Serological Surveillance of Anti HCV Antibody Among Nepalese Males

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Abstract

Introduction Hepatitis C virus is responsible for the vast majority of non-A, non-B post transfusion hepatitis and sporadic cases of non-A, non-B hepatitis. It causes acute and chronic hepatitis, chronic carrier state, liver cirrhosis and hepatocellular carcinoma.

Objective To determine the prevalence of HCV infection and carriers among healthy Nepalese male population within the age range of 16-50 years.

Methods 2585 blood samples from healthy Nepalese male population seeking job abroad were collected randomly during July - September 1999 and tested for the presence of anti HCV antibody using third generation ELISA kits.

Results Anti HCV antibody was detected in 0.35 percent (9) of the total subjects. Prevalence of anti HCV antibody was found varied in different development regions, the greatest prevalence was in mid western development region (0.69%) followed by eastern (0.39 %), western (0.35 %) and central (0.18 %) development region. However, the difference in prevalence was not significant statistically ($X^2 = 0.97$). The occurrence of anti HCV antibody was found almost equal in terai and hilly regions (0.38% and 0.37% respectively) whereas, no case was detected in mountainous region (0%), (and was not significant $X^2 = 0.617$). In 41-45 years age group, high (2.27 %) occurrence of anti HCV antibody was found, however, it could not be associated with a particular age group ($X^2 = 10.688$). Alanine aminotransferase (ALT) value was obtained elevated in 44.4 percent (4/9) of the anti HCV antibody positive subjects clearly showing the association of elevated ALT with HCV infection ($X^2 = 43.44$. $P < 0.001$). And only 2 out of 150 negative subjects had elevated ALT.

Conclusion The result suggests that the prevalence of HCV infection is low in healthy Nepalese males but people should be educated about the infection and its sequel.

Key words Alanine aminotransferase, Anti HCV antibody, Healthy, Nepal, Prevalence.

Introduction

First reported by Prince and co-workers in 1975[1] as non-A, non-B virus and identified by Choo and co-workers in 1989[2] as hepatitis C virus, is responsible for the vast majority of (at least 80 %) non-A, non-B post transfusion hepatitis cases and over 50 percent of sporadic cases of non-A, non-B hepatitis[3]. It causes acute and chronic hepatitis, chronic carrier state, liver cirrhosis and hepatocellular carcinoma (HCC). Vast majority of infected persons (up to 90%) do not show abnormal sign, even those with chronic disease. The outcome of HCV infection may be self-limiting infection to cirrhosis and hepatocellular carcinoma, but fulminant disease with HCV infection is rare[4].

Approximately 25 percent of the acute hepatitis C is icteric and the mortality during the infection is less than 1 percent[5]. Similar to chronic Hepatitis B and D virus infection, the chronic hepatitis C infection may lead to carrier state with no apparent liver disease or to mild or severe chronic hepatitis which may progress to cirrhosis or HCC[6, 7]. It has been reported that 50-70 percent of the acute hepatitis is manifested to chronic hepatitis[5, 8, 9] and 20-25 percent of chronic hepatitis infection leads to cirrhosis and HCC[8, 9]. Yet another report showed that 20 percent of the chronic hepatitis infection developed cirrhosis after 16 years of blood transfusion[10]. Association of HCV has been reported with 13.83 percent (78 of 564) and 9 percent

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of the chronic liver disease patients and acute viral hepatitis patients respectively in India[11].

The world's 1 percent population is symptom free carrier of HCV [4] and 100 million are chronic carriers[8]. In Europe and North America 0.5 percent to 2 percent of the population are infected with HCV, whereas in India 0.9 percent of the normal healthy population and less than 6.1 percent voluntary blood donors are positive for anti HCV antibody[12]. Yet another study by Thursz suggests that 240 million (1990) people worldwide are infected with virus[13]. It has emerged as second most common indicator of liver cirrhosis related with the chronic vial hepatitis.

Nepal is a small country divided administratively into five development regions: Eastern, Central, Western, Mid Western and Far Western Development Regions. Each region extends from north bordering China to south bordering India. The Eastern and Far Western Development Regions have India in its east and west respectively. Furthermore, topographically Nepal has three different regions; terai the plain belt (0-300 m above the sea level), hilly (600- 3000 m above the sea level) and mountainous region (3000 m above the sea level). Most Nepalese people are unaware of the virus, its transmission and the sequel. Since the majority of HCV infected persons do not show the abnormal signs even with chronic disease; fulminant disease with HCV infection is rare and association of male with fibrosis is high. Thus, this research study was aimed to find out the prevalence of anti HCV antibody among healthy Nepalese male population.

Methods

Blood samples from 2585 healthy Nepalese male population seeking jobs abroad, exhibiting no sign and symptom of liver inflammation within age range between 16-50 years were collected randomly during July to September 1999. Sera were separated and tested on the same day for the presence of anti HCV antibody by using third generation ELISA kit viz, LG HCV ELISA kit, (Korea) detecting the antibody against core, E1, E2, NS3, NS4, and NS5. Serum sample positive with this kit was retested for confirmation by using Genedia HCV ELISA kit, (Korea) that detects antibody against core, NS3, NS4, and NS5. To find out the validity, positivity, negativity and cutoff value of the test samples, absorbance of the wells was measured at 450 nm by using ELISA reader ELX 800G, America. For each test, standard methodology as recommended by the manufacturers of the kits was followed. Enzymatic SGPT kit of Ranbaxy laboratories, India, was used to determine the ALT enzyme in all anti HCV antibody positive subjects and 150 negative subjects. According to the kit any value above 49 IU/Lit was regarded as elevated value of ALT. The tests were performed by using autoanalyser Biotron 810, America.

The data of the study was analyzed by using chi square test. The age group 46-50 was not included in the statistical calculation due to small sample size, (n = 18). Consent from the subjects was taken for the test and privacy was assured.

Results

Of 2585 cases tested 0.35 percent (9) was found to be positive for anti HCV antibody. The prevalence was greatest 0.69 percent (1/144) in mid-western development region followed by eastern 0.39 percent (4/1036), western 0.35 percent (3/851) and central 0.18 percent (1/550). The prevalence of anti HCV antibody in different development region was not significant statistically ($X^2 = 0.977$). (Table No. 1)

Table 1: Prevalence of HCV infection in different development regions.

Development regions	No	positive cases	(%)
Eastern	1036	4	(0.39)
Central	550	1	(0.18)
Western	851	3	(0.35)
Mid Western	144	1	(0.69)
Far Western*	4	0	(—)
Total	2585	9	0.347 ~0.35

*Not included in statistical calculation due to small sample size.

Of nine cases positive for anti HCV antibody, 44 percent (4/9) were from terai region, that is 0.38 percent (4/1065) of the total terai population was anti HCV antibody positive. Similarly, 56 percent (5/9) were from hilly region indicating that 0.37 percent (5/1351) of the total hilly region population was anti HCV antibody positive. None of the positive cases were detected in mountainous region (Table No. 2).

Table 2: Occurrence of HCV infection in different regions of 4 development regions.

	Eastern			Central			Western			Mid western			Total	
Region	Tot	No.	%	Tot	No.	%	Tot	No.	%	Tot	No.	%	Total	%
Terai	542	2	0.37	348	0	0	136	1	0.73	39	1	2.56	1065	0.38
Hilly	390	2	0.51	185	1	0.54	671	2	0.30	105	0	0	1351	0.37
Mount	104	0	0	17	0	0	44	0	0	-	-	-	165	0
Total	1036	4	0.39	550	1	0.18	851	3	0.35	144	1	0.69	2581	0.35

However, no association was observed between different regions and anti HCV antibody positivity ($X^2 = 0.617$).

The occurrence of anti HCV antibody was seen highest 2.27 percent (2/88) in 41-45 age group followed by 31-35, 16-20, 21-25 and 26-30 (Table No. 4).

Table 4: Relation of anti HCV antibody positivity with age group

Age group	No. Tested	HCV positive (%)	
16-20	201	1	(0.5)
21-25	873	3	(0.34)
26-30	652	1	(0.15)
31-35	388	2	(0.52)
36-40	233	-	(-)
41-45	88	2	(2.27)
46 –50*	18	-	(-)

* Not included in statistical analysis due to small sample size.

This finding was not significant statistically showing no association of age group with HCV positivity ($X^2 = 10.688$). In age group 36-40 and 46–50 anti HCV antibody was not detected. The age group 46-50 was not included in the statistical calculation due to small sample size 18.

ALT enzyme was determined in samples positive for anti HCV antibody. Of 9 positive subjects, 44.4 percent (4) showed elevated values of ALT enzyme showing chronic infection or the liver damage in them (Table No.3).

Table 3: Association of elevated ALT with positivity of anti HCV antibody

	ALT Elevated		ALT Normal	
HCV	Number	%	Number	%
Positive	4	44.4	5	55.5
Negative	2	1.33	148	98.66

Whereas out of 150 subjects negative for anti HCV antibody, only 2 had elevated ALT indicating statistically significant association of HCV infection with elevated ALT ($X^2 = 43.44$, $P < 0.001$).

Discussion and conclusion

The prevalence of asymptomatic HCV infection in healthy Nepalese male population was quite low (0.35 %) compared to the stated 1percent infection in the world population[4] and also to that of India, the neighboring country with similar cultural, socioeconomic, educational values, where 0.9 percent of healthy male[12], 1.85 percent[11] and 2.5-4 percent[8] of the blood donors had been reported infected. The subject group 2585 of present study was 0.023 percent (2585/11,167,503) of the total Nepalese male population and 0.0115 percent (2585/22,367,048) of the total Nepalese population in the year 1999[14]. This finding of low prevalence of anti HCV antibody was similar to the prevalence reported by Shrestha et al., where 0.6 percent of the healthy Nepalese adults had been found positive to anti HCV antibody[15].

The seroprevalence of anti HCV antibody among healthy Nepalese male population in present study was obtained highest in mid-western development region and lowest in central development region. The prevalence in eastern development region (0.39%) in present study was consistent with the finding of Rai et al in the same region (0.33%)[16]. In a similar

study conducted by Manandhar and Shrestha to find out prevalence of HBsAg in healthy Nepalese male population in all five-development regions, 3.97 percent prevalence was reported[17].

The prevalence of anti HCV antibody was almost equal in terai and hilly region and no subject was positive in mountainous region, perhaps due to small sample size in the later. According to Nakashima et al, the HCV infection had been in increasing trend. In their ten years study in two Nepalese villages (Bhadrakali and Kotyang), in previous study anti HCV antibody positivity was 0.1 percent in 1987[18], which rose to 1.7 percent in 1996[19] clearly indicating that the anti HCV antibody positivity differ with time and population involved. It is an established fact that anti HCV antibody positivity increases with age. In studies performed among elderly population a higher prevalence can be anticipated. In present study of 106 subjects in the age group 41-50, 2 was positive making the prevalence 1.89 percent. In 41-45 age group alone HCV was prevalent in 2.27 percent (2/88) cases. However, the age group 46-50 was not included in the statistical calculation in present study due to small sample size.

Regarding ALT enzyme, the index of liver damage was found high in 44.4 percent (4/9) of anti HCV antibody positive subjects, indicating that they were chronically infected and 20-25 percent of them could have to live with the sequel of infections like cirrhosis and hepatocellular carcinoma in their life time. ALT enzyme test can indicate the stage of infection. It is raised only in chronic and acute infections and not in carrier stage. Therefore, the subject with elevated ALT could have been chronically infected but not acutely, since the ALT value was not as high (10-20 times) as in acute infections and none had any symptoms of infection. The positive cases with normal ALT can either be the carriers or the case of chronic inflammation or recovered HCV infected person. To differentiate these groups HCV RNA should be detected which is positive only in carriers and in chronic inflammation. But this was not in the objective of this study. In a similar study to find out the prevalence of HBsAg carriers in healthy males in Nepal, abnormal ALT value has been reported in 16 percent of HBsAg positive subject[17], which give the indication that there is great chance of liver damage in HCV infection than in HBV infection.

Une et al suggested that in males, excessive alcohol consumption and acquisition of HCV infection after 40 years of age were associated with more severe fibrosis[20] and the finding that among subjects of HCV infection male population seemed likely to develop chronic hepatitis than female was also supported by Tassopaulos et al[21] and Tomimatsu et al[22]. Furthermore, the chronicity in male could be due to the history of drinking and drinking habit[23]. There may be some other unknown factors responsible for the chronicity in males. This indicates that the damage imparted in liver by HCV infection especially in male population is dangerous and the population should be made aware of the mode of transmission and its consequence in health.

Since, the subjects in this research study were all healthy people who did not have history of hospitalisation or transfusion, the mode of transmission in them could be from mother to the child or by parenteral drug abuse or by some other unknown causes. A further study is required to find out the mode of transmission.

Prevalence for anti HCV antibody is low in Nepalese male population. However, almost half of the infected had elevated ALT showing the liver damage. Therefore, it is necessary that people should be educated and be made aware of the infection, mode of infection and its sequele.

References

1. Prince AM, Brotman B, Grady GF et al Long incubation post transfusion hepatitis without serological evidence of exposure to hepatitis B virus. *Lancet*.1974; 2: 241-6
2. Choo QL, Kuo G, Weiner AJ, Houghton M et al. Isolation of cDNA clone from a blood borne non A non B viral hepatitis genome. *Science*. 1989; 244:329-2
3. Kumar VK, Cotran RS, Robbins SL. Basic Pathology, Prism Books, India, 1992; 523-67.
4. Hallauer J, Kane M, McCloy F. eds. Viral Hepatitis. *Viral Hepatitis Prevention Board*. 1996; 5: 1-11
5. Esteban JI, Genesca J and Alter JH. Hepatitis C: Molecular Biology, Pathogenesis, Clinical features and prevention. *Prog Liver Dis*.1992; 10:253-82
6. Brillanti S, foli M, Gaiani C, et al. Persistent Hepatitis C viremia without liver disease. *Lancet*. 1993; 1: 464-5.
7. Bukh J, Miller RH, Kew MC, et al. Hepatitis C virus DNA in Southern African blacks with hepatocellular carcinoma. *Proc Natl Acad Sci USA*. 1993; 90: 1848-51

8. Kohli V, Diagnostic Tests for Hepatitis C Virus. In: *Pulse Diagnostics*, Sept. 2000, vol. 7.
9. Alter HJ, Hoofnagle JH. NonA, NonB. Observation on the first decade. In: Vyas GN, Dienstag JL, Hoofnagle JH, Eds. Viral hepatitis and liver disease. *Orlando, FL: grune and Stratton*; 1984:345-55
10. Koretz RL, Abbey H, Coleman E, et al. Non A non B Post-transfusion hepatitis : looking back in the second decade. *Ann Intern Med*.1993; 119: 110-5
11. Panigrahi AK, Panda SK, Dixit RK et al. Magnitude of Hepatitis C Virus in India: Prevalence in healthy blood donors, acute and chronic liver diseases. *Journal of Medical Virology*.1997; 51:167-74
12. Tandan BN, Acharya SK, Dasarathy S et al. Viral hepatitis in India. *Viral hepatitis and liver disease.* 1994; 300-2.
13. Thursz M, Yallop R, Goldin R, et al. Influence of MHC class II genotype on outcome of infection with Hepatitis C Virus. *Lancet*, 1999, 354:2119 -24.
14. Statistical Year Book of Nepal 1999, 7 th ed, HMG National Planning Commission Secretariat, Central *Bureau of Statistics*, Nepal
15. Shrestha SM, Subedi NB, Shrestha S, et al. Epidemiology of Hepatitis C Virus Infection in Nepal. *Trop. Gastroenterol*. 1998; July-Sept 19 (3): 102-4
16. Rai SK, Shibata H, Satoh M, et al. Seroprevalence of Hepatitis B & C Viruss in Eastern Nepal. *Kansenshogaku Zasshi*.1994; Dec.68 (12): 1492-7
17. Manandhar K and Shrestha B. Prevalence of HBV infection among healthy Nepalese males: a serological survey. *Journal of Epidemiology.* 2000; 10:6:410-13.
18. Nakashima K, Kasiwagi S, Noguchi A, et al. Humam T Lymphotropic Virus Type- I, and Hepatitis A, B and C virus in Nepal: A Serological Survey. *J Trop Med Hyg*. 1995 Oct. 98 (5): 347-50.
19. Sawayama Y, Hayashi J, Arimaya I, et al. A TenYear Serological Survey of Hepatitis A, B & C Virus Infections in Nepal. *J Epidemiology*. 1999; Nov. 9 (5) : 350-4.
20. Une H, Esaki H, Shigematsu T et al. Prevalence of antibodies to hepatitis C virus among Japanese workers with a history of blood transfusion. *Journal of Epidemiology*. 1995; 5: 75- 80
21. Tassopoulos NC, Hartzakis A, Delladetsima I, et al Role of Hepatitis C in acute nonA nonB hepatitis in Greece: A five year prospective study. *J. Gastroenterology,* 1992; 102: 969-72
22. Tomimatsu M, Ishiguro N, Taniai M et al Hepatitis C virus antibody in patients with primary liver cancer (hepatocellular carcinoma,cholangiocarcinoma and combined hepatocellular cholangiocarcinoma) in Japan. *Cancer*. 1993; 72: 683-88.
23. Yamachi M, Nakahara M, Malzawa Y et al. Prevalence of hepatocellular carcinoma in patients with alcoholic cirrhosis and prior exposure to hepatitis c. *Am J Gastroenterology.* 1993; 88: 39-43.