



Full Length Article

Passive Surveillance of Anti-Hepatitis C Virus Antibodies in Human Subjects of Four Medical Units of Balochistan, Pakistan

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ABSTRACT

Hepatitis C virus is a major public health problem and a causative agent of chronic liver disease worldwide. Hepatitis C virus infection is indolent and asymptomatic disease in human that can lead to liver cirrhosis and hepatocellular carcinoma. A cross sectional study was conducted in six selected districts of Balochistan. Blood samples of 1800 subjects (both genders aged between 18-60) were collected and sera/plasma were screened for anti-hepatitis-C virus (HCV) antibodies by rapid immunochromatography test (ICT). Out of 1800 subjects 1259 (70.0%) male and 541 (30.0%) female were screened for anti-HCV antibodies. A total of 161 (8.9%) subjects were found positive for anti-HCV antibodies. Gender and area were not found statistically associated ($p > 0.05$) with the prevalence of anti-HCV antibodies. However, age group 41-50 years was found most susceptible to HCV. Keeping in view the alarming level of anti-HCV antibodies, routine screening of subjects through rapid ICT is suggested, which is an important and economical tool and can help in reduction of HCV transmission. © 2012 Friends Science Publishers

Key Words: Anti-HCV antibodies; Prevalence; Hepatitis-C virus; Screening; Immunochromatography; Balochistan; Pakistan

INTRODUCTION

Hepatitis C virus (HCV) is an infectious disease and a major public health problem in the world. HCV is one of the major causative agents of non-A and non-B hepatitis and identified in 1989 (Choo *et al.*, 1989). Acute hepatitis C is often asymptomatic, which leads to chronic hepatitis and could be a major cause of morbidity and mortality (Shepard *et al.*, 2005; Hafez, 2011). The infection with hepatitis C virus is often asymptomatic, but become persistent, it can cause chronic liver diseases and progress to liver cirrhosis and hepatocellular carcinoma throughout world (Alter, 2007; Ahmad).

HCV infection has shown worldwide distribution, occurring among patients of all ages, gender, race, regions and affecting viral immune response. In most individuals anti-HCV antibodies appear 2 to 8 weeks after exposure, although it can last for as long as nine months (Bowen & Walker, 2005; Alter *et al.*, 1992). It is estimated that approximately 170 million people are infected chronically with HCV, which is 3% of the global population. HCV prevalence reported from different regions of the world is 1.7% from America, 1.03% from Europe, 3.9% from Western Pacific, 2.15% from south Asia and 5.3% from Africa (Lavanchy, 1999). However, the highest prevalence rate of HCV infection (10-20%) is reported in general

population from Egypt (Waked *et al.*, 1995). It has been estimated that Chronic HCV infection is responsible for 250000 to 350000 deaths per year in the world and 3-4 million people with HCV infection as a new case are diagnosed each year (Chevaliez & Pawlotsky, 2007). Although 50% to 85% of infected individuals progress from acute to chronic and 15% clear the HCV infection spontaneously (Seeff, 2002). It is difficult to determine the exact prevalence and incidence of acute HCV infection because most of the patients with acute infections are asymptomatic. The chronic infection once established can progress to scarring of the liver (fibrosis), cirrhosis and hepatocellular carcinoma (Chevaliez & Pawlotsky, 2007).

In Pakistan majority of the studies conducted have focused on the prevalence of anti-HCV antibodies which is informative about the active HCV infection. However, prevalence of anti-HCV antibodies is not well known in Balochistan, Pakistan. Prevalence of anti-HCV antibodies in the population of Pakistan have been recorded as 4-10 % (Akbar *et al.*, 2009; Shah & Dar, 2004; Hussnain *et al.*, 2007). Hospital-based studies have revealed prevalence rates of anti-HCV antibodies that is 9% in Mardan and 17.77% in Faisalabad (Khan *et al.*, 2004; Nafees *et al.*, 2007).

Several research workers have used ICT method for screening of anti-HCV antibodies. A study was conducted

to screen the blood donors for HCV antibodies by ICT method in Nigeria. They found 7.6% of the blood donors positive for the anti-HCV antibodies (Chukwurah *et al.*, 2005). Similarly another study was conducted in Pakistan, to screen the subjects for the presence of anti-HCV antibodies and hepatitis B surface antigens (HbsAg) by using ICT method. They found prevalence rate of 1.48% for anti-HCV antibodies and 2.46% for HbsAg respectively (Tanveer *et al.*, 2008).

Currently significant improvement is obtained in the efficacy of antiviral therapy combination with IFN- α and ribavirin for chronic HCV patients (Kato, 2001). The most widely used tests for the diagnosis of HCV infection are anti-HCV antibodies by ICT and confirmation by ELISA or HCV ribonucleic acid (RNA) with PCR (WHO, 2001). However, ICT or ELISA can not differentiate between acute and chronic HCV disease and resolved infection. The HCV RNA test with PCR can be used to confirm or prove false positive anti-HCV antibodies results (Strader *et al.*, 2004).

The present study was carried out to determine the prevalence of anti-HCV antibodies by screening selected samples from different regions of Balochistan, Pakistan.

MATERIALS AND METHODS

Study plan and design: This study was conducted at the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan Quetta from August 2010 to July 2011. A cross sectional study was designed, selected a total of 1800 subjects from both gender (male & female) with age of 18-60 years from six different districts/areas for screening (ACON Laboratories, Inc. USA).

Blood sampling: Disposable sterile syringes were used to collect blood samples from 1800 subjects visiting the different medical units (Bolan Medical Hospital, Civil Sandeman Hospital, Tariq Hospital & A-one laboratory Quetta). Five ml of whole blood was collected from each subject and allowed to clot. The blood samples were then centrifuged at 3000 rpm and serum/plasma was separated. The samples were tested for anti-HCV antibodies or stored at -20°C until needed for the analysis.

Data collection: The detailed demographic information was obtained either by interviewing the subjects or from hospital record at the time of sampling. A standard consented questionnaire was developed before conducting the study. The demographic information was obtained from subjects including age, gender and area. All 1800 subjects were grouped according to gender, age and area. The study population was divided into four different age groups such as group 1 (18-30 years), group 2 (31-40 years), group 3 (41-50 years) and group 4 (51-60 years). Male subjects were 1259 (70.0%) and female were 541 (30.0%) respectively. The subjects were from six different areas (Table I).

Inclusive criteria:

1. Age: 18-60 years
2. Gender: male and female
3. Area: districts of Quetta, Kalat, Sibi, Jaffarabad, Killah Saifullah and Panjgur

Exclusive criteria:

1. Below 18 and above 60 years of age
2. Patients who are known to be HCV, HBV and HIV infected
3. Family history (HCV, HBV & HIV infected)
4. History of liver disease or jaundice.

Immunochromatographic test: All serum samples were tested for anti-HCV antibodies by using Anti-HCV One Step Immunochromatographic method (Chukwurah *et al.*, 2005; Nafees *et al.*, 2007; Abou *et al.*, 2009). The ACON one-step anti-HCV test strip (ACON Lab. INC. USA) is a qualitative membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is coated with recombinant HCV antigen on the test line region of the strip. During the testing the serum or plasma react with the protein A coated particles. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a two colored lines. Presence of these colored lines indicates positive results, while absence indicates negative results. Relative sensitivity, specificity and accuracy of the test was greater than 99.9, 98.6 and 99.3%, respectively and the precision 98%.

Test procedure: The test procedure was conducted following the manufactures protocol. The test strips, buffer and specimens were allowed to stand at room temperature prior to the test. The test strip was then removed from the foil pouch and placed on a clean and leveled working surface. The disposable specimen dropper was held vertically and specimen was drawn. It was transferred to the specimen wells of the test strip. Two drops of buffer were also added. The results were read after 10-15 min. Two distinct red lines appeared one line on the control (C) region and another on test region (T) indicates positive results. One line on control (C) region with no other red or pink line in the test (T) region was taken as negative result, while control line which failed to appear was considered invalid result.

Statistical analysis: The results were statistically analyzed by using SPSS-PC version 16.0. The Chi-square Fishers exact test was also used to find relationship between the various variables such as age group, gender and area. $P < 0.05$ was accepted statistically significant and $P > 0.05$ was considered as insignificant.

RESULTS

The results indicated that 161 (8.9%) subjects in both sexes had anti-HCV antibodies by ICT (Table I). The results showed significantly increased prevalence in age group 3 (40-50 years) (11.5%) ($P < 0.05$). It was also noted that prevalence rate of anti-HCV

Table I: Prevalence of HCV seropositivity among gender, different age group and area in Balochistan, Pakistan

Characteristics	No. of Subjects	Male Tested No.	Male Positive No. (%)	Female Tested No.	Female Positive No. (%)	Total Positive No. (%)	P Value
Age Group (Years)							
18-30	386	247	14 (5.7%)	139	7 (5.0%)	21 (5.4%)	NS
31-40	363	245	21 (8.6%)	118	8 (6.8%)	29 (8.0%)	NS
41-50	434	299	37 (12.4%)	135	13 (9.6%)	50 (11.5%)	0.015
51-60	617	468	49 (10.5%)	149	12 (8.1%)	61 (9.9%)	NS
Total	1800	1259	121 (9.61%)	541	40 (7.39%)	161 (8.9%)	NS
Area							
Quetta	400	281	26 (9.3%)	119	10 (8.4%)	36 (9.0%)	NS
Kalat	257	183	17 (9.3%)	74	5 (6.8%)	22 (8.6%)	NS
Sibi	335	230	24 (10.4%)	105	7 (6.7%)	31 (9.3%)	NS
Jaffarabad	340	237	25 (10.5%)	103	7 (6.8%)	32 (9.4%)	NS
Killahsaifullah	253	178	16 (9.0%)	75	6 (8.0%)	22 (8.7%)	NS
Panjgur	215	150	13 (8.7%)	65	5 (7.7%)	18 (8.4%)	NS
Total	1800	1259	121 (9.61%)	541	40 (7.39%)	161 (8.9%)	NS

P>0.05: non-significant; P<0.05: significant; NS: non-significant; ICT: Immunochromatography test

antibodies with age group 4 (51-60 years) (9.9%) was higher to age group 1 (18-30 years) and age group 2 (31-40 years) (5.4% & 8.0%) respectively (Table I). The association was not observed between males and females for the prevalence of anti-HCV antibodies when compared both genders. There was no statistical significance observed within areas but higher prevalence of anti-HCV antibodies was found in Jaffarabad, Sibi and Quetta (9.4%, 9.3%, (9.0%) when compared with areas Kalat, Killah Saifullah and Panjgur (8.6%, 8.7% & 8.4%), respectively (Table I).

DISCUSSION

Hepatitis C virus infection is endemic not only in Pakistan but also all over the world (Shah *et al.*, 2002), although prevalence of HCV slightly varies from 1.18-4.8% in some regions (Hamid *et al.*, 2004). Prevalence rates of HCV antibodies vary in different geographical regions and various groups of the same areas (Idrees & Riazudine, 2008). Several studies on anti-HCV antibodies are conducted among the blood donors in various regions of Pakistan (Mumtaz *et al.*, 2002; Asif *et al.*, 2004; Mujeeb & Pearce, 2007). A cross sectional study has been conducted among volunteer blood donors at Quetta Balochistan and it has been observed that the seroprevalence rate of anti-HCV antibodies among blood donors was 1.85% but during the study rejection rate was 8.2% among the blood donors on the basis of interview (Khan *et al.*, 2007).

Earlier the highest prevalence of HCV infection in population has been reported in Pakistan and around the world (Frank *et al.*, 2000; Tanveer *et al.*, 2008). The prevalence of anti-HCV antibodies is studied in both sexes and it was found that the prevalence of HCV was 8.9% in adult males as compared to young males 6.66% (Ali *et al.*, 2009). Similarly, the other studies have reported higher percentage among males as compared to females (2.5, 0.9% & 3:1) (Ahmed *et al.*, 2002; Tanveer *et al.*, 2008). The high prevalence of anti-HCV antibodies in males could be attributed to their exposure status to HCV risk factors, which is obvious from the life style and history of the individuals.

The studies have shown the prevalence of anti-HCV antibodies in Pakistan could be due to reuse of contaminated syringes, use of barber shops for shaving, contaminated blood products, unsafe blood transfusion, dental procedures, hemodialysis and use of surgical instrument without proper sterilization (Chaudry *et al.*, 2005). The possibility of nonparenteral HCV transmission is reported as it is present in several body fluids and may be detected in chronically infected patients (Fariás *et al.*, 2010).

The prevalence of anti-HCV antibodies was not significantly increased within area but found increased significantly in age group 3 (40-50 years) (Table I). Our results are in agreement with the result reported by some workers among middle age group 40 to 50 years of age (Muhammad & Jan, 2005). The highest prevalence of anti-HCV antibodies increasing with age group and other risk factors may be due to unawareness of disease transmission from contaminated syringes, barber shops, use of contaminated instruments, unsafe blood transfusion or poor socio economics (Janjua & Nizamy, 2004).

Some studies have reported highest prevalence rate of HCV antibodies in patients more than 40 years of age (Muhammad & Jan, 2005; Nafees *et al.*, 2007). In the present study prevalence rate of anti-HCV antibodies was observed lower than the prevalence rate of HCV infection reported from different countries such as 12.5% in 40-59 years of age, 65% in 30-49 years of age in United State and 13.3% in above 50 years of age (Sherman *et al.*, 2002; Khan *et al.*, 2004; Chaudry *et al.*, 2005).

There is a wide variation in the prevalence of anti-HCV anti-bodies worldwide. It is estimated that 8-10 million people are living with chronic HCV infection in Pakistan (Hamid *et al.*, 2004). The global prevalence of HCV is 3% and there may be more than 170 million patients with chronic HCV infection in the world, and approximately 3 to 4 million individuals are diagnosed for HCV infection as new cases each year (Alter, 2007). The present study results are comparable with the studies reported with in country that is 9.0% in Mardan and 17.77% in Faisalabad (Khan *et al.*, 2004; Nafees *et al.*, 2007) and also from other countries such

as Egypt which is 10-20% (Frank *et al.*, 2000). The ICT tests are used for screening of anti-HCV antibodies in most clinical or diagnostic laboratories of the world. It gives qualitative results of anti-HCV antibodies. However, this test does not tell about the disease presence in patients.

Several studies have reported a high HCV infection in developing countries in animals and humans, where resources are very limited or unavailable for the diagnosis, disease prevention and treatment (Ander, 2000; Ahmad *et al.*, 2011). The anti-HCV screening facility is being provided only in some tertiary care hospitals and is not available at primary or secondary levels due to inadequate resources for health care and significant lack of public health awareness and poor attitude about the disease in Pakistan (Talpur *et al.*, 2007). Serological tests in immuno-compromised patients could be negative even in the form of positive viral load due to a weak immune response. These patients can spread the disease despite the low level of Viremia (Fabrizi *et al.*, 2007).

The one step device ICT test kits are quite suitable for underdeveloped countries like Pakistan due to lack of suitable facilities such as infrastructure and limited resources. Consequently, supplement test may be used further to confirm anti-HCV antibody test result such as ELISA and PCR (WHO, 2001). There is a lack of routine screening of anti-HCV antibodies in our provincial hospitals prior to invasive procedures which is one of the major risk factors responsible for spreading of viral disease such as hepatitis C virus (Ahmad *et al.*, 2006). Moreover, globally protective vaccine for HCV is not available (Ray, 2002). Consequently, public health interventions including screening of blood and blood products, destruction of disposable needles, adequate sterilization such as syringes, promotion of health education on HCV infection and its prevention are available to prevent the infection (Perz *et al.*, 2006).

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