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HCV GENOTYPE 3A;
HAVE MILD TO MODERATE GRADES OF STEATOSIS IN HYDERABAD.

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ABSTRACT… The aim of this study was to see the correlation between hepatic steatosis in chronic HCV infection and HCV genotypes. **Study Design:** It was a prospective observational study done. **Setting:** Isra University Hospital and histopathology laboratory. **Period:** May to November 2014. **Material & Methods:** The study was conducted on 87 liver biopsy specimens of patients infected with chronic HCV. The biopsy samples were collected from department of Pathology, Isra University. H&E stained sections of liver biopsies were evaluated to determine Grade and Stage of chronic hepatitis C and grade of steatosis. Blood samples of those patients were collected from Isra University hospital and Asian Institute of Medical Sciences (AIMS), Hyderabad to determine the HCV genotype, platelet count and liver function test including ALT, AST and GGT. **Results:** It was found that majority of the patients 38 (43.6%) had genotype 3a infection followed by 3b, 1a, 1b and Un-typeable genotypes. Hepatic steatosis was divided into categories according to Brunt's classification. It was found that 39 (44.8%) patients had grade 0 steatosis while 48 out of 87 patients presented with steatosis. It was found that 29 (33%) presented with grade 1 steatosis followed by grade 2 and 3. Steatosis was most frequently seen with genotype 3a (26.4%) and presented with mild to moderate grade. **Conclusions:** The present study concludes that hepatic steatosis is more frequent in genotype 3a and presents with mild to moderate grade.

Key words: Hepatitis C genotypes, Hepatic steaosis.

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INTRODUCTION

Hepatitis C virus (HCV) belongs to the genus Flavivirus having a single stranded positive sense RNA which is inherently unstable.[1] Depending upon the nucleotide arrangement and 67% similarity in them HCV is categorized into seven major genotypes.[2,3] and they also differ in terms of prevalence in various regions. Most of infections in North America, South America, and Europe are caused by genotype 1.[4] Whereas in Pakistan the most frequently found HCV genotype is genotype 3a and after that genotype 3b and 1a[5] The chronic infection of liver caused by HCV is a worldwide challenge that has influenced about 170 million population [6]. The hepatitis C infection is heralding as sooner or later it leads to the fatty liver (Steatosis), scarring of liver, cirrhosis and hepatocellular carcinoma.[1] An abnormal accumulation of lipids (triglycerides) in the hepatocytes is termed as Steatosis[7] and is known to have strong association with the inflammation, fibrosis and cirrhosis of liver.[8] The incidence of steatosis associated with chronic HCV infection in Pakistan is reported to be 65.7%.[6] The severity of fibrosis corresponds with the degree of steatosis.[9] Broadly speaking, HCV induced steatosis may be due to hold back in the lipid metabolism at one of these levels: 1. Defective secretion of lipid. 2. Escalated formation. 3. Defective breakdown[7] and 4. Declined VLDL secretion[10] It has been reported in numerous in vitro studies that this hepatic steatosis is inferred from HCV core protein specifically by genotype 3 proteins as compared to other genotypes.[11] This steatosis, caused by HCV genotype 3, is believed to be due to its cytopathic effect of its replication.[12] Furthermore, HCV genotype 3 induced steatosis settles down after complete annihilation of HCV by antiviral treatment but it is not the case in patients infected with genotype 1 virus.[7] The grounds for these

genotype differences are indistinct. The genotype 3 HCV infection might require lower levels of expression to induce steatosis.[13] Whereas complications like cirrhosis and hepatocellular carcinoma develop more frequently in patients infected with genotype 1 as compared to other genotypes.[14] Now-a-days the determination of HCV genotype is imperative before starting the treatment and to evaluate the chronically infected Patients.[15] The aim of the present study was to evaluate the effect of HCV genotypes on hepatic steatosis.

MATERIALS AND METHODS

This study was conducted in Isra university Hospital and histopathology laboratory from May to November 2014. The liver biopsy samples were received in the histopathology laboratory (Department of Pathology), Isra University and blood samples of those patients were collected from Isra university hospital and Asian Institute of Medical Sciences, Hyderabad (AIMS) which are the major centers where most of the cases of hepatitis from around the Sindh are treated. Study population comprised of 87 patients with chronic HCV infection selected by simple random technique. Informed consents were obtained from all patients and attendants. The Ethical Committee at Isra University, Hyderabad approved the study.

Inclusion criteria

Serum HCV-RNA positive patients of both genders fulfilling the criteria for liver biopsy and including ages between 20 and 60 years.

Exclusion criteria

HBsAg positive, known cases of Diabetes mellitus, hemophilia, patients, alcoholics and patients with other concomitant illnesses such as autoimmune hepatitis, hemochromatosis, alpha-1 antitrypsin deficiency and Wilson's disease.

Procedure

Viral genotype determination was done by using Abbott m2000 real-time PCR system, which uses the automated extractor *m*2000sp and the *m*2000rt device for automated real-time PCR amplification and detection of PCR products. HCV RNA was extracted from 500 μl of serum and levels were expressed in IU/ml. Serum levels of Alanine aminotransferase, Aspartate aminotransferase, Gamma glutamyl transpeptidase and platelet counts were determined.

The liver biopsy samples were fixed in 10% formaline and stained with hematoxyline and eosine. The slides were studied under light microscope and histopathological findings were recorded like necro-inflammation, steatosis and fibrosis. The Portal and lobular necro-inflammatory activity scores and the fibrosis stage were assessed using the Scheuer score[16] and steatosis was graded according to Brunt's Classification.[17] The data was analyzed on SPSS version 21.0 for windows release (IBM, incorporation, USA). Continuous and categorical variables were analyzed using students t-test and chi-square test respectively. Continuous variables were presented as mean ±SD and categorical variables as frequency and percentage.

RESULTS

Age of study population ranged between 20 and 60 years.Mean ± SD of study subjects was noted as 39±11.3 years. The study population was divided into two age groups i-e <40 and >40 years which is shown in table-I. There was insignificant difference between both groups (p=0.061).

Male population predominated compared to female as table-II shows that 57 (65.5%) were male and 30 (34.4%) were female with significant differences (p=0.001).

65.5% patients had normal platelet counts as shown in table-III and serum levels of Aspartate Aminotransferase (AST), Alanine Amino transferase (ALT), Gamma Glutamyl transferase (γ-GT) are shown in table-IV

Frequency of HCV Genotypes is shown in table-V and the most frequent genotype was genotype 3a (43.6%). Necro-inflammation grades and fibrosis stages were assessed according to the method of Scheuer et al[16] and are shown in tables-VI and

VII respectively. Histologic Activity Index (HAI) is categorized as chronic persistent hepatitis (CPH) and chronic active hepatitis (CAH) - mild, moderate, and severe. CAH-mild type was the most frequently noticed in present study (48.2%), followed by CPH (35.6%), CAH-moderate (12.6%) and least frequency was noted for the CAH-severe category (3.4%). Fibrosis stage 4 (cirrhosis) was note in 15 (17.2%) of patients. 20 (22.9%) patients showed no fibrosis. Frequency of steatosis is shown in table-VIII. Steatosis was divided into categories according to Brunt's classification[17] as Grad 0 to Grade3. Grade 0- no steatosis was noticed in 39 (44.8%) of patients, severe steatosis in 3 (3.4%), while mild and moderate comprised major part; noted in 29 (33.3%) and 16(18.3%) of patients respectively. Steatosis was common finding in HCV genotype 3a- (26.4%), followed by 3b (12.6%), 1a (8%), 1b (6.8%) and Untypable (2.2%) as shown in table-IX.

Age	No. of Pt.	%	p-value
<40 years	39	44.8	
>40 years	48	55.17	0.061

Table-I. Distribution of study population by age (n=87)

Gender	No. of Pt.	%	p-value
Male	57	65.5	
Female	30	34.4	0.001

Table-II. Gender distribution of study population (n=87)

	No. of Pt.	%
Normal Platelet counts	57	65.5
Low Platelet counts	28	32.1
High Platelet counts	2	2.29

Table-III. Platelets counts of study population (n=87)

Enzymes	No. of Pt.	%	p-value
AST			
Normal	40	45.9	
High	47	54.0	0.03
ALT			
Normal	28	32.18	
High	59	67.81	0.0001
G-GT			
Normal	54	62.06	
High	33	37.93	0.001

Table-IV. Aspartate transaminase (AST) (IU/L) (n=87)

Genotype	No. of Pt.	%
3a	38	43.6
3b	28	32.1
1a	8	9.1
1b	5	5.74
Untypable	8	9.1

Table-V. Frequency of HCV Genotype (n=87)

HAI	No. of Pt.	%
CPH	31	35.6
CAH-Mild	42	48.2
CAH-Moderate	11	12.6
CAH-Severe	3	3.4

Table-VI. Necro-inflammation (Histologic Activity Index) (n=87)

	No. of Pt.	%
Stage 1. (No fibrosis)	20	22.9
Stage 2. (Fibrous Portal expansion with intact lobular architecture)	25	28.7
Stage 3. (Septal fibrosis with lobular architecture distortion)	27	31.0
Stage 4. Cirrhosis	15	17.2

Table-VII. Fibrosis Stage

	No. of Pt.	%
Grade 0. Nill (=<2% hepatocyte)	39	44.8
Grade 1. Mild (3-29% hepatocyte)	29	33.3
Grade 2. Moderate (30-59% hepatocyte)	16	18.3
Grade 3. Severe (>60 hepatocytes)	3	3.4

Table-VIII. Frequency of hepatic steatosis (n=87)

HCV Genotypes	Hepatosteatosis	%
3a	23	26.4
3b	11	12.6
1a	7	8.0
1b	6	6.8
Untypable	2	2.2

Table-IX. Association of HCV genotypes with hepatosteatosis

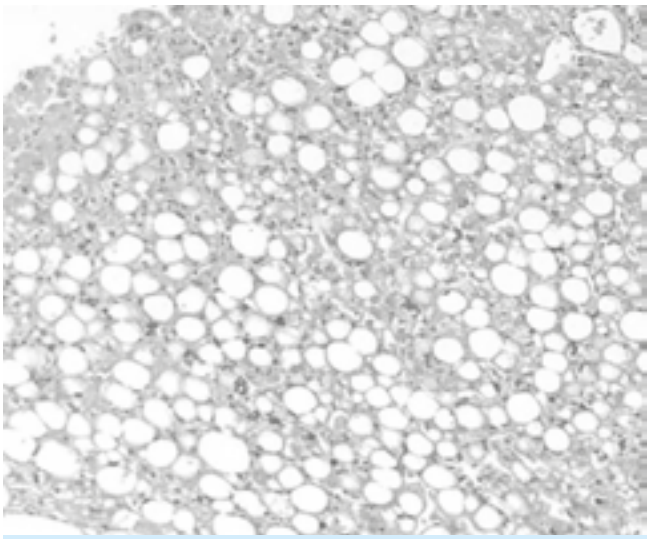

Figure-1. H&E stained section of liver biopsy showing grade 3 hepatic steatosis at magnification x 200.

DISCUSSION

The present study is an original research work conducted on liver biopsy specimens of patients with chronic HCV infection to see the correlation between HCV genotypes and steatosis.

It was found that most of the patients with chronic HCV infection were more than 40 years of age. This result contrasts with the study reported in India by Rajani and Jais[18] who reported that the seroprevalence of HCV was most frequent in age group 11-20 years and least frequent in more than 40 years of age.

In our study most of the patients infected with chronic HCV infection were males. Similar results were reported in other study conducted in Islamabad.[1]

Regarding HCV genotypes, it was found that genotypes 3a, 3b, 1a, 1b and Untypable were present in 38 (43.6%), 28 (32.1%), 8 (9.1%), 5 (5.7%) and 8 (9.1%) respectively. These findings of HCV genotypes are consistent with previous studies[19,20] which had reported genotype- 3a as most common in Southeast Asia including Pakistan. In contrast Safi S et al[1] reported the presence of genotype 2a in eight (6.7%) patients and genotype 4 in one (0.8%) out of one hundred and twenty six patients of chronic HCV infection. A systemic review by ATTAULLAH, S, et al[21] reported findings of 34 studies from all over Pakistan. Genotype 3a was the most common genotype calculated at a rate of 55.10%, followed by genotype 3b, 1a and mixed genotypes respectively. Genotypes 4, 5 and 6 were rare.[21] These findings are consistent with present study as genotypes 4, 5 and 6 were not noticed in our study population.

Hepatitis C virus induced hepatic steatosis is proposed to be a direct effect of the virus itself, and most commonly reported with genotype 3a.[22,23,24] The findings of present study are in full agreement with previous reports as mentioned. . However, in this study no association of ALT, AST and GGT was found with Histological Activity Index (HAI) and Fibrosis stage. These results are in agreement with the study of Fecury AA et al.[26]

CONCLUSION

In this study the predominant HCV genotype was 3a in the population of Hyderabad and majority of the patients infected with genotype 3a were males.

Moreover, Steatosis was found significantly associated with genotype 3a and mostly presented with mild to moderate grade.

This study has got certain limitations. The sample size was very small. It is recommended that in future the study should be conducted on larger population size to obtain more conclusive results.

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PREVIOUS RELATED STUDY

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"Minds are like parachutes - they only function when open "

Thomas Dewar

AUTHORSHIP AND CONTRIBUTION DECLARATION

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