FREQUENCY OF NON-ALCOHOLIC FATTY LIVER DISEASE IN NEWLY DIAGNOSED CASES OF HEPATITIS C VIRUS INFECTION AND ITS CORRELATION WITH GENOTYPES IN NORMAL WEIGHT PATIENTS

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ABSTRACT

Objective: To determine the frequency of non-alcoholic fatty liver disease (NAFLD) in newly diagnosed cases of hepatitis C virus (HCV) infection and its association with genotypes in normal weight patients.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Departments of internal medicine and diagnostic imaging, Combined Military Hospital Okara, from Oct 2013 to Mar 2014.

Material and Methods: We included 211 patients from Okara through consecutive sampling who were found positive for anti HCV antibodies and HCV RNA after informed consent. The sampled patients were evaluated for liver echotexture through ultrasonography and genotype analysis by polymerase chain reaction. Variables were defined qualitatively and quantitatively and frequencies, percentages, means, and standard deviations were calculated. For the association of ultrasonographic findings with the genotypes, Pearson's Chi-square or Fischer's exact tests were applied where appropriate. All the data were analyzed using statistical package for social sciences version 20. A *p*-value <0.05 was considered significant.

Results: The mean age was 32 ± 6 years with a range of 21 ± 47 years. Most (85.3%, n=180) were married. The majority (62.1%, n=131) hailed from the Punjab province and from the age-group of ≤32 years (55.9%, n=118). The findings seen on ultrasonography were normal echotexture in 93 (44.1%), NAFLD in 112 (53.1%), and chronic liver disease in 6 (2.8%) individuals. NAFLD was commonest among HCV RNA genotype 3 positive cases.

Conclusion: Fifty-three percent patients with positive HCV RNA had NAFLD identified on ultrasonographic examination. The genotype 3 of HCV RNA was particularly affiliated with NAFLD.

Keywords: Genotype, Hepatitis C virus, Non-alcoholic fatty liver disease.

INTRODUCTION

Seroprevalence of hepatitis C virus (HCV) among the general adult Pakistani population is 6.8%[1] and genotype 3a is the most prevalent (66.7%)[2] genotype. Non-alcoholic fatty liver disease (NAFLD), on the other hand, is defined as fat accumulation in the liver exceeding 5% to 10% by weight, as determined by the percentage of fat laden hepatocytes by light microscopy[3] in individuals who do not consume alcohol at all or consume alcohol only in quantities generally considered not harmful to the liver. Spectrum of NAFLD consists of isolated hepatic macrovesicular steatosis at one end and steatohepatitis at the other. NAFLD is frequently seen in individuals infected by HCV. About 50% of patients infected with HCV infection have steatosis[4]. Hepatic steatosis is important to document as it also adversely affects the virologic response rates to anti-HCV therapy[5].

In most cases of NAFLD, obesity has been identified as the risk factor. Prevalence of NAFLD due to any cause is 33.6% in general population of the world[6], whereas, in obese population, its prevalence rises up to 74%[7]. Whether HCV infection directly contributes towards fatty infiltration of liver or is it because of co-existing obesity, the query has been answered by multiple studies, which have proved that the genotype 3 of HCV is an independent risk factor for NAFLD. In

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populations where HCV genotype 3 is common, the prevalence of hepatic steatosis exceeds the expected value of prevalence[8]. Other genotypes e.g. genotype 1 are not associated with NAFLD and in such cases if NAFLD is found, it is most probably because of co-existing obesity or other components of metabolic syndrome[9]. Liver biopsy is the gold standard for the diagnosis of NAFLD but it can also be diagnosed non-invasively with the help of ultrasonography[10].

According to the national health survey of Pakistan conducted in 2006, obesity was present in 25% of Pakistani population[11]. We intended to find the frequency of NAFLD in newly diagnosed HCV infected cases after excluding the confounding effects of obesity. The patients were of normal weight and waist circumference and were free from diseases leading to fatty infiltration of liver. The correlation of NAFLD frequency with different genotypes of HCV was the secondary goal.

Table-I: Ultrasound variable and scores used for diagnosis of chronic liver disease

Variables	Score 0	Score 1	Score 2
Liver parenchymal echotexture	Homogenous/fine	Coarse	Highly nonhomogeneous/coarse
Liver surface	Smooth	Irregular	Nodular
Liver edge (inferior margin right lobe)	Sharp (acute)	Blunted	Rounded

Table-II: Demographic properties of the sample (n=211)

Variables	n (%)	Variables	n (%)
Marital status		Age group	
Married	180 (85.3)	Age ≤32	118 (55.9)
Single	31 (14.7)	Age >32	93 (44.1)
Ethnicity based on provinces		Ultrasonographic evaluation results	
Punjab	131 (62.1)	Normal echotexture	93 (44.1)
Sindh	50 (23.7)	NAFLD*	112 (53.1)
Khyber Pakhtunkhwa	12 (5.7)	Chronic liver disease	6 (2.8)
Balochistan	6 (2.8)		
Azad Jammu and Kashmir	12 (5.7)		

*Non-alcoholic fatty liver disease

MATERIAL AND METHODS

This cross-sectional study was carried out in the departments of internal medicine and diagnostic imaging, Combined Military Hospital Okara, from October 2013 to March 2014. Through consecutive sampling, we included 211 patients from Okara and the surrounding areas, who were found positive for anti HCV antibodies through 4th generation ELISA and HCV RNA through polymerase chain reaction. The other standards were: body mass index (BMI) <24.9 kg/m^2, waist circumference <90 cm, and no previous history of obesity, diabetes mellitus (DM), hypertension, chronic hepatitis B or C, and drug treatment affecting liver functions. Informed written consent was taken from each patient and permission from the hospital ethical committee was also sought. For BMI assessment, the body weight was measured in light clothing to the nearest 0.1 kg using SALTER 920 digital weighing scale (Salter Ltd, Tonbridge, UK). Waist circumference was mea-sured at the midway between iliac crest and the lower rib margin at the end of normal expiration using a plastic flexible tape to the nearest 0.1 cm. For blood pressure (BP) recording, each patient was asked to sit quietly in a chair with his or her back supported for 5 minutes in a private, quiet, and warm room before taking the measure- ment. BP was measured three times by the same physician using Catetek CM-3620 mercury

sphygmomano-meter (Catetek, Zhejiang, China). For the purpose of analysis, the mean of the three measured values was considered. Use of the correct cuff size with the air bladder encircling at least 80% of the arm was ensured. Centre of the cuff was placed at the heart level. Width of the cuff was kept equal to a minimum 40% of the arm circumference. The rate of deflation was fixed at 2 mmHg/sec. All participants underwent assessment of levels of fasting blood glucose, serum triglycerides, alanine transaminase, and high-density lipoprotein cholesterol using automated analyser i.e. Microlab 300 (ELI Tech Group, Puteaux, France). Those who fulfilled the inclusion criteria were reviewed for HCV RNA genotype through PCR and for liver echotexture by ultrasonography machine Toshiba Nemio 30 (Toshiba Medical Systems Corporation, Tochigi, Japan). Ultrasound examination of all cases was done by a single radiologist having at least seven years of experience in performing abdominal sonography. For the purpose of study, NAFLD was defined by the presence of bright hepatic echoes, increased hepatorenal echogenicity, vascular blurring of portal or hepatic vein, and subcutaneous tissue thickness[12]. For the diagnosis of chronic liver disease (CLD), a modified ultrasonographic criteria with a sensitivity of 90.3% used by Afzal *et al* was used (table-I)[13]. A minimum score of 2 was considered suggestive of CLD. All the data were analysed using statistical package for social sciences version 20. The frequencies and percentages for categorical variables and means and standard deviations for quantitative variables were calculated. For association of ultrasonographic findings with the genotype for HCV RNA, Pearson's Chi-square or Fischer Exact tests were applied where appropriate. A *p*-value <0.05 was considered significant.

Table-III: Association of results of ultrasonographic evaluation with age group, marital status, ethnicity based on provinces and HCV RNA Genotype on PCR

Variables	Normal echotexture	NAFLD*	Chronic liver disease	Total	*p*-value
	n (%)	n (%)	n (%)	n (%)	
Marital Status					
Married	80 (44.4)	94 (52.2)	6 (3.3)	180 (100)	0.54
Single	13 (41.9)	18 (58.1)	0	31 (100)	
Age group					
Age ≤32	51 (43.2)	63 (53.4)	4 (3.4)	118 (100)	0.85
Age >32	42(45.2)	49(43.8)	2 (33.3)	93 (100)	
Ethnicity based on provinces					
Punjab	52 (39.7)	75 (57.3)	4 (3.1)	131 (100)	0.53
Sindh	27 (54)	22 (44)	1 (2)	50 (100)	
Khyber Pakhtunkhwa	7 (58.3)	4 (33.3)	1 (8.3)	12 (100)	
Balochistan	3 (50)	3 (50)	0	6 (100)	
Azad Jammu and Kashmir	4 (33.3)	8 (66.7)	0	12 (100)	
HCV RNA Genotype on PCR					
Genotype 1	8 80)	0 (48.6)	2 (20)	10 (100)	<0.001
Genotype 2	3 (100)	0	0	3 (100)	
Genotype 3	29 (21.6)	104 (77.6)	1 (0.7)	134 (100)	
Genotype 4	3 (60)	1 (20)	1 (20)	5 (100)	
Mix and untypable	50 (84.7)	7 (11.9)	2 (3.4)	59 (100)	

*Non-alcoholic fatty liver disease

RESULTS

Considering 211 individuals finally included, the mean age was 32 ± 6 years with a range of 21-47 years. Most (85.3%, n=180) were married. The majority (62.1%, n=131) hailed from the Punjab province and from the age group of ≤32

years (55.9%, n=118). The findings seen on ultrasonography were normal echotexture in 93 (44.1%), NAFLD in 112 (53.1%) and CLD in 6 (2.8%) individuals (table-II). Fifty-nine (27.7%) cases had either mixed genotype or non-typeable HCV RNA on PCR. The commonest single genotype among all 211 cases was found to be genotype 3 (63.5%, n=134) followed by genotype 1 (4.7%, n=10), genotype 4 (2.4%, n=5), and genotype 2 (1.4%, n=3). Those with a genotype 3 for HCV RNA were significantly more likely to have NAFLD on ultrasonography (p<0.001) than those who had either another single genotype 1, 2 or 4 or mixed/untypable genotypes (table-III). Marital status, age-group, and ethnicity based on provinces did not significantly affect the ultrasonographic findings (p=0.54, 0.85, and 0.53 respectively) (table-III).

DISCUSSION

Hepatic steatosis is present in many liver diseases including chronic HCV infection[14]. There are many factors that can lead to development of steatosis in chronic HCV infection namely viral factors (HCV genotype-3), host factors (alcohol consumption, overweight, hyperlipidemia, DM, and insulin resistance), and drug therapy (corticosteroids, amiodarone, methotrexate[15]). In our study, we only considered viral factors and excluded remaining host factors and drug therapy by careful selection of study population. In the western world, steatosis occurs more frequently in patients with chronic HCV infection than in general population of the adults (50.9% and 55% in chronic HCV infection cases against 20-30% among general population)[14,16] and these results correlate with our study in which 53.1% of HCV infected people had NAFLD. Although we excluded overweight and obese people and hence a smaller percentage of NAFLD was expected yet the results showed similar trends indicating strong influence of HCV itself in the pathogenesis of NAFLD. Few international studies have termed such fat as "viral fat" rather than metabolic fat[17] that is produced by genotype 3 viremia.

Genotype 3 frequency was 63.5% in our study which is also supported by another study carried out in Pakistan by Idrees *et al*[18] where its prevalence was found to be 66.7%. In our study, 77.6% of the patients infected with genotype 3 had NAFLD as compared to genotype 1, 2, and 4 which is again compatible with other studies[19]. We incidentally found 2.8% to have advanced fibrosis and meet ultrasound criteria of CLD[13].

There is no study in Pakistan yet to determine the frequency of NAFLD in HCV patients and our study might be the first one. It is important because hepatic steatosis is an independent risk factor for poor drug response to treatment[20]. However, compared with other predictors of treatment failure in HCV, such as genotype, viral load, and ethnicity, the relationship between steatosis and treatment failure is less well understood. Correspondingly, we could not identify significant correlation of NAFLD with age-group, marital status or ethnicity based on provinces.

Our study had few limitations. Firstly, it was exclusive for the male gender and thus the results could not be applied to the whole population. Secondly, we excluded cases that were either overweight or obese or had metabolic syndrome. Thus, our study sample was highly selective and actual frequency of NAFLD in HCV population could have been quite higher than our estimate. Thirdly, although ultrasound is quite sensitive in diagnosing fatty liver, it is not the gold standard and thus the actual frequency could have been little higher or lower than found in our study.

CONCLUSION

The frequency of NAFLD identified on ultrasonographic examination in HCV positive patients was 53.1%. The genotype 3 of HCV RNA was particularly affiliated with NAFLD. Marital status, age-group, and ethnicity based on provinces were not associated with NAFLD.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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