

## Seroprevalence of antibodies to hepatitis C virus among injecting drug users from Kathmandu

Shrestha IL<sup>1</sup>

<sup>1</sup>Siddhi Polyclinic, Pathology Lab, Kathmandu, Nepal

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### Abstract

**Objective:** To determine the seroprevalence of antibodies to hepatitis C virus among injecting drug users (IDU) from Kathmandu. **Design:** Retrospective cohort study from January 1997 to December 2002. **Setting:** Pathology Lab, Siddhi Polyclinic, Dillibazaar, Kathmandu. **Materials and methods:** Blood samples were collected from 400 injecting drug users and 400 healthy young adults. Serum samples were examined in duplicate along with negative and positive controls for the presence of antibodies to hepatitis C virus using third-generation enzyme-linked immunoassay (EIA 3.0) kit in fully automated, USA FDA approved, Bio-Rad EIA analyzer. Samples positive for anti HCV were re-examined for confirmation. **Results:** 342 out of 400 (85.5%) serum samples from IDU were found to be positive for anti HCV compared to 3 out of 400 (0.75%) serum samples from young adults without the history of IDU.

### Conclusions:

1. Injecting drug use is an important risk factor in the spread of hepatitis C virus.
2. Sharing of needles should be stopped.
3. Education and health counselling should be given to the people at risk to bring about behavioural change.

**Keywords:** antibodies to Hepatitis C virus, seroprevalence study, EIA third generation, Injecting drug users.

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Hepatitis C virus (HCV) is a new virus identified in the year 1989<sup>1,2</sup>. Screening assay for antibody to HCV became available late in 1990 and their use has subsequently become widespread. According to WHO estimations, about 3% of the world population may be infected with the hepatitis C virus. The relative prevalence of subtypes of this virus varies in different geographic areas. The main known routes of transmission are parenteral, intravenous drug abuse, contaminated injection devices and receipt of unscreened blood or blood products<sup>5</sup>. Intravenous drug use is by far the most important mode of transmission of HCV. It affects an estimated 170 million people worldwide<sup>11</sup>.

Most of the people infected by hepatitis C virus are asymptomatic at the beginning. Persons who develop acute HCV infection rarely recover completely, more than 80% of them remain HCV infected. The virus can stay in the body for many years, eventually leading to chronic hepatitis, cirrhosis of liver in 15-20%, hepatic failure and in 0.7-1.3% of the cases hepatocellular carcinoma (HCC) after 20-30 years<sup>12,13,14</sup>. There is no vaccine against this virus till

today. The genomes of hepatitis C virus display significant sequence heterogeneity. Six types (1 to 6) and many subtypes have been identified<sup>3</sup>. Presence of various genotypes have epidemiologic and therapeutic implications. Seroprevalence of anti HCV in general population of Nepal has been estimated to be from 0.1%-1.7%<sup>8,9</sup> and in IDU 94%<sup>10</sup> in the previous studies.

### Material and methods

Between the year January 1997 to December 2002 blood samples were collected from four hundred IDU to determine anti HCV. As the control group for the same period, blood samples from four hundred young adults who did not have a history of IDU or other risk factors were collected at Siddhi Polyclinic Pathology Laboratory for anti HCV assay.

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### Correspondence

Dr Iswar Lal Shrestha.  
Pathology Lab  
Siddhi Poly clinic. Dillibazaar, Kathmandu  
E mail: [ishrestha@healthnet.org.np](mailto:ishrestha@healthnet.org.np)

After informed consent, five ml of blood was collected aseptically from each participant and serum separated. Each sample were given code number and refrigerated at  $-20^{\circ}\text{C}$  till the time of assay. Third-generation Enzyme linked immunosorbent assay (ELISA) anti HCV screening kit (SD HCV ELISA 3.0) was used. The microplate were pre-coated with recombinant HCV antigen core, NS3, NS4 and NS5 on well. Optical density (OD) reading was measured in fully automated full plate ELISA reader using bichromatic filters. Cut off value (mean absorbance of the negative controls + 0.400) was determined and samples showing OD more than the cutoff values were considered as reactive to anti HCV. Each sample was tested in duplicate. Both negative and positive controls were run during the assay. Serum samples found positive for anti HCV was re-examined for confirmation. Sample that gave repeatedly reactive result was considered as positive for anti HCV. Serum alanine aminotransferase (ALT) was measured in all the samples.

## Results

Antibodies to hepatitis C virus were detected in 85.5%(342/400) samples of injection drug users. Whereas 0.75% (3/400) samples of serum from control group of young adults had anti HCV. The OD reading ranged from 0.8 to 2.5 for the samples that were positive compared to cutoff reading of OD 0.4. Serum ALT measured 80-200 U/l (normal 0-40 U/l) in samples positive for anti HCV and those samples which were negative for anti HCV had ALT less than 40 U/l. Majority (88%) of anti HCV positive cases were males. 73% of anti HCV positive cases were between 20-29 years of age. Statement of sharing of needle more than once, was given by all anti HCV positive person. There were no other risk factors.

Among the control group, no one gave history of drug abuse or sharing of needle or syringes.

**Table showing the results of ELISA test for anti HCV**

Total number screened for anti HCV	Anti HCV positive n (%)
Injecting Drug Users n=400	342 (85.5%)
Control group of young adults n=400	3 (0.75%)

**Table showing the results of serum ALT test**

	ALT (U/l)
Those who were Anti HCV positive	80-200
Those who were Anti HCV negative	10-40

## Discussion

Among the six different causes of viral hepatitis, hepatitis C virus is the most common cause among the injecting drug users. Currently the most common route of transmission of hepatitis C virus is intravenous drug use. Worldwide 60-90% of intravenous drug users are infected with HCV. In 1993, a retrospective survey in England and Wales between 1990 and 1993, revealed that the seroprevalence of antibody was highest 67%, (222/331) among injecting drug users and recipients of blood or blood products 34%, (189/548)<sup>6</sup>. In a study carried among a cohort of injecting drug users in Victoria, Australia, anti HCV was detected in 68%, (206/303)<sup>7</sup>. Since IV drug use is a highly efficient mode of spreading HCV infection many IV drug users become anti HCV positive within months of

beginning their drug use. Similarly, people who receive blood or blood products, not screened for anti HCV, are also the likely candidates for HCV infection. The risk of transmitting HCV through blood transfusion has been significantly reduced because all blood donors are now screened for anti HCV. Other modes of HCV transmission are, long term haemodialysis, health care workers who come in contact with blood, accidental needle stick pricks, contaminated equipment used in tattooing, piercing nose and ear, sharing personal items such as razors, toothbrushes with the HCV infected person. There is no vaccine against this virus till today. Various genotypes and subtypes involved in hepatitis C virus are the main hindrance in the development of vaccine.

The HCV genotype distribution varies among the different exposure categories, with HCV 1 being more frequent among blood donors, haemophilic and haemodialysis patients. A high frequency of HCV 3 was observed in cirrhotic patients and injecting drug users. In a study carried out in Brazil, HCV1 was the most prevalent (72.0%), followed by type 3 (25.3%), HCV 2 (2.0 %) and HCV 4 (0.7%)<sup>4</sup>.

The future impact of this virus is greatly dependent on the trends of intravenous drug use as well as the possible emergence of increased late morbidity among present asymptomatic carriers during the next few decades.

The present study employs basic screening assay for detecting anti HCV antibodies by ELISA that is currently available in Kathmandu. Detection of HCV-RNA using reverse transcription polymerase chain reaction (RT-PCR) and their genotypes by restriction fragment length polymorphism (RFLP) analysis and confirmation tests of nucleotide sequencing and phylogenetic analysis of the E1 gene or NS5B gene are not available at present in Nepal.

Those who were positive for anti HCV among the IDU group, each and every one had the history of using injection drug and sharing of needles in the past. Among the healthy young adults none gave a history of using injection drug. This study shows the seroprevalence of anti HCV among the healthy population to be 0.75% whereas the seroprevalence of anti HCV among IDU in Kathmandu is 85.5%. This underlines the role of IDU as an important risk factor in the spread of HCV. Effort must be directed to give health counselling to the people at risk to bring about behavioural change. Sharing of needles should be stopped as far as possible. IDU should not donate blood or body organs so that spread of hepatitis C virus may be prevented. Preventive measures should be taken as early as possible to reduce late complications like chronic hepatitis, cirrhosis, liver failure and hepatocellular carcinoma due to chronic hepatitis C virus infection in the future.

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**Correspondence**

Dr Iswar Lal Shrestha.

Pathology Lab

Siddhi Poly clinic. Dillibazaar, Kathmandu

E mail: [ilshrestha@healthnet.org.np](mailto:ilshrestha@healthnet.org.np)