ORIGINAL ARTICLE

Histomorphological changes in hepatitis C non-responders with respect to viral genotypes

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Abstract

Objective: To evaluate the distinct histopathological changes of chronic hepatitis C (CHC) non-responders in association with viral genotypes.

Methods: This cross-sectional study was conducted at the histopathology section of the Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences in collaboration with Sarwar Zuberi Liver Centre, Civil Hospital, Karachi from September 2009 to August 2011. Seventy-five non-responders (end-treatment-response [ETR] positive patients) from a consecutive series of viral-RNA positive CHC patients with known genotypes were selected. Their genotypes and pertinent clinical history was recorded. They were subjected to liver biopsies which were assessed for grade, stage, steatosis, stainable iron and characteristic histological lesions.

Results: Majority of the patients (63, 84%) had genotype 3 while 12(16%) cases had genotype 1. The genotype 1 patients had significantly higher scores of inflammation ($p<0.03$) and fibrosis ($p<0.04$) as compared to genotype 3. Steatosis was significantly present in all genotype 3 patients in higher scores ($p<0.001$) compared to genotype 1. Stainable iron scores were generally low in the patients in this study, however, it was more commonly seen in genotype 3. The distribution of characteristic histological lesions was noteworthy in both the groups, irrespective of genotype.

Conclusion: In this series, the predominant genotype was 3. However, genotype 1 patients were more prone to the aggressive nature of the disease with significantly higher scores of inflammation and fibrosis. Steatosis was characteristically observed in genotype 3 group. Stainable iron could not be attributed as a cause of non-response.

Keywords: Hepatitis C non-responders, Genotype, Pakistan. (JPMA 63: 358; 2013)

Introduction

Hepatitis C is a major health problem with an estimated global prevalence of 2.2-3.0%, infecting more than 170 million people worldwide.[1] In a developing country like Pakistan, the estimated prevalence rate of 5.7% is alarming.[2] The most frequent and efficient route of hepatitis C transmission is through parenteral exposure to the hepatitis C virus (HCV).[3] In Pakistan unsterile and used needles and surgical equipments are major causes of disease spread.[4]

HCV is intrinsically unstable and exists as multiple genotypes and subtypes, with different regional distribution worldwide.[3] The predominant HCV genotype in Pakistan is type 3a, followed by 3b, 1a, 2a and 1b.[4] Genotyping is important as it dictates the duration of treatment and is the strongest predictor of response to interferon (IFN) and ribavirin (RBV) combination therapy.[5]

HCV can cause both acute and chronic infections. More than 80% of the patients develop chronic hepatitis C (CHC), which induces hepatic inflammation and associated fibrosis. The related morbidity and mortality is due to progression into chronic active hepatitis, cirrhosis and hepatocellular carcinoma.[3]

The histomorphological changes seen in the liver biopsy of CHC as well as other hepatotropic viruses, referred to as elementary lesions by Rosai,[6] include portal inflammation, periportal interface hepatitis, focal (spotty) lytic necrosis, confluent necrosis, fibrosis and cirrhosis. Furthermore, some of the histological changes like steatosis, increased amounts of hepatic iron, portal lymphocyte aggregates and bile duct damage are considered more characteristic of CHC, whereas Mallory bodies, apoptotic bodies and sinusoidal lymphocytic infiltration can be noticed in hepatic inflammation associated with other causes as well.[6,7]

It has been established that certain virological and host factors depict the histological picture in CHC. In this regard, genotypes are considered more important and distinct genotypes are reported to be associated with characteristic histological findings.[8,9]

Liver biopsy is considered the "gold standard" for assessing liver disease status and is advocated for diagnostic and prognostic purposes. Furthermore, its role is established for the decision of initiating re-treatment in non-

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responders.[5] Recently, there has been a debate in performing liver biopsy routinely in genotypes 2 and 3 because of their much better treatment response compared to other genotypes. The surrogate serum markers of fibrosis as well as ultrasound-based methods are more preferred in the management of CHC patients due to their non-invasive nature. This crucial issue needs to be addressed at length; though the current non-invasive markers can detect the presence or absence of advanced fibrosis but cannot definitely replace the liver biopsy.

With the currently recommended antiviral treatment, viral clearance occurs in more than 80% of genotype 2 and genotype 3, and in less than 50% of genotype 1 infected patients. Negative HCV RNA by a sensitive test after 24 or 48 weeks of therapy refers to end-of-treatment response (ETR). Patients who fail to clear HCV RNA and show a positive ETR are termed "non-responders" (NR) and are a great challenge for the clinicians all over the world. Infection with HCV genotype other than genotype 2 and genotype 3, a baseline viral load of >600,000 IU/ml, age >40 years, male gender, advanced fibrosis, steatosis, increased body mass index (BMI), hepatic iron deposition, and race are some of the factors reported to be associated with non-response to treatment.[5,10-12] Research is underway to introduce effective novel therapies to improve the treatment response and to retreat the non-responders.

The increasing proportion of non-responders in our population with a comparatively easy to treat prevalent genotype, is a matter of great concern. This significant pool of non-responders has resulted due to the initial use of IFN monotherapy for the treatment of CHC in Pakistan – RBV was later added.[2] Patient noncompliance to prescribed treatment and inappropriate dose reduction for the management of adverse effects are additional factors associated with non-response.[5] The non-response to therapy is an issue to be addressed on the national level as it brings about grave emotional and economic strain not only to the affected families, but also the entire state at large. The histological features associated with non-response to therapy have been reported from western countries, having a diverse ethnic and genetic profile than ours. Therefore, we decided to report the histological features seen in non-responders of our local population. Furthermore, the genotype specific histomorphological changes of CHC reported in the Western literature might not be pertinent to our population as the frequency of genotype distribution in our country is entirely different from the West. Hence, we need to determine these histological changes in our own genetically diverse population, which is more susceptible with largely existing nutritional deficiencies in the background as compared to the developed countries. This study will generate baseline data that will definitely add into the existing body of information. Such a study in Pakistan has not been done before to our knowledge.

Materials and methods

This cross-sectional study was carried out over a period of about two years from September 2009 to August 2011, at the Histopathology section of Dow Diagnostic Research and Reference Laboratory (DDRRL) in collaboration with Sarwar Zuberi Liver Centre (SZLC), Civil Hospital Karachi, with the approval of the Ethics Review Board of Dow University of Health Sciences (DUHS). A sample size of 75 was calculated by using statistical software OpenEpi.com taking 5.7%[2] of prevalence and ± 3% bound of error, with 95% confidence interval.

We closely monitored all HCV RNA-positive CHC patients visiting SZLC for their disease management. Their genotyping was done prior to starting treatment. Genotype 2 and genotype 3 patients received treatment for 6 months whereas non-2 and 3 genotypes patients received treatment for one year. At the end of treatment, the ETR was evaluated. We only selected ETR-positive patients i.e. non-responders for this study and they were subjected to liver biopsy. We carried out direct patient interviews on the biopsy days and recorded the genotypes and pertinent clinical information on especially designed proformas. Informed consent was taken. CHC responders, patients with any other type of viral hepatitis or liver disease, human immunodeficiency virus co-infection, decompensated liver disease, and alpha-1 antitrypsin deficiency were excluded.

Histopathological evaluation

Case by case, liver biopsies at DDRRL were grossly examined, fixed with formalin and processed on automation. After paraffin embedding, 3-4mm thick sections were cut. They were stained with hematoxylin and eosin (H&E), periodic acid-Schiff ± diastase, Masson's trichrome, Reticulin and Perl's iron stains.

The grade and stage were evaluated by Ishak modified histological activity index (HAI).[13] This system includes four categories to assess the necroinflammation: (a) periportal or periseptal interface hepatitis, (b) confluent necrosis, (c) focal (spotty) lytic necrosis, and (d) portal inflammation. Consecutive scores for these separate categories are added together to reach the activity grade, the maximum possible score being 18. The modified staging includes architectural changes, fibrosis and cirrhosis with the maximum possible score of 6.

For statistical convenience the grade and stage were

categorised as mild (grade 0-6; stage 0-2), moderate (grade 7-12; stage 3-4) and severe (grade 13-18; stage 5-6). For statistical significance, the grade and stage were further grouped into two major categories as mild (group 1) and moderate to severe (group 2). Steatosis was assessed on a scale of 0-3,[6] depending on the percentage of hepatocytes involved; grouped into two as grade 0 and 1 (group 1) and grade 2 and 3 (group 2). Stainable hepatic iron was graded on a scale of 0-4, modified from Silva et al,[14] (Grade 0: no detectable iron; grade 1: iron visible only at 400x; grade 2: iron visible at 200x; grade 3: iron visible at 100x; and grade 4: iron granules visible at 40x). It was categorised as grade 0-1 (group 1) and grade 2, 3 and 4 (group 2). The presence or absence of other histological parameters like portal lymphocyte aggregates, bile duct damage, mallory and apoptotic bodies, and sinusoidal lymphocytic infiltration were noted as previously described.[6,7]

The data were subjected to statistical software SPSS version 16 and described in terms of mean ± SD and percentages. The association of demographic and histological parameters with genotypes was evaluated by using chi-square (χ^2) test. p-value < 0.05 was considered significant.

Results

Among 75 non-responders, majority 63(84%) had genotype 3. No significant difference was seen in the mean age of genotype 3 and genotype 1 patients. The male to female ratio in genotype 3 was 1:1, whereas in genotype 1 it was 1:3.

The demographic features and elementary histological lesions of patients in relation to genotypes are depicted in Table-1. Liver biopsy in majority of genotype 3 patients showed milder scores of the four necroinflammatory categories which were summed up to reach the final Grading HAI. Similarly, the Staging HAI was also mild in a greater proportion of genotype 3 patients compared to genotype 1.

Table-2 illustrates other histological changes in CHC non-responders in relation to genotypes. It shows that steatosis was present in all of the genotype 3 patients, and a greater proportion had higher steatosis grades 2 and 3. In contrast, steatosis was either absent or mild, grade 1 steatosis was seen in genotype 1 patients.

Table-2 also includes the confounding factors associated with steatosis. Greater proportion of genotype 1 patients were overweight/obese and had a history of diabetes mellitus (DM). History of alcohol intake was absent in both genotype groups.

Overall low frequency of stainable iron was observed in our set of series. Although stainable iron was more common in genotype 3 group, higher grades (grade 2-4) were seen in greater proportion of genotype 1 patients compared to genotype 3.

Table-3 reveals the correlation of age, gender and histological parameters with the viral genotypes, using the chi-square test. A significant association of moderate to severe necroinflammatory score (p<0.03) was seen in

Table-1: Demographic features and elementary histological lesions in chronic hepatitis C non-responders with respect to viral genotypes.

Demographic & histological parameters	Genotype 3 n (%)	Genotype 1 n (%)
Total number of patients	63 (84)	12 (16)
Mean age (Years)	38.3 ± 7.3	38.1 ± 6.9
Gender		
Male	33 (52.4)	3 (25)
Female	30 (47.6)	9 (75)
A. Periportal or periseptal interface hepatitis (piecemeal necrosis)		
Absent	6 (9.5)	1 (8.3)
Mild (focal, few portal areas)	28 (44.4)	2 16.7)
Mild / moderate (focal, most portal areas)	16 (25.4)	2 (16.7)
Moderate (continuous around <50% of tracts or septa)	10 (15.9)	4 (33.3)
Severe (continuous around >50% of tracts or septa)	3 (4.8)	3 (25)
B. Confluent necrosis		
Absent	52 (82.5)	3 (25)
Focal confluent necrosis	1 (1.6)	2 (16.7)
Zone 3 necrosis in some areas	1 (1.6)	3 (25)
Zone 3 necrosis in most areas	5 (7.9)	0
Zone 3 necrosis + occasional P-C bridging	4 (6.3)	3 (25)
Zone 3 necrosis + multiple P-C bridging	0	1 (8.3)
Panacinar or multiacinar necrosis	0	0
C. Focal (spotty) lytic necrosis, apoptosis, and focal inflammation		
Absent	1 (1.6)	0
One focus or less per 10 x objective	8 (12.7)	0
One to four foci per 10 x objective	42 (66.7)	10 (83.3)
Five to ten foci per 10 x objective	11 (17.5)	2 (16.7)
More than ten foci per 10 x objective	1 (1.6)	0
D. Portal inflammation		
None	0	0
Mild, some or all portal areas	14 (22.2)	2 (16.7)
Moderate, some or all portal areas	29 (46.0)	5 (41.7)
Moderate / marked, all portal areas	11 (17.5)	4 (33.3)
Marked, all portal areas	9 (14.3)	1 (8.3)
I. Ishak modified HAI grading		
Mild (1-6)	42 (66.7)	4 (33.3)
Moderate (7-12)	12 (19.0)	6 (50)
Severe (13-18)	9 (14.3)	2 (16.7)
II. Ishak modified HAI staging		
Mild (1,2)	36 (57.1)	3 (25)
Moderate (3,4)	20 (31.7)	7 (58.3)
Severe (5,6)	7 (11.1)	2 (16.3)

SD= Standard deviation
HAI= Histological activity index
P-C= Portal central.

Table-2: Histological changes and confounding factors in chronic hepatitis C non-responders with respect to viral genotypes.

Histological parameters		Genotype 3 n (%)	Genotype 1 n (%)
Steatosis			
Grade 0		0	5 (41.7)
Grade 1 (<33% hepatocytes involved)		31 (49.2)	6 (50)
Grade 2 (33-66% hepatocytes involved)		26 (41.3)	1 (8.3)
Grade 3 (>66% hepatocytes involved)		6 (9.5)	0
Confounding factors associated with steatosis			
BMI	18.5-24.9 (normal)	44 (69.8)	8 (66.7)
	25-29.9 (overweight)	14 (22.2)	2 (16.7)
	>30 (obese)	5 (7.9)	2 (16.7)
Diabetes mellitus	Present	4 (6.3)	1 (8.3)
	Absent	59 (93.7)	11 (91.7)
History of alcohol intake		0	0
Stainable iron			
Grade 0		46 (73.0)	9 (75)
Grade 1		8 (12.7)	0
Grade 2		2 (3.2)	1 (8.3)
Grade 3		5 (7.9)	1 (8.3)
Grade 4		2 (3.2)	1 (8.3)
Portal lymphocyte aggregates		21 (33.3)	4 (33.3)
Bile duct damage		38 (60.3)	8 (66.7)
Mallory bodies		28 (44.4)	3 (25)
Apoptotic bodies		25 (39.7)	7 (58.3)
Sinusoidal lymphocyte infiltration		34 (54)	9 (75)

genotype 1 patients. In addition, moderate to severe fibrosis was seen in greater proportion of genotype 1 patients compared to genotype 3, with a significant association ($p<0.04$). Steatosis was significantly associated with genotype 3 ($p<0.001$). Age, gender, stainable iron and other histological lesions showed no significant association with genotypes of the patients.

Discussion

The genotype distribution in our study reflects the expected pattern established by other local studies with predominance of genotype 3.[2,4] This study thus highlights the genotype variation in our distinct population from the Western countries, where their prevalent genotypes have received more attention in terms of studies carried out on histological parameters as well as clinical behaviour.

Majority of the patients in our series, both males and females, were 40-50 years of age. Our findings are in agreement with the reported literature[5,10] where age less than 40 years is documented as one of the predictors of sustained response to antiviral therapy. Thus, age more than 40 years might be an important factor in the non-response to therapy in our patients. In addition, some of the studies have documented an association of advanced disease with different genotypes attributable to older age

Table-3: Correlation of Demographic and Histological parameters in chronic hepatitis C non-responders with viral genotypes.

Demographic/Histological parameters		Genotype 3 n (%)	Genotype 1 n (%)	P-value (chi-square, χ^2)
Age (Years)	20-29 (gp.1)	8 (12.7)	1 (8.3)	
	30-39 (gp.2)	18 (28.6)	4 (33.3)	0.88
	40-50 (gp.3)	37 (58.7)	7 (58.3)	
Gender	Female	30 (47.6)	9 (75)	0.08
	Male	33 (52.4)	3 (25)	
Modified HAI Grading	Mild (gp.1)	42 (66.7)	4 (33.3)	0.03*
	Moderate –severe (gp.2)	21 (33.3)	8 (66.7)	
Modified HAI Staging	Mild (gp.1)	36 (57.1)	3 (25)	0.04*
	Moderate – severe (gp.2)	27 (42.9)	9 (75)	
Steatosis	Absent-Mild (gp.1)	31 (49.2)	11 (91.7)	0.00*
	Moderate-severe (gp.2)	32 (50.8)	1 (8.3)	
Stainable iron	Absent-Mild (gp.1)	54 (85.7)	9 (75)	0.35
	Moderate-severe (gp.2)	9 (14.3)	3 (25)	
Portal lymphocyte aggregates	Present	21 (33.3)	4 (33.3)	1
	Absent	42 (66.7)	8 (66.7)	
Bile duct damage	Present	38 (60.3)	8 (66.7)	0.67
	Absent	25 (39.7)	4 (33.3)	
Mallory bodies	Present	28 (44.4)	3 (25)	0.21
	Absent	35 (55.6)	9 (75)	
Apoptotic bodies	Present	27 (42.9)	7 (58.3)	0.32
	Absent	36 (57.1)	5 (41.7)	
Sinusoidal lymphocytic infiltration	Present	34 (54)	9 (75)	0.17
	Absent	29 (46)	3 (25)	

* = Significant P-value
HAI= Histological activity index

and longer duration of infection.[8] We did not find any relationship of age with the histomorphological differences in the two genotype groups as the mean age of both our genotype 3 and 1 patients was almost the same. We, however, could not determine the duration of infection because many of our patients presented with history of exposure to more than one risk factor for HCV acquisition and could not correctly recall the event.

The significant correlation of male gender with fibrosis progression and non-response of treatment has also been reported by some researchers,[5] however, we did not find any significant association in this respect.

The unexpected finding of this study was that majority of our patients presented with mild inflammation and fibrosis, which was definitely in contrast to the Western literature that reports higher inflammatory and fibrosis scores as the hallmark of non-response.[15] Extensive fibrosis and cirrhosis in genotype 2/3 non-responders compared to responders has been reported both internationally[10] and in the local literature.[16] Therefore, we suggest that the lack of response to treatment in our patients could be more related to several other factors including racial differences, and HLA genes,[11] which need to be evaluated in later studies.

The most imperative finding of our research work is the statistically significant association of grade and stage with distinct genotypes. We found that a greater proportion of our genotype 3 patients had mild, whereas genotype 1 patients had moderate to severe inflammation. We concur with Fontain et al[17] as they reported an association of genotype 1 with more aggressive disease and poor response to IFN therapy. There are certain contradictory reports as well in this respect as Vigani et al[9] observed more severe disease and cirrhosis in genotype 3, compared to genotype 1 patients. A recent local study[18] reported no association of liver histology with the genotypes, however, the results displayed higher scores of grade and stage in genotype 1. Most of the studies in the last decade compared the post-treatment histology in responders with non-responders without taking into account the viral genotypes. However, Tassopoulos et al[19] observed the histological features of 20 non-responder patients; higher scores of necroinflammatory grade were seen in genotype 1 patients compared to type 3 showing similarity to our findings.

On the international level, some of the studies documented an improvement in the grade and stage of the disease after receiving treatment, even in the non-responder patients.[20] However, controversies exist in this regard as Ikawa et al[21] reported no significant histological change in non-responders after treatment. We documented an association of histological lesions with genotypes in CHC non-responders with a limitation of having no pre-treatment biopsies. Therefore, we could not compare the existing morphological status with the baseline information. Hence, the possibility still exists that lower scores of grade and stage in genotype 3 in our series could be related to post-treatment improvement in the necroinflammation and fibrosis. According to the most recent guidelines for management of CHC,[5] pre-treatment biopsies are not required in standard protocols especially in genotype 3, which shows good response to treatment. This approach is followed at most of the health care centers in Pakistan and currently IFN/RBV therapy is initiated in all the CHC patients irrespective of their disease status.

The pretreatment viral load is another factor which is considered as a predictor of response to therapy. A viral load >600,000 IU/L is associated with unfavourable response.[5] The viral load of majority of our patients was not available; we could not include this parameter in our study due to financial constrains.

The significant association of steatosis with genotype 3 in our series is supported by the existing literature.[22] Moreover, we observed that a greater proportion of our genotype 1 patients had raised BMI and DM, compared to type 3. Therefore, the mild steatosis seen in genotype 1 could be due to the confounding factors of DM and raised BMI rather than the viral genotype; in agreement with an international series.[23] However, a large number of patients in this regard would be more conclusive.

Stainable iron was present in low frequency in our series. The presence of stainable iron more commonly in genotype 3 group compared to type 1 is in concordance with Sebestiani et al.[24] We, however, do not agree with the studies that document the association of excessive iron deposition in CHC with the non-response to treatment.[12] Moreover, iron deposition in CHC is associated with alcohol intake.[25] Therefore, the low frequency of iron accumulation in our series might be due to the absence of history of alcohol intake in our set of patients.

The characteristic histological lesions related to hepatitis C were present in patients and as described in previous published studies.[6,7] The portal lymphocyte aggregates in our series were present in exactly the same proportion in both the genotype groups. We found an increased frequency of bile duct lesions in the genotype 1 group. Our data, differs from Mihm et al[8] who reported a greater frequency of portal lymphocyte aggregates and bile duct lesions in genotype 3. Since there was no significant variation in the distribution of the other histologic lesions among the two genotype groups, therefore, it appears that these changes are notably present in CHC irrespective of genotypes.

Conclusively, our data shows variation, as fibrosis is not the hallmark of non-response in our patients as is projected. Genotype 1 shows more aggressive disease with higher inflammatory and fibrosis scores compared to genotype 3. Steatosis is significantly associated with genotype 3 in particular. The mild scores of steatosis in genotype 1 patients could be due to the confounding factors of raised body mass index (BMI) and DM. Iron overload cannot be attributed as a cause of non-response in our patients. Hence, the histomorphological manifestations of HCV infection are associated with distinct genotypes in our patients. It is important to document these significant and consistent histological features and address these parameters in future on large population scale.

Furthermore, our study highlights the importance of pre-treatment biopsy to exactly determine the tissue status at the inception of treatment. We advocate liver biopsy as still the gold standard because the insight into the histological picture of treatment naive and non-responders appears to be the best guidance of disease progression and prognosis.

We recommend that if the histomorphology of HCV non-responders is not aggressive, the unidentified factors need to be explored as the cause of non-response. Advance molecular studies are desirable to understand the pathogenetic mechanisms to determine any existing sequencing variability in our prevalent HCV genotypes among responders and non-responders.

Conclusion

Genotype 3, was presenting the majority of the patients in this study. Steatosis was predominant in the genotype 3 group. Genotype 1 patients had more severe inflammation and fibrosis. In both genotype 1 and 3, stainable iron was not a cause of non-response.

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