg % and among these 06 cases were of genotype 1a, 02 cases were of genotype 2a and one case of untyped. 55 cases shows serum ALT level between 50-100 mg % and among this majority of cases belongs to untyped category. 209 cases shows serum ALT level between 100-200 mg % and among this majority of cases belongs to genotype 3a. 71 cases shows serum ALT level >200 mg % and among this majority of cases belongs to genotype 3a. **Conclusions:** The data in the current study indicates the strong correlation between HCV genotypes and serum ALT levels. The genotypes 3a, 3b and 2 were found associated with high serum ALT levels and the genotype 1 was found associated with low levels of serum ALT.

Key words: HCV RNA, HCV Genotypes, ALT, Chronic Hepatitis

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INTRODUCTION

The liver is the second richest source of ALT. It is not surprising that any marked necrosis of liver cells leads to an escape of this enzyme into the blood. In acute hepatitis the level increases some times 10 or 100 folds than the normal, but in cirrhosis and obstructive jaundice the rise is less pronounced than in acute necrosis[1,2].

The hepatitis C virus has been affected approximately ten million populations of Pakistan[3], and millions of people worldwide[4]. Some patients with chronic hepatitis C have normal serum alanine aminotransferase (ALT) levels, even when tested on multiple occasions. In this and other situations in which the diagnosis of chronic hepatitis C may be questioned, the diagnosis should be confirmed by testing for HCV RNA. The presence of HCV RNA indicates that the patient has ongoing viral infection despite normal ALT levels. The ALT levels frequently fluctuate over time and may be normal or significantly elevated in

same patients measured at different times, where as other have persistently normal or persistently elevated ALT level[5]. Approximately 20% of patients with chronic HCV infection have persistently normal ALT levels. Although most of these patients have some degree of inflammation on liver biopsy[6], but some researchers reported up to 10% of cases with fibrosis[3]. However in non alcoholics, the rate of progression of fibrosis is very slow in patients with normal serum ALT levels[7]. Although the virologic response to interferon monotherapy appears to be the same as in patients with elevated ALT levels, treatment increases the serum ALT levels in about half of patients, and this increase can persists[7,8], so whether increase in ALT level is associated with a change in the rate of progression is not known[9]. There is no correlation between the serum ALT level and liver histology has been found in some studies[10], and thus should not be used to guide therapy[11], on the other hand successful therapy is associated with clearance of serum HCV, RNA, normalization of serum ALT levels, and improvement in liver histology[12].

It has been documented that there are significant clinical differences among the various genotypes and serum ALT levels and very few data are available from the literature on the relationship between ALT level and genotype[13].

Keeping all the above facts in view, we conduct a study on this controversial issue to determine any correlation between HCV genotypes and serum ALT levels in various patients of chronic hepatitis induced by hepatitis C virus, in our setup. This study shows the data from whole interior of Sindh, as the cases were collected from all the teaching hospitals attached with all the medical colleges of Sindh.

MATERIALS AND METHODS (METHODOLOGY)

This study was conducted at Research Medical Center LUMHS Jamshoro, Pathology Department Peoples University of Medical & Health Sciences Nawabshah and Biotechnology Department university of Karachi, during August 2006 to December 2009.

This study was a multi centric study covering all the interior of Sindh. The blood samples from 344 patients were collected from various medical wards of Liaquat University Hospital Jamshoro and Hyderabad, Peoples University of Medical & Health Sciences Hospital Nawabshah, Chandka Medical College Hospital Larkana, Civil Hospital Sukkur and Muhammad Medical College Hospital Mirpurkhas. The patients included in the study were having ages between 18 – 55 years, with evidence of presence of HCV-RNA in serum of patient by PCR and histological evidence of chronic hepatitis with either fibrosis or inflammatory activity by biopsy.

The suspected patients of chronic hepatitis were informed about the study, they signed a consent form and ELISA test for the presence of HCV antibodies was performed by ELISA kit of Biokit Spain. Than a sample of 05.0 ml of blood was collected in a tube with separating gel, to obtain serum, which was stored at room temperature for the determination alanine aminotransferase by using UV enzymatic kinetic method[12]. A sample of 10.0 ml of blood was collected in a tube with separating gel, to obtain serum, which was stored at -80°C for determination of hepatitis C virus by extracting HCV-RNA from plasma, amplified using reverse transcription and detected through the use of fluorescent reporter dye probes specific for HCV in the smart cycler® (cephid) and for HCV genotyping, which was performed by Anagen Kit that determines 12 HCV genotypes by simply electrophoresing HCV 5"NCR & Core region genes of type-specific sequence length amplified by Reverse-Transcription Polymerase Chain Reaction (RT-PCR) and features accurate and simple determination of HCV genotypes. The Kit can correctly genotype HCV RNA in serum or plasma at concentration equal to or greater than 100IU/ml.

RESULTS

In this prospective study (Table-I) a total of 344 HCV-PCR positive patients with different genotypes were evaluated (239 men and 105 women). Their ages rang from 18-55 years with a mean age of 35.14 years. The duration of infection

was evaluated in all the patients; it was below 2 years in 140 (40.69%), between 3 and 5 years in 196 (56.9%) and above five years in 8 patients (2.32%). The age at infection was below 20 years in 42 (12.20%) patients, between 21 & 40 years in 221 (64.25%) and above 40 years in 81 patients (23.55%).

Study Design Type Chronic hepatitis C patients With +ve HCV-PCR and genotype	 Prospective 344
Mean age	35.14 years
Sex Male Female	 239 (69.47%) 105 (30.52%)
Age at infection	
≤ 20 years 21-40 years > 40 years	42 (12.20%) 221 (64.25%) 81 (23.55%)
Duration of infection in year	
≤ 2 years 3-5 years > 5 years	140 (40.69%) 196 (56.9%) 08 (2.32%)

Table-I. Characteristics of the study population

Estimation of alanine aminotransferase (ALT)

The ALT is the major liver enzyme and is used as a marker of liver infection or hepatitis was estimated in 344 patients and was 4.4 times higher (mean level= 154.5 U/L) than normal in 335 (97.38%) patients, while 09 (2.61%) patients show ALT with in normal limit.

HCV Genotypes

The genotype (Table-II) was performed on 344 blood samples of chronic hepatitis C patients with positive hepatitis C virus RNA. The results of genotyping indicated (Table-II) that HCV genotype- 3a was found to be a major genotype in 242 (70.34%) cases, followed by genotype 3b in 19 (5.52%) cases, genotype 1a in 10 (2.61%) cases, genotype 1b in 5 (1.45%) cases, genotype 2 in 4 (1.16%) cases, genotype 5 in 3 (0.87%) cases and genotype mixed in 9 (2.61%). However no other genotype could be identifiable by this technique in 52 (15.11%) cases so classified as such remained untyped.

Genotypes	No.	%age
1a	10	2.9
1b	05	1.5
2	04	1.2
5	03	0.9
3a	242	70.3
3b	19	5.5
Mixed	09	2.6
Untypable	52	15.1
Total	344	100

Table-II. Prevalence of HCV Genotype in Chronic Hepatitis "C" patients

HCV Genotypes and alanine aminotransferase (ALT) level

As described in (Table-III), when HCV genotypes were correlated with serum ALT levels, 09 cases were found < 50 mg % and among these 06 cases were of genotype 1a, 02 cases were of genotype 2a and one case of untyped. 55 cases shows serum ALT level between 50-100 mg % and among this majority of cases belongs to untyped category. 209 cases shows serum ALT level between 100-200 mg % and among this majority of cases belongs to genotype 3a. 71 cases shows serum ALT level >200 mg % and among this majority of cases belongs to genotype 3a.

DISCUSSION

In current when we correlate the serum ALT levels with various genotypes of hepatitis C virus, the majority, ie eight cases out of nine of cases of genotype 1 show ALT levels < 100 U/L and no any patient show ALT level more than 100 U/L this finding is in consistent with another study held in Italy[14].

We observed that the majority of the cases of genotype 3a, 3b, and 2 have serum ALT levels between 100-200 U/L, and cases above 200 U/L also belong to this class. In these genotypes no any case was observed < 50 U/L, this finding is also observed by other workers[14]. The most

Genotypes	Serum ALT level				Total
	<50 U/L	50-100 U/L	100-200 U/L	>200 U/L	
1a	06	04	-	-	10
1b	02	03	-	-	05
2	-	01	01	02	04
3a	-	04	182	56	242
3b	-	03	10	06	19
5	-	01	01	01	03
Mixed	-	03	05	01	09
Untypable	01	36	10	05	52
Total	09	55	209	71	344

Table-III. Genotype in Correlation with Serum ALT Level

prevalent genotype in Pakistan is 3a followed by 3b & 1a[15].The patients of genotype 3 which also the most prevalent genotype in Pakistan[16-18], had significantly higher ALT values; this finding was also reported in two other studies from the USA[19,20]. Few data are available from the literature on the relationship between ALT level and genotype: Mita et al[21] found no correlation, but included only 8 patients with genotype 3. Zeuzem et al[22] reported a trend of lower ALT levels in patients with previous intravenous drug abuse where genotype 3 was prevalent; also in this study the number of patients was small.

CONCLUSIONS

The data in the current study indicates the strong correlation between HCV genotypes and serum ALT levels. The genotypes 3a, 3b and 2 were found associated with high serum ALT levels and the genotype 1 was found associated with low levels of serum ALT.

REFERENCES

1. A Forbes. **Hepatic failure and jaundice.** Walter JB, Talbot IC eds in Walter and Israel General Pathology 7th ED. Churchill Livingstone, New York, N.Y.10011.1996 Page 790.
2. Alan G, Johnson, Sir Robert S. **The Liver.Mann CV, Russel RCG eds in Baily & Love's Short Practice of Surgery21st ED.** Chapman & Hall, London, 1991; 1006.
3. Raja NS, Janjua KA. **Epidemiology of hepatitis C virus infection in Pakistan.** J Microbiol Immunol Infect 2008;41(1):4-8.
4. Bashir MF, Haider MS, Rashid N and Riaz S. **Distribution of Hepatitis C virus HCV genotypes in different remote cities in Pakistan.** African J Microbiol Res 2012;6(22):4747-51.
5. Shiffman ML, Stewart CA, Hofmann CM, et al. **Chronic infection with hepatitis C virus in patients with elevated or persistently normal serum alanine aminotransferase levels: Comparison of hepatic histology and response to inferferon therapy.** J Infect Dis 2000;182:1595-1601.
6. Marcellin P, Levy S, Benhamou LP, et al. **Management of the asymptomatic HCV carrier with normal ALT levels.** Virol Hep Rev 1996;2:277-83.
7. Mathurin P, Moussalli J, Canadranel JF, et al. **Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transferase activity.** Hepatology 1998;27:868-72.
8. Serfaty L, Chazouilleres O, Pawlotsky JM, et al. **Interferon alfa therapy in patients with chronic hepatitis C and persistently normal alanine transferase activity.** Gastroenterology 1996;110:291-5.
9. Di-Bisceglie AM. **Chronic hepatitis C viral infection**

in patients with normal serum alanine aminotransferase. Am J Med1999;107:53S-5S.

10. Jamal MM, Sony A, Qunin PG, et al. **Clinical features of hepatitis C infected patients persistently normal alanine transferase levels in the south western United States.** Hepatology 1999;30:1307.

11. Umer M, Khaar H, eds in **Hepatitis C in Pakistan 1st ED SAF Publishers, Islamabad, Pakistan.** 2006; page 122.

12. Camp ME, Rossol S, Chokshi S, et al. **Hepatitis C virus-specific T-cell reactivity during interferon and ribavirin treatment in chronic hepatitis C.** Gastroenterology 2000;118:346-55.

13. Ahmed W, Ijaz B, Javed FT, et al. **HCV genotype distribution & possible transmission risks in Lahore Pakistan.** World J Gastroentrol 2010;16(34):4321-8.

14. Saracco, Sostegni, Ghisetti, et al. **Hepatitis C virus genotypes in a non-cirrhotic Italian population with chronic hepatitis C: correlation with clinical, virological and histological parameters. Results of a prospective multicentre study.** J Viral Hepatitis 2000;7(2):124-9.

15. Schulman; S. N. Kinnman, P. Linmarker and M.A. von Sydow. **Randomized study of alph-interferon plus ribavirin for 6 months or 12 months for the treatment of chronic hepatitis C in patients with bleeding disorders.** Haemophilia 2002; 8(2): 129-135.

16. Bushra I, Waqar A, Fouzia TJ, et al. **Any correlation to HCV genotype/viral load, ALT, AST, ALP, bilirubin, gender, age to disease progression.** Virol J 2011;8:361.

17. Idrees M, Riazuddin S. **Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission.** BMC Infec Dis 2008;8:69.

18. Waqar A, Bushra I, Fouzia TJ, et al. **HCV genotype specific correlation with serum markers.** High predictability of genotype 4a. Virol J 2011;8:293.

19. Mahaney K, Tedeschi V, Maertens C, et al. **Genotypic analysis of hepatitis C virus in American patients.** Hepatology 1994;20:1405-14.

20. Lau JY, Mizokami M, Kolberg JA, et al. **Application of six hepatitis C virus genotyping systems to sera from chronic hepatitis C patients in the United States.** J Infect Dis 1995;171:281-9.

21. Mita E, Hayashi N, Kanazawa Y, et al. **Hepatitis C virus genotype and RNA titer in the progression of type C chronic liver disease.** J Hepatol 1994;21:468-73.

22. Zeuzem S, Teuber G, Lee JH, Ruster B, Roth WK. **Risk factors for the transmission of hepatitis C.** J Hepatol 1996;24(2):3-10.

PREVIOUS RELATED STUDY

Dilshad Muhammad, Khalid Amin, Amin Anjum, Masood Javed. CHRONIC HEPATITIS C VIRUS INFECTION; ASSOCIATION WITH TYPE 2 DIABETES MELLITUS (Original) Prof Med Jour 17(4) 557-562 Oct, Nov, Dec 2010.

Shaukat Ali, Syed Khurram Shahzad, Atiq ur Rehman Slehria. CHRONIC HEPATITIS C; RESPONSE TO INTERFERON AND RIBAVIRIN COMBINATION (Original) Prof Med Jour 17(4) 563-567 Oct, Nov, Dec 2010.