INTRODUCTION

The HCV is an RNA virus that belongs to the family of flaviviruses\(^1\). The natural targets of HCV are hepatocytes and possibly B lymphocytes\(^2\). It has estimated that more than 10 trillion virus particles are produced per day, even in the chronic phase of infection\(^6\). The HCV is inherently unstable, giving rise to multiple genotypes and more than 50 subtypes have been identified on the basis of molecular relatedness\(^2\). Like other viruses, the substantial heterogeneity of HCV genome is the result of mutations that occur during viral replication\(^7\). HCV circulates as a population of divergent genome exhibiting a quasispecies distribution\(^8\) which result from the rapid development of mutations in a hyper variable region (HVR1) within one of envelope proteins. Patients infected with HCV, mount a humeral immune response to epitopes of HVR1, but sequential changes in the consensus sequence of HVR1 during infection results in the generation of variants that are not recognized by pre-existing antibodies\(^7\). Specifically over time several dozen mutant strains can be detected with in one individual and mapped as derivates strains of the original HCV strain infecting that individual\(^2\). Study of viral diversity is crucial to elucidate the role of the different HCV genotypes in the pathogenesis and progression of disease. Viral genotyping might also be of clinical relevance, since numerous studies have reported a relation-
ship between the HCV genotype and the response to treatment. At present correct HCV genotyping is of great importance as part of the pretreatment evaluation of patients with chronic HCV infections. This is of great interest that significant differences and correlations between various genotypes and histological grade and stage have been observed in some studies. Staging, in the broadest sense, is the determination of the position of the patient on the continuum of disease progression between its initiation and its end stage. Grading is the assessment of the activity of a disease, which is the rate at which the disease stage is changing. In the natural history of most chronic diseases, the stage of the disease generally increases with time, although relapsing or remitting diseases may be examples of disease as a disease flares and subsides, or may remain static throughout the disease. Therapeutic intervention typically has most of its effect on disease activity, so histological activity is important for the patient and the clinician because it provides a measure of severity of the hepatitis at the time of biopsy.

Keeping all these above facts in view, we conduct this study in which the genotyping of HCV infection is studied in interior of Sindh in correlation to grading and staging of chronic hepatitis induced by hepatitis C virus. 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 interior Sindh.

MATERIALS AND METHODS

This study was conducted at Research Medical Center LUHMS Jamshoro, Pathology Department Nawabshah Medical College for Girls Nawabshah and Biotechnology Department of University of Karachi, during August 2006 to June 2008.

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, Nawabshah Medical College 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 persistent abnormal alanine aminotransferase levels, and evidence of presence of HCV RNA in serum of patient by PCR.

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.

The anti-HCV positive patients were be submitted to a laboratory protocol. The first composed of questionnaire with clinical and epidemiological data (sex, age at biopsy, routes of contamination, age of infection, consumption of alcohol, and estimated duration of infection, defined as the time elapsed between the presumed date of infection and date of biopsy). The following biochemical examinations were included in the laboratory protocol: ALT and AST determination, number of times above normal level, gamma-GT (number of times above normal level), serum protein, serum albumin, serum bilirubin, blood urea, glucose, uric acid, cholesterol, triglycerides, serum electrolytes, coagulation (BT, CT, PT, APTT) and haematological (haemoglobin, TLC, DLC, Platelets) examinations.

HCV genotyping: A sample of 10.0 ml of blood was collected in a tube with separating gel, to obtain serum, which was stored at -80OC for determination of hepatitis C virus RNA 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.

Histopathological evaluation: After completing the above mentioned protocol the patients were submitted to a percutaneous hepatic biopsy. A hepatic biopsy was performed in clinically indicated patients and the biopsy fragments were submitted to conventional histopathological procedures. The sample was taken and placed in 10% formalin, embedded in paraffin, cut into 4μm sections and stained with hematoxylin and eosin stain, observed under microscope, histological diagnosis was made, and results were tabulated.

Interpretation of histopathological results: The samples were considered adequate for analysis when at least eight portal areas were seen. The criteria used for the chronic hepatitis classification included staging of fibrosis and grading of inflammatory activity. The stage of fibrosis was evaluated as the following:
F0 = no fibrosis.
F1 = portal fibrosis without septa.
F2 = few septa.
F3 = numerous septa delineating nodules without cirrhosis.
F4 = cirrhosis.

The grading of activity was performed by taking into account the inflammatory activities in the portal tract, and in the periportal and lobular regions:

A0 = no histological activity.
A1 = minimal lesion.
A2 = mild activity.
A3 = moderate activity.
A4 = severe activity.

RESULTS

In this prospective study 344 HCV-PCR positive patients with different genotypes were evaluated. The demographic characteristics are given in Table-1, Grading in Table-2 and staging in Table-3, while Table-4 & 5 shows their correlation with genotypes.

Table-1: Characteristics of the study population

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Prospective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type: Chronic Hepatitis C patients</td>
<td>344</td>
</tr>
<tr>
<td>With +ve HCV-PCR and genotype</td>
<td></td>
</tr>
<tr>
<td>Mean Age</td>
<td>35.14 years</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>239 (69.47%)</td>
</tr>
<tr>
<td>Women</td>
<td>105 (30.52%)</td>
</tr>
<tr>
<td>Age at Infection</td>
<td></td>
</tr>
<tr>
<td>≤ 20 years</td>
<td>42 (12.20%)</td>
</tr>
<tr>
<td>21-40 years</td>
<td>221 (64.25%)</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>81 (23.55%)</td>
</tr>
<tr>
<td>Duration of infection in year</td>
<td></td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>140 (40.69%)</td>
</tr>
<tr>
<td>3 - 5 years</td>
<td>196 (56.9%)</td>
</tr>
<tr>
<td>&gt; 5 years</td>
<td>08 (2.32%)</td>
</tr>
</tbody>
</table>

DISCUSSION

Knowledge of HCV genotypes is not required for diagnosis, but it is now useful parameter in the assessment of patients before consideration of treatment\(^\text{18}\). There may be differences in disease severity associated with different genotypes, but this has been difficult to prove. It is important to point out that severe and progressive liver disease has been found in association with each of the well-characterized genotypes, so that there is little evidence that any variants of HCV are completely nonpathogenic\(^\text{18}\).

The genetic diversity of HCV may be responsible for high prevalence of chronic infection, and the tendency toward rapid mutations allowing HCV to constantly escape immune recognition\(^\text{19,20}\).

In current study when we correlate the various genotypes with histological grading and staging, no any case of any genotype was observed in grade A0 and most of the cases fall in advanced grade, while least (4.06%) cases were observed in stage F0 and most of the cases in stage F2 (35.17%) and F1 (29.65%).

We detect 242 cases of genotype 3a which is more prevalent in Pakistan\(^\text{21-23}\), and out of these cases majority were presented with necro-inflammatory grades A2, A3 and stages F2, F1, this find-

<table>
<thead>
<tr>
<th>Inflammatory Activity(Grading)</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (A0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minimal (A1)</td>
<td>38</td>
<td>11.04</td>
</tr>
<tr>
<td>Mild (A2)</td>
<td>151</td>
<td>43.89</td>
</tr>
<tr>
<td>Modrate (A3)</td>
<td>117</td>
<td>34.01</td>
</tr>
<tr>
<td>Severe (A4)</td>
<td>38</td>
<td>11.04</td>
</tr>
<tr>
<td>Total</td>
<td>344</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage of Fibrosis</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Fibrosis (F0)</td>
<td>14</td>
<td>4.06</td>
</tr>
<tr>
<td>Portal Fibrosis (F1)</td>
<td>102</td>
<td>29.65</td>
</tr>
<tr>
<td>Few Septa (F2)</td>
<td>121</td>
<td>35.17</td>
</tr>
<tr>
<td>Many Septa (F3)</td>
<td>77</td>
<td>22.38</td>
</tr>
<tr>
<td>Cirrhosis (F4)</td>
<td>30</td>
<td>8.72</td>
</tr>
<tr>
<td>Total</td>
<td>344</td>
<td>100</td>
</tr>
</tbody>
</table>
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ing is in consistent with other studies\textsuperscript{11-13} who show poor grade in genotype 3 but an advanced stage in genotype 1, we found no any case in grade A0 and 22 cases in grade A4 but these studies show no any case in grade A4, some cases in grade A0, and majority of cases in stages F0 and F1.

We found 10 cases of genotype 1a and 05 cases of genotype 1b, almost of these cases were in grade A3, A4, no any case in grade A0 and stage F3, this finding is again differs from the other studies, which observed majority of cases of genotype 1 in a low grade and a low stage\textsuperscript{11-13}.

The above mentioned controversies in the observation are due to early detection and treatment facilities in developed countries as these studies were conducted in United States and Italy, in comparison to Pakistan and especially in rural Sindh where this study was conducted. The

\begin{table}[h]
\centering
\caption{HCV Genotype and Grading of Chronic hepatitis C patients.}
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Genotype & Histological Necro-inflammatory (Grading) & Total \\
 & A0 & A1 & A2 & A3 & A4 \\
\hline
1a & 0 & 0 & 1 & 6 & 3 & 10 \\
1b & 0 & 0 & 0 & 3 & 2 & 5 \\
2 & 0 & 0 & 2 & 2 & 0 & 4 \\
3a & 0 & 36 & 122 & 62 & 22 & 242 \\
3b & 0 & 2 & 9 & 7 & 1 & 19 \\
5 & 0 & 0 & 3 & 0 & 0 & 3 \\
Mixed & 0 & 0 & 1 & 4 & 4 & 9 \\
Untypable & 0 & 0 & 13 & 33 & 6 & 52 \\
\hline
Total & 0 & 38 & 151 & 117 & 38 & 344 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{HCV Genotype and Staging of Chronic Hepatitis C patients.}
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Genotype & Histological fibrosis (Staging) & Total \\
 & F0 & F1 & F2 & F3 & F4 \\
\hline
1a & 0 & 1 & 1 & 6 & 2 & 10 \\
1b & 0 & 0 & 0 & 5 & 0 & 5 \\
2 & 0 & 2 & 2 & 0 & 0 & 4 \\
3a & 14 & 91 & 81 & 43 & 13 & 242 \\
3b & 0 & 3 & 7 & 6 & 3 & 19 \\
5 & 0 & 0 & 3 & 0 & 0 & 3 \\
Mixed & 0 & 1 & 3 & 1 & 4 & 9 \\
Untypable & 0 & 4 & 24 & 16 & 8 & 52 \\
\hline
Total & 14 & 102 & 121 & 77 & 30 & 344 \\
\hline
\end{tabular}
\end{table}
peoples of rural Sindh which are mostly residing in villages have little knowledge about the disease and take treatment from quakes, they seek for medical assessment from specialist doctor when disease advances, and at time of diagnosis shows a higher grade and stage.

CONCLUSION

There is no correlation of genotype with grading and staging of the disease in chronic hepatitis C.

REFERENCES


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