

FREQUENCY OF HCV INFECTION AND ITS GENOTYPES AMONG PATIENTS ATTENDING A LIVER CLINIC AND VOLUNTARY BLOOD DONORS IN A RURAL AREA OF PAKISTAN

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ABSTRACT

Objectives: To determine the frequency of Hepatitis C virus (HCV) infection and its genotypic distribution in a rural area of Sindh, Pakistan.

Methodology: Retrospective study of patients attending the Free Liver Clinic (FLC), and investigated for detectable HCV antibodies ($n=1638$), and those screened for HCV infection prior to voluntary blood donation ($n=804$) at a teaching hospital, located in rural Sindh. All patients had HCV antibodies tested by ELISA. A total of 1022 patients, who tested 'reactive' to HCV antibodies, and who could financially afford to have HCV RNA tested by PCR, had their results analysed. A total of 200 patients also had their HCV genotyped and analysed.

Results: Patients at FLC had a higher chance of being reactive for HCV antibodies, compared to voluntary blood donors (20% VS 14% - $p = 0.004$). HCV RNA was detectable in 904/1022 (88%) patients. Among typeable genotypes, 125/166 (75%) had a single genotype, and 7 patients (4%) were infected with genotype 1, either alone ($n=4$) or in combination with 3a.

Conclusions: One out of every five people tested in our FLC, and 14% of "healthy" voluntary blood donors were seropositive for HCV antibodies. Genotype 1 is very rare in our region.

KEYWORDS: Hepatitis C, Seroprevalence, Genotype, Blood donors.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major health problem. World Health Organization estimates that globally 170 million persons are chronically infected with HCV, and 3 to 4 million persons are newly infected each year.¹ In the USA, it accounts for about 15 percent of

acute viral hepatitis, 60 to 70 percent of chronic hepatitis, and up to 50 percent of cirrhosis, end-stage liver disease, and liver cancer.² In our centre, it is responsible for 69% of all medical ICU admissions, and also for 69% of all deaths.³ After initial exposure, 60% to 85% patients develop chronic infection.⁴ There are 6 main genotypes of HCV. Although they do not determine the outcome of infection, they may affect the treatment outcome and duration of therapy.⁵ Generally speaking, patients with genotype two and three should be treated for 24 weeks, whereas most patients with genotype one need treatment for 48 weeks as currently accepted standard duration. Improved

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responses are found in patients infected with genotype two and three as compared to patients infected with genotype one. Major guidelines in the world recommend that genotype should be determined in all HCV infected patients prior to treatment.^{5,6}

Accurate epidemiological information regarding HCV in Pakistan is still scanty. Based on data from studies largely from urban centres, Pakistan Society of Gastroenterology & GI Endoscopy (PSG&GIE) estimates 6% of community as a whole, and 3-4% of voluntary blood donors may be infected with HCV. It also suggests "as large majority of our patients are infected with HCV genotype three, testing for HCV genotypes may not be necessary as a routine prior to initiation of therapy in our patients".⁷ However, a recent large study involving 3351 patients from many cities/towns of Pakistan, showed that genotype one was found in over 22% of all HCV infected patients. Moreover, there was quite a large variation in genotype one prevalence among different parts of the country.⁸ Anecdotally, the seroprevalence of HCV in our local rural population may be much higher than estimated by PSG & GIE's, and predominantly of genotype three.

It is therefore important to find out the burden of the disease in a population, and also to find out the viral characteristics, especially genotype. This clearly will have clinical and also financial implications, as the eradication therapy for HCV is expensive, and out of reach for a majority of Pakistani population, particularly those residing in financially poor rural areas. We undertook this study to answer these questions in our population.

METHODOLOGY

Retrospective study of the data of patients attending the free liver clinic (FLC) of our teaching hospital, located at rural Sindh province of Pakistan was done. Data was collected from May 2005 for three years. In addition, data from voluntary blood donors for the same period was obtained. All serological tests were performed by ELISA at the hospital's laboratory. HCV RNA testing by Polymerase Chain Reaction (PCR), was arranged at another labora-

tory for 1022 patients. These patients were already known to be anti-HCV 'reactive'. HCV RNA was extracted from sample, amplified using Real-Time Amplification and detected using fluorescent reporter dye probes in the Smart Cycle ® (Cepheid). Sensitivity and specificity of the assay was 98% and 97% respectively.

Among those who turned out to have HCV RNA detected by PCR, 200 agreed to have their HCV genotyped. Chi-Square test was done to test significance and *p* value of less than 0.05 was considered significant. Odds Ratio (OR) with 95% Confidence Intervals (CI) was calculated for a significant *p* values. Approval was taken from the hospital's Research Ethics Committee to conduct this study.

RESULTS

A total of 1638 patients were investigated for the presence of HCV antibodies in FLC. Out of these, 324 (20%) tested 'reactive' (males = 210 [65%]; average age 34 years). In addition, 804 voluntary donors (males = 725[90%]) were screened for HCV antibodies, of which 121 (14%) tested 'reactive' (males = 110 [91%], average age 31 years). A chi-square test found a significant association between being 'reactive' for HCV and whether they attended the FLC or were blood donors (test statistic = 8.1, *p* = 0.004). The odds ratio was 1.39 with 95% CI: 1.11 - 1.75, i.e. those attending the FLC had increased odds of 1.39 of HCV being 'reactive' compared to the blood donors.

Out of a total of 1022 patients tested, 904 (88%) had HCV RNA detected by PCR (males = 510[56%] average age 36 years). Out of 200 patients investigated further, 166 were found to have a typeable HCV genotype. Of them, 125 /166 (75%; 95% CI = 68.7% - 81.9%. Estimated odds = 3.05) patients were infected with a single genotype. Of them, 115/125 patients (92%) had type 3a, 6/125 (5%) had type 3b, and 4/125 (3%) had type 1a. Of the 166 typeable genotypes, 41 patients (25%; 95% CI = 18.1% - 31.3%. Estimated odds = 0.328) were found to be infected with a mixed genotype (two genotypes in one patient). Of these, 35/41 (85%; 95% CI = 79.8% - 90.9%. Estimated

odds = 5.84) had infection with types 3a/3b. Three patients each were infected with type 3a/2a and 3a/1a.

One hundred fifty nine out of 166 (96%) were infected with only "non-1" genotypes - (approximate 95% CI = 92.7% - 98.8%), with estimated odds being 22.7. Only seven patients (4%) were infected with genotype one, either alone (n=4) or in combination with 3a (approximate 95% CI = 1.2% - 7.3%), with estimated odds of being infected with this genotype being 0.044.

DISCUSSION

This is perhaps the first study done in a rural area of Pakistan. It shows that many more people are infected in this region of Pakistan than what was previously suggested by reports from urban areas and was estimated by the PSG&GIE for Pakistan as a whole. A review of literature published recently, showed that data published over last 5 years indicated a variation of HCV prevalence in Pakistan among different individual cities, ranging between 2.2% (Karachi) to 13.5% (Lahore). In fact, different centres within the same city have reported different figures.⁹ Nearly 2/3 of Pakistani population (66%) resides in the rural areas.¹⁰ Anecdotally, diseases like hepatitis C infection are more prevalent in rural than urban areas. It is therefore likely that previously reported prevalence figures, mainly from urban areas, may not be representative of other areas, particularly rural areas.

There are potential explanations of higher frequency of HCV prevalence in rural areas. Lack of knowledge, poverty and poor awareness among the people are generally blamed to be responsible for the spread of HCV infection.⁹ These factors are significantly more prevalent in rural areas. Moreover, the mode of transmission of HCV in developing countries still appears to be unsterilized injection equipment and unscreened blood transfusions.^{1,8} Our unpublished data also showed the figures to be 69 and 10% respectively. Thus, the spread of HCV infection is likely to be higher among the rural population for the higher occurrence of above factors. Significantly

higher seroprevalence found in our study (done in rural setting) than most reports published previously (mainly from urban areas) is therefore not surprising.

We found that HCV was more prevalent in males (65%). This is in keeping with previous reports.^{8,9} Anecdotally, in our society, males are at higher risk for this infection because of the lifestyle involving exposure to barbers and thus to potentially contaminated razors. They also take part in sports and other activities which put them at higher risk of injuries and thus requiring healthcare facilities involving exposure to injections, blood transfusions and surgical procedures - adding to the risk of acquiring infection.

Patients presenting at FLC in our study, were found to have higher risk of being infected with HCV compared with people screened for voluntary blood donation, - 20% VS 14%. This was statistically quite significant - ($p = 0.004$; OR = 1.39 - 95% CI = 1.11 - 1.75). This could be because the patients presenting at FLC would be expected to have higher chances of being infected than the apparently healthy voluntary blood donors. It has been shown that healthy voluntary blood donors are less likely to be infected than those requiring healthcare services.¹¹ It is therefore possible that prevalence seen in FLC patients and voluntary blood donors may over-estimate and under-estimate the real situation respectively. So, the true prevalence of HCV infection in general population of our region may lie somewhere between 14% (voluntary blood donors) and 20% (patients attending FLC). National survey regarding prevalence of HBV & HCV conducted by Pakistan Medical Research Council presented in 2009 has showed the overall prevalence of HBV as 2.5% and HCV as 4.9%. This survey has also highlighted certain districts in all the four provinces which are at high risk where prevalence of HBV & HCV is much more.

A massive 96% of all type able genotypes in our study turned out to be genotype 3 (a or b), and only 4% had genotype one. This is in contrast to a previous study which showed prevalence of genotype one alone to be over 11.5% in Pakistan overall - alone or in com-

bination with another genotype. In the province of Sindh, 12.97% had genotype one alone, and a further 5.02% had this genotype mixed with another genotype. Genotype three alone contributed to at least 67.36% of all isolates.⁸ It would therefore appear that substantial regional differences do exist, not only among the countries, but also within Pakistan, and even within the same province.

The finding that the predominant genotype in our region is three has obvious clinical and financial implications. This genotype requires shorter duration of treatment as compared with genotype one, with its associated reduced cost and side effects. The fact that genotype one was only found in 4% of our patients, supports the recommendation by PSG&GIE that genotype testing may not be necessary in this part of the world prior to initiating treatment.⁷ However, this recommendation may not be suitable for other parts of Sindh, and indeed rest of Pakistan for that matter.

Another limitation of the study is that we were not able to separate different kinds of patients among the 'un-type-able' genotype group because of the retrospective nature of the study. A proportion of patients will have their genotype reported "un-type-able" (5.99% in one report).⁸ Most of our patients already were found to have detectable HCV RNA by PCR, and were tested further for genotype on samples taken at a later stage. Some patients had HCV antibodies 'reactive', and then jumped on to have genotype straightaway without first having the RNA checked, presumably to cut the "unnecessary" cost of doing two expensive tests. Hence we may have found more un-type able genotypes than would have done otherwise. We therefore only analysed the type able data.

In conclusion, 20% of all patients presenting at FLC and 14% of all voluntary blood donors tested to be seropositive for HCV antibodies in our region, which is a much higher figure than estimated for Pakistan as a whole by PSG&GIE. In our region, genotype three appears to be much more prevalent than that reported in other parts of the country.

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