HEPATITIS C VIRUS GENOTYPES IN BAHAWALPUR

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This study was conducted at Medical Unit-II Bahawal Victoria Hospital / Quaid-e-Azam Medical College Bahawalpur from May 1st, 2005 to December 31st 2005. The objective of this study was to determine hepatitis C virus (HCV) genotypes in Bahawalpur, Pakistan. In consecutive 105 anti-HCV (ELISA-3) positive patients, complete history and physical examination was performed. Liver function tests, complete blood counts and platelet count, blood sugar fasting and 2 hours after breakfast, prothrombin time, serum albumin, serum globulin and abdominal ultrasound were carried out in all the patients. Trucut biopsy was performed on 17 patients. We studied HCV RNA in all these patients by Nested PCR method. HCV RNA was detected in 98 patients and genotyping assay was done by genotype specific PCR. Among total of 105 anti-HCV positive patients, HCV-RNA was detected in 98 patients. Out of these 98 patients there were 57 (58.2%) males and 41 (42.8%) females. Their age range was 18-75 years. The age 18-29 years 26 (26.5%), 30-39 years 35 (35.7%) and 40-75 37 (37.8%), while 10 (10.2%) patients were diabetics and 34 (34.7%) patients were obese. Liver cirrhosis was present in 10 (10.2%) patients. Forty two (43.9%) patients were symptomatic while 56 (57.1%) were asymptomatic. Out of 98 patients 11 (11.2%) were un type-able and 87 (88.8%) were type-able. 70/98 (71.4%) were genotype 3; 10/98 (10.2%) were genotype 1; 03/98 (3.1%) were genotype 2; 03/98 (3.1%) were mixed genotype 2 and 3; 01/98 (1%) were mixed genotype 3a and 3b. Genotype 3 is the most common HCV virus in our area which shows that both virological and biochemical response will be better. Because HCV genotype 3 is more frequent among the drug users which points towards unsafe injection practices in our area.

INTRODUCTION
Hepatitis C infection has been recognized as a major public health problem all over the world. Worldwide prevalence is 3%, while the prevalence in Pakistan is 4-7%. Hepatitis C infection is asymptomatic not only in chronic but also in acute stage. It leads to chronic liver disease in 60% and liver cirrhosis in 10-20 years. Hepatocellular carcinoma is an important complication of liver cirrhosis developing at the rate of 1-4% per year. Hepatitis C infection has been recognized as a major public health problem all over the world. It is asymptomatic both in chronic and acute stage. Worldwide prevalence is 3%, while the prevalence in Pakistan is 4-7%. Hepatitis C virus is an important cause of chronic liver disease and cirrhosis in Pakistan. Hepatitis C virus (HCV) is the major causative agent of non-A, non-B post-transfusional hepatitis, possessing a positive-stranded RNA genome. High variability of HCV genome appears not only in its highly variable region (HVR) of E2 protein, but also in the most highly conserved’ NCR. This has led to a classification of the virus into a series of genotypes that show distinct geographical distribution in various parts of the world. On the basis of phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of hepatitis C virus (HCV) have been identified. Up till now, 6 major genotypes and 80 subtypes of hepatitis C have been identified. However, little is currently known about which HCV variants are present in Pakistan, India, Bangladesh and Burma. We have analyzed patients from Bahawalpur for HCV genotyping by using type-specific PCR.

PLACE AND DURATION OF STUDY
The study was conducted at Medical Unit-II, Bahawal Victoria Hospital / Quaid-e-Azam Medical College, Bahawalpur from May 1st, 2005 to December 31st 2005.

OBJECTIVES
To study frequency of hepatitis C virus (HCV) genotypes in Bahawalpur, Pakistan.

MATERIALS AND METHODS
In 105 anti-HCV (ELISA-3) positive patients, complete history and physical examination was carried out. Liver function test, blood complete...
plus platelet count, blood sugar fasting and 2HPP, prothrombin time, serum albumin, serum globulin and abdominal ultrasound were carried out in all the patients. Liver biopsy was performed on 17 patients. We studied HCV RNA in all these patients by Nested PCR method which is intended to reduce the contaminations in products due to the amplification of unexpected primer binding sites. Nested PCR is based on five major processes: extraction of HCV RNA from serum sample, reverse transcription of target RNA to generate cDNA, two rounds of PCR amplification and detection (The sensitivity and specificity of this HCV RNA PCR assay is 97% and 99% respectively). And genotyping assay was done by genotype specific PCR in those cases where HCV RNA was detected. HCV RNA was isolated from 150µl serum of the patient and was reverse transcribed into cDNA with Molony-murine leukemia virus reverse transcriptase enzyme. The cDNA was subjected to two rounds of PCR amplification. The 1st round utilized the outer primers specific for the core region. The 2nd round was performed with one universal inner-sense and 11 genotype-specific anti-sense primers. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide and evaluated on UV transilluminator. The HCV genotype for the sample was determined by identifying the genotype-specific cDNA band.

RESULTS
Among a total of 105 anti-HCV positive patients, HCV-RNA PCR was detected in 98 (93.33%). Out of these 98 patients, 57 (58.2%) were male and 41 (42.8%) were females. Ages range was 18-75 years. Age between 18-29 years 26 (26.5%) patients, 30-39 years 35 (35.7%) patients and 40-75 years 37 (37.8%) patients, while 10 (10.2%) patients were diabetics and 34 (34.7%) patients were obese. Acute hepatitis was in 04 (4.0%) patients, while chronic hepatitis was present in 84 (85.7%) patients and 10 (10.2%) were having liver cirrhosis. Forty two (43.9%) patients were symptomatic while 56 (57.1%) were asymptomatic. Out of 98 patients 87 (88.8%) were typeable and 11 (11.2%) were untypeable .Among typeable patients 70 (71.4%) were genotype 3; 10 (10.2%) were genotype 1; 03 (3.1%) were genotype 2; 03 (3.1%) were mixed genotype 2 and 3; and 01 (1%) were mixed genotype 3a and 3b.

Table 1:

<table>
<thead>
<tr>
<th>AGE</th>
<th>Percentage</th>
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<tr>
<td>18-29 Yrs</td>
<td>26/98 (26.5%)</td>
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<tr>
<td>30-39 Yrs</td>
<td>35/98 (35.7%)</td>
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<tr>
<td>40-75 Yrs</td>
<td>37/98 (37.8%)</td>
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Table 2:

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<th>GNOTYPE AND SEX</th>
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<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>III a</td>
</tr>
<tr>
<td>III b</td>
</tr>
<tr>
<td>I a</td>
</tr>
<tr>
<td>Un-typified</td>
</tr>
<tr>
<td>Mixed</td>
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<td>II a</td>
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<tr>
<td>Total</td>
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DISCUSSION
HCV infection is causing significant morbidity and mortality worldwide. In an infected individual the HCV genome population circulates as a quasi-species distribution of closely related yet heterogeneous RNA sequences. HCV evades host immune surveillance and establishes and maintains persistent infection because of these quasi-species\[10,11\]. Genetic heterogeneity of HCV is great, and this may have important implications in diagnosis, pathogenesis, treatment, and vaccine development. The determination of HCV genotypes, subtypes and isolates has been helpful in understanding the evolution and the epidemiology of the virus, and is an important factor in the pre-treatment evaluation\[12,13\]. Genotyping can be performed by different methods; direct sequence analysis, reverse hybridization, the use of restriction fragment length, the Trugene HCV 5'NC Genotyping Kit, the line-probe assay.\[1\] There are six major HCV genotypes\[14-16\]. Genotype 1 is most common (60 to 70 percent of isolates) in the United States and Europe\[17\]; genotypes 2 and 3 are less common in these areas, whereas genotypes 4, 5, and 6 are rare: Genotype 5 is most common in the Far East and Australia\[18-21\]. Genotype 4 is most
common in Africa and the Middle East. Genotype 5 is most common in South Africa and Genotype 6 is most common in Hong Kong22-24. Response to therapy with INF and Ribavirin depends not only upon host factors but also viral factors and among the viral factors HCV genotype is most consistently identified predictor. The sustained virologic response to pegylated interferon plus ribavirin ranges from about 40 to 50 percent with genotype 1 (including 1a and 1b) to as high as 70 to 80 percent with genotypes 2 and 325,26. More severe progression of chronic hepatitis C is seen in patients showing genotype 1b compared with those with genotype 2 and 3. Patients who have a high body mass index and hepatic steatosis are at increased risk for the development of fibrosis; the risk of fibrosis in those with steatosis seems to be increased significantly with even moderate alcohol intake29-30. Hepatitis C virus genotype 1 and 3 are associated with lower response31.

The majority of the patients in our study (70/98) are having genotype 3 while genotype 1 is the second highest genotype. We did not detect genotype 4, 5 and 6. Majority of the patients were young below the age of 40. Genotype 1 was more common in males as compared to females. Diabetes was present in 10/98 cases. Hepatitis C virus infection is said to be associated with diabetes mellitus33-37. Ten patients were having liver cirrhosis. Hepatitis C Virus genotype 3 seems to be high in Bangladesh, Pakistan, India and Thailand38,39. HCV genotype 3 patients are easy to treat as compared to genotype 1 and 4. IRES (Internal Ribosome Entry Site) efficiency in vitro correlates with the treatment response with HCV genotype 340. There is a strong evidence that HCV genotype 3 is a modulator of hepatic steatosis which appears to be a direct action on virus on hepatic lipid homeostasis42. Nonalcoholic fatty liver disease is now considered part of the metabolic syndrome, and, with alcohol and hepatitis C, is the most common cause of chronic liver disease in the United States43-46. Nonalcoholic fatty liver disease can also be caused by the HCV infection42. HCV genotype 3 is more common among the IV drug users and in our country it may be due to unsafe injection practices. High prevalence of HCV genotype 3 in Pakistan is a good hope for cure as well as control of Hepatitis C infection. HCV genotype should be determined in all HCV-infected persons prior to treatment in order to determine the duration of therapy and likelihood of response1.

In conclusion it is our observation that genotype 3 is the most common HCV virus in our area which predicts not only short duration therapy but also better biochemical as well as virological response. HCV genotype 3 is more frequent among the drug users in Europe and America but in our country it may be due to unsafe injection practices.

REFERENCES
2. 38th EASL, The European Association for the Study of Liver, July 2003.
intake on liver fibrosis progression in chronic hepatitis C. Am J Gastroenterol 2002; 97: 1807.


