

SEROPREVALENCE OF HBsAg AND ANTI-HCV IN GENERAL HEALTHY POPULATION OF SWAT DISTRICT WITH FREQUENCY OF DIFFERENT HCV GENOTYPES

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ABSTRACT

Objective: The main purpose of this study was to find out the seroprevalence of HBsAg and anti HCV in apparently healthy population of district Swat. The other objective was to find out the different types of genotype prevalent in this part of the country.

Methodology: This is a descriptive, observational study which is community based and was conducted by the Hepatology section of Medical Department, Saidu Teaching Hospital, Saidu Sharif from May 2007 to August 2008. Screening camps were held in all the four sectors to which the district was arbitrarily divided. Sera were checked by 3rd generation Elisa technique for HBsAg and anti HCV antibody. Positive cases for anti HCV were recalled in batches and the blood samples taken for HCV RNA testing by PCR and Genotyping.

Results: Data was available on a total of 4680 healthy men and women. There were 2870 male and 1810 female participants. We found 3871 (82.7%) subjects Negative. One hundred sixty four (3.5%) were positive for HBsAg and 645(13.8%) were positive for anti-HCV antibodies. Only 15 volunteers (0.3%) were infected with both HBV and HCV. Two hundred twenty cases, who were positive for HCV RNA BY PCR testing were checked for genotyping. One hundred nine (49.5%) cases had genotype 3a, 74(33.7%) cases were having genotype 3b, 19 cases (8.7%) had mixed, 10 cases (4.5%) had 1b and eight cases(3.6%) were untypeable.

Conclusion: In district Swat of NWFP, prevalence of hepatitis B surface antigenemia is 3.5% and anti Hepatitis C antibody is 13.8% with a combined prevalence of 17.3%. Genotype three is the commonest genotype (83%) prevalent in this area and six months combination antiviral therapy can be undertaken without checking for genotype in routine.

KEY WORDS: HBsAg, anti-HCV, Seroprevalence, Genotypes.

Pak J Med Sci October - December 2009 (Part-I) Vol. 25 No. 5 744-748

How to cite this article:

Ahmad A, Ahmad B, Ali A, Ahmad Y. Seroprevalence of HBsAg and anti-HCV in general healthy population of Swat district with frequency of different HCV Genotypes. Pak J Med Sci 2009;25(5):744-748.

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- * Received for Publication: January 29, 2009
- * Accepted: July 28, 2009

INTRODUCTION

Whereas, chronic Hepatitis B and C infections are now recognized as important cause of chronic ill health, non-specific general symptoms, overall poor quality of life and psychosomatic symptoms have emerged as the most common cause of chronic liver disease and cirrhosis in this part of the world. It seems that we are now living in a society where every ninth or tenth person is a carrier of these deadly viruses.¹

The exact prevalence of chronic hepatitis B and C infection is unknown in Pakistan. Various studies done in different parts of the country in different sub groups of patients have presented different figures. These studies have shown a prevalence rate 4.8-25% for HCV and 1.5-10% for Hepatitis B infections.²⁻⁸ It is also now well documented that these viral infections are the commonest causes of chronic liver disease, cirrhosis and Hepatocellular carcinoma in this part of the world. The analysis of 30 published studies in different Pakistani journals from different regions of Pakistan related to chronic liver disease including chronic active Hepatitis and cirrhosis of from 1992-2004 shows a cumulative prevalence of 40%. Some of the studies have shown as high figures as 79%,⁹ 68%,¹⁰ and upto 86%.¹¹ It has been demonstrated in 11 studies from different regions of Pakistan that there is cumulative prevalence of 50-80% of HCV and 20-30% of HBV in patients with hepatocellular carcinoma (HCC).¹¹

Hepatitis C virus belongs to Flaviviridae family and is a single stranded RNA virus. It has got at least six different genotypes labeled as one to six and numerous sub types.¹² The ability of HCV to undergo high rates of mutation allows it to escape the effects of immune system and to resist the impact of antiviral therapy.¹³ Identification of a particular HCV genotype does not predict the natural history of the disease but does have important ramifications for the likelihood of the response, intensity and duration of therapy.¹⁴ Genotyping enables Physician to determine length and type of treatment, identify a mixed infection and will identify if a strain has mutated during course of treatment. Patients with genotype two and three generally respond better to treatment and do not need as long a course of chemotherapy as compared to genotype one.¹⁵ These genotypes have different geographic distributions. In addition to the epidemiological importance, these genotypes may influence the disease pattern and response to treatment, and so it is important to identify the virus genotype. Multiple studies have confirmed that the genotype three is the predominant genotype in Pakistan, with

prevalence of 70-90%.¹⁶⁻¹⁸ Among this genotype, subtype 3a is the commonest followed by 3b. Patients with genotypes two and three generally respond better to treatment and do not need as long a course of therapy. Patients with HCV genotype one have lower rates of response and require a longer duration of therapy.¹⁹

The main purpose of this study was to investigate the prevalence of HBsAg and anti HCV in apparently healthy population of district Swat which is situated in remote northern part of NWFP. The other objective was to find out the different types of genotype prevalent in this part of the country.

METHODOLOGY

Ethical issues: Informed signed consent was collected from all volunteers who participated in the study, after the purpose, nature and risks of the participation were fully explained to them verbally and in writing. The individual laboratory results were kept confidential and given to the participants at the completion of the project. Also, the positive cases were given / provided with information on limiting the spread of these infections and referred to central registry for further work up and treatment. *Subjects:* A total of 5415 healthy men and women of different age group were screened.

Inclusion Criteria: Age 16-60 years. Apparently healthy men and women not suffering from any acute or chronic illness. Clinical history of volunteers was noted, especially jaundice, blood transfusion, exposure to syringes, surgical and dental procedures.

Exclusion Criteria: Apparently unhealthy or malnourished individuals were excluded.

Study Plan and Design: The study was carried in May 2007 and completed by August 2008. To obtain enough volunteers, four screening camps were organized at different locations of Swat trying to incorporate all the four sectors to which Lower Swat was arbitrarily divided to get maximum representation. This included Aman Kot, Kabal, Matta and Madyan. Announcements were made in the local mosque and information leaflets were distributed amongst the local government councilors. Camps were held by

Table-I: Prevalence of HbsAg, and ANTI-HBs and anti-HCV antibodies in 4680 volunteers. Mean age was 35years $\pm$ 10.

<i>HbsAg-positive</i>	<i>Anti-HCV antibodies positive</i>	<i>Negative</i>	<i>Mixed infection: HBV & HCV</i>
164 (3.5%)	645(13.8%)	3871 (82.7%)	15 (0.3%)

two teams at different interval and target of fifteen hundred samples collection given to each team. Every camp followed an awareness lecture on "Hepatitis infection, their consequences, and preventive measures", to create awareness about the importance of regular health check-ups and hepatitis screening. After submitting their signed consents, volunteers were subjected to health checkups by a medical doctor and blood samples collected.

Methods: From every volunteer 10 ml of blood was taken. Serum was separated by Centrifugation and stored at -20°C . Laboratory testing of these samples for HBsAg and HCV antibodies were done by using 3rd generation ELISA (IMX, Abbott, USA), at the Pathology Department of Saidu Medical college. All those cases who were found positive, were then called to the central registry at Saidu Teaching Hospital, Saidu Sharif in different batches.

Blood samples were taken and serum separated, and stored at -15 degree Celsius. These were sent to Centre for applied molecular Biology lab at Lahore at regular programmed interval as 20 cases per week to get their genotyping and quantitative PCR testing as per prior arrangement. HCV RNA Quantification was performed using state of the art Smart Cycler II (Cepheid, FDA approved) Real-time amplification and detection system using fluorescent reporter dye probes specific for HCV (cy3) or HCV (FAM). Dynamic range of the assay is 125

to 12,500,00000.00 IU per ml sample. HCV genotyping was performed using nested type-specific primers and probes specifically designed for Pakistani HCV isolates. Sensitivity of genotyping assay is 500 IU per ml.

RESULTS

Some samples were lost and other were not suitable for lab testing. Data was available on a total of 4680 healthy men and women. There were 2870 male and 1810 female participants. We found 3871 (82.7%) subjects Negative. One hundred sixty four (3.5%) were positive for HBsAg, and 645(13.8%) were positive for anti-HCV antibodies. Only 15 volunteers (0.3%) were infected with both HBV and HCV (Table-I). Characteristics of these positive cases are shown in Table-II. As per protocol samples of anti HCV positive by ELISA were sent for PCR testing and Genotyping. Results of all these investigations are shown in Table-III.

DISCUSSION

There have been numerous studies from all over Pakistan on prevalence of chronic Hepatitis B and C infection. Over 350 reports, papers and presentations estimate the combined prevalence of hepatitis B and C in various parts of Pakistan at 8-10%. These studies have looked into specific at risk groups such as blood recipients, paid blood donors, patients suffering from liver disorders, haemodialysis patients, health care workers and voluntary blood donors.²⁰⁻²³ For example, the prevalence of HBsAg has been variously reported as 9.97%, 10%, 3.1%, 0.99%, 1.11%, 4%, 3%, 3.2%, 3%, 4.3% and 6.5%, respectively, in different groups of Individuals. On the other hand, seroprevalence of HCV antibodies has been variously reported as 4%, 16.3%, 4.8%, 2.2%, 3.3%, 16% and 11.3%, respectively.^{2-8, 22,24} A large scale study from Islamabad has reported

Table-II: Biochemical & Serological profiles of HBsAg and anti HCV positive cases. Values are given as mean $\pm$ SD

	<i>HbsAg Positive (n=164)</i>	<i>Anti HCV Positive (n=645)</i>
Sex (F/M)	64/100	252/393
Age (yrs.)	28.5 $\pm$ 3.2	36.5 $\pm$ 5.5
Total serum bilirubin (mg/dl)	0.91 $\pm$ 0.8	0.88 $\pm$ 0.6
ALT (U/L)	50 $\pm$ 3.5	59 $\pm$ 5

Table-III: Genotypic Distribution of HCV. Total number tested: 220

<i>Genotype</i>	<i>3a</i>	<i>3b</i>	<i>3a & 3b or 1 mixed infection</i>	<i>1b</i>	<i>Untypeable</i>
Number	109(49.5%)	74(33.7%)	19(8.7%)	10(4.5 %)	8(3.6%)

on young male healthy population seeking employment abroad.²⁵ Another study by Noor Mohammad et al was general population based undertaken at District Buner, showing prevalence of anti HCV at 4.5%.²⁶ We have previously published a study on more than 40,000 voluntary blood donors from Swat showing HBsAg positive in 1.1% and anti HCV in 2.3% of this highly selective healthy male population.² The strength of our present study is the fact that it is community based, involving both male and female healthy population and all the four sectors of the district Swat have been screened with a representative samples. Thus it gives us the overall true seroprevalence of HBsAg and anti HCV in general healthy population of this district.

The importance of genotyping of Hepatitis C cannot be over emphasized. All Current treatment regimen and duration of treatment are entirely based upon the particular genotype prevalent in an area. In Pakistan, studies from Islamabad, Lahore and Karachi have shown that genotype 3b, 3a and two are the commonest.^{16,18,19} Ours study is also in keeping with these figures showing genotype three to be present in almost 83% of cases. (3a to be commonest followed by 3b), in this part of country so as adding to our national epidemiological data. Pakistan society of Gastroenterology does not recommended to test for genotyping in routine except for research purposes or some selected patients.²⁷ The other benefit obtained from conducting this study and holding these camps was creating mass awareness in community regarding spread of hepatitis B and C infection and the availability of treatment for those found positive and arranging vaccination program for Hepatitis B prevention.

CONCLUSION

Chronic Hepatitis B and C infection are common in asymptomatic healthy population of

district Swat, NWFP like elsewhere in Pakistan. Prevalence of hepatitis B surface antigenemia is 3.5% and anti Hepatitis C antibody is 13.8% with a combined prevalence of 17.3%. These are possibly candidates for chronic liver disease and potential sources of spread of infection both horizontally and vertically. Genotype three is the commonest genotype (83%) prevalent in this area and six months combination antiviral therapy can be undertaken without checking for genotype in routine.

It is imperative to create mass awareness program on different aspects of these deadly infections in the community. Mass screening program should be undertaken to define the infected population properly so that they may be managed as per guidelines and further spread of the disease may be prevented.

ACKNOWLEDGMENTS

We are grateful to Prime Minister's program for prevention and treatment of Hepatitis, who established a sentinel site at Saidu Teaching Hospital and all the above work was undertaken with their logistic and financial support. PCR testing and Genotyping was partly done through Pakistan Baitul Mal support and partly through self help and some charity organization in Swat. We are grateful to Dr. Hayatullah, Senior Pathologist and all the technical staff of Pathology department, Saidu Teaching Hospital for their continued cooperation, support and help in sample collection, camping, and laboratory services throughout the study period. This work is dedicated to the Late Mr. Rehmat Ali, (died recently in bomb blast) Senior clerk in central registry of Hepatology who has tremendous contribution in establishing the registry and keeping proper record of all the patients.

Conflict of interest: None

REFERENCES

- Butt AA. Hepatitis C virus infection: the new global epidemic. *Future Drugs* 2005;3(2):241-9.
- Malik IA, Tariq W. The prevalence and pattern of viral hepatitis in Pakistan (editorial) *J Coll Physicians Surg Pak* 1995;5:2-3.
- Ahmad A. Frequency of HBV surface Antigen and Anti-HCV in Healthy Voluntary Blood donors in Swat district. *JPMI* 2006;20(2):187-90.
- Khattak MF, Salamat N, Bhatti FA, Qureshi TZ. Seroprevalence of Hepatitis B,C and HIV in blood donors in North Pakistan. *J Pak Med Assoc* 2002;52:398-402.
- Asif N, Khokhar N, Illahi F. Seroprevalence of HBV, HCV and HIV infection among voluntary non remunerated and replacement donors in northern Pakistan. *Pak J Med Sci* 2004;20:24-8.
- Najmul Hassain S, Ghulam Shabir. A review of published literature on hepatitis "B" and "C" virus prevalence in Pakistan. *J Coll Physicians Surg Pak* 2002;12(6) 368-71.
- Farooq MA, Iqbal MA, Rariq WZ, Hussain AB, Ghani I. Prevalence of hepatitis B and C in healthy Pakistani adults. *Pak J Pathol* 2005;16:42-46.
- Hakim ST, Kazmi SU, Bagasra O. Seroprevalence of Hepatitis B and C Genotypes Among Young Apparently Healthy Females of Karachi-Pakistan. *Libyan J Med, AOP*: 071123.
- Shah F, Salih M, Malik IA, Hussain I. Increasing prevalence of chronic hepatitis and associated risk factors. *Pak J Med Res* 2002;41(2):46-50.
- Khan AA, Khalil R, Haider Z, shafqat F. Seromarkers of Hepatitis B and C in patients with Cirrhosis. *J Coll Physicians Surg Pak* 2002;12(2):105-7.
- Umer M, Bushra HT, Shuaib. Spectrum of chronic liver disease due to Hepatitis C virus infection. *J Coll Physicians Surg Pak* 2000;10(10):380-3.
- Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of Hepatitis C virus: quasi species and genotypes. *Semin Liver dis* 1995;15:15-32.
- Sallie R. Replicative Homeostasis: A fundamental mechanism mediating selective viral replication and escape mutation. *Virology* 2005;2:10-23.
- Trepo C. Genotype and viral load as prognostic indicator in the treatment of hepatitis C. *J Viral Hepat* 2000;7:250-57.
- Idrees M. Detection of six serotype of HCV in anti-HCV positive patients and rate of ALT/AST normalities. *Pak J Microbiol* 2001;2:61-5.
- Khokhar N, Asif N, Khokhar OS. Serotype 3 is most common hepatitis serotype in Pakistan, however, significant numbers are untypeable. *Hepatology* 2003;38:270-1.
- Azhar MA, Bukhari MH, Ghanni U, Khan A, Malik JL, Shah AH. Prevalence of hepatitis C virus and its serotypes in Bhawalpur division. *Biomedica J* 2003;19:18-22.
- Mumtaz K, Hamid S, Moatter T, Abid S, Shah HA, Jaffri N, et al. Distribution of Hepatitis C virus Genotypes and response to treatment in Pakistani patients. *JPMI* 2005;19(1):S 61.
- Umer M, Bushra HT. Hepatitis C and Genotypes. In: *Hepatitis C in Pakistan*. 1st ed. Islamabad: SAF 2006;83-90.
- Shaikh MA, Shaikh MW, Salangi AG, Abro H. Frequency and transmission mode of Hepatitis C virus in Northern Sindh. *J Coll Physician Surg Pak* 2003;13:691.
- Gul A, Iqbal F. Prevalence of hepatitis C in patients on maintenance hemodialysis. *J Coll Physicians Surg Pak* 2003;13:15-17.
- Rehman K, Khan AA, Haider Z. Prevalence of seromarkers of HBV and HCV in health care personnel and apparently healthy blood donors. *J Pak Med Assoc*. 1996;46:152-4.
- Zakaria M, Ali S, Tariq GR, Nadeem M. Prevalence of anti hepatitis C antibodies and hepatitis B surface antigen in healthy male naval recruits. *Pak Armed Forces Med J* 2003;53:3-5.
- Moatter T, Hussain AS, Hamid S, Ahmad Z, Siddiqui S. Comparative analysis of viral titers and histologic features of Pakistani patients infected with hepatitis C virus type 3. *Int J Infect Dis* 2002;6(4):272-6.
- Khokhar N, Gill ML, Malik GJ. General Seroprevalence of Hepatitis B and C virus infection in population. *J Col Phy Surg Pak* 2004;14(9):534-36.
- Mohammad N, Jan A. Frequency of Hepatitis C in Buner, NWFP. *J Col Phy Surg Pak* 2005;15(1)11-4.
- Hamid S, Umer M, Alam A, Siddiqui A, Qureshi H, Butt I. PSG consensus statement on management of hepatitis C virus infection. (2003). *J Pak Med Assoc* 2004;54(3):146-50.