ORIGINAL ARTICLE

Seroprevalence of Hepatitis C Virus in General Population of Balochistan, Pakistan

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ABSTRACT

Background: Hepatitis C virus is an important public health problem and major etiological agent of chronic liver disease all over the world. Chronic hepatitis C virus infection is indolent and asymptomatic disease in human can lead to liver cirrhosis, fibrosis and hepatocellular carcinoma.

Methods: A cross sectional study was designed comprising of 2800 serum samples of general population of Balochistan. The samples were tested for anti-HCV antibodies by enzyme linked immunoassay (ELISA) and for positive samples alanine aminotransferase (ALT) levels were also evaluated. The results were statistically analyzed by using SPSS version 16.0. Chi-square, Fishers exact test was used to find the relationship between the various variables such as age group, gender and area.

Results: Out of 2800 subjects 1872 (66.8%) male and 928 (32.2%) female were tested for anti-HCV antibodies. Among 2800 subjects 196 (7.0%) were found positive for anti-HCV antibodies. Association between anti-HCV antibodies for gender and area was also observed. There was no significant difference between genders by ELISA. Only anti-HCV antibodies with age group 41-50 years was significantly different at p-value (<0.05) by ELISA. The highest ALT (alanine aminotransferase) level mean value 77.3 IU/L was observed for the age group of 41-50 years.

Conclusions: In this study, it was aimed to determine the prevalence of anti-HCV antibodies in general population of Balochistan, Pakistan and concluded that the prevalence of anti-HCV infection is 7% in the studied population. We suggest routine tests of anti- HCV antibodies by ELISA, which can help in reduction of HCV infection transmission.

Keyword: Seroprevalence, Anti-HCV antibodies, ELISA, ALT, Balochistan

INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem in the world. Acute hepatitis C is often asymptomatic which leads to chronic hepatitis and could be a major cause of morbidity and mortality[1,2]. Firstly, HCV discovered in 1989 through molecular biological techniques as the major cause of non-A, non-B hepatitis[3]. Hepatitis C virus is well known causative agent of chronic liver diseases that develop liver cirrhosis and hepatocellular carcinoma, which is uncontrollable and fatal all over the world[4,5]. The prevalence rate of chronic HCV infection is affected by a person's age, gender, race, and viral immune response. It is estimated that approximately 170

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million people are infected chronically with HCV, which is 3% of the global population[5]. The highest prevalence rate of HCV infection (10-20 %) in general population is reported from Egypt[6].

It has been estimated that chronic HCV infection is responsible for 250000 to 300000 deaths per year in the world and 3-4 million people with HCV infection as a new case are diagnosed each year[7,8,9]. However, 50% to 85% of infected individuals progress from acute to chronic and 15% clear the HCV infection spontaneously[10]. However, it is difficult to determine the exact prevalence and incidence of acute HCV infection because most patients with acute infections are asymptomatic. The chronic infection once established can progress to scarring of the liver (fibrosis) and cirrhosis[11,12].

The majority of the patients have comparatively mild disease with slow progression of HCV infection. Some studies have shown that the hepatitis C virus infection causes chronic infection leading to serious complications such as liver cirrhosis, fibrosis and liver failure with end stage of liver cancer[11]. The chronic HCV infection accounts for 27% of liver cirrhosis and

25% of hepatocellular carcinoma (HCC) but liver cirrhosis is considered irreversible[12,13].

Seroprevalence of anti-HCV antibodies is not well known in Balochistan, Pakistan due to lack of studies and also there is varying reports regarding HCV prevalence. It is reported that HCV seroprevalence is 4-10% in Pakistan[14]. Some studies have shown the seroprevalence of HCV infection is varying in different regions of Pakistan, such as approximately 9% in Mardan[15], 13.33% in D I Khan[16], 17.77% in Faisalabad[17] and 4-6% in Karachi[18]. HCV is endemic in Pakistan and all over world. Prevalence of anti-HCV antibodies is high in Pakistan this could be due to reuse of contaminated syringes, use of razor blade in barber shops for shaving, contaminated blood products, and unsafe blood transfusion, dental procedures, hemodialysis and use of surgical instrument without proper sterilization[19,20]. Primary source of hepatitis C virus infection are infected blood, blood products and body fluids[9-21]. Anti-HCV antibody assay system has been developed for the diagnosis of HCV infection and considerable progress has been made regarding identification of anti-HCV antibodies in past decades. The most widely used tests for the diagnosis of HCV infection are anti-HCV antibodies by ICT, ELISA or HCV RNA with PCR[22]. An anti-HCV antibody by immunochromatograpghy test (ICT) and enzyme-linked immunosorbent assay (ELISA) does not differentiate between acute and chronic HCV cases or resolved infection. This assay can be used to confirm or prove false positive anti-HCV results[7]. The present study was carried out to determine the prevalence of anti-HCV antibodies by ELISA in general population of Balochistan.

RESULTS

Anti-HCV antibodies were determined in both sexes. Out of total number of general population 196 subjects (7.0%) were found positive for anti-HCV antibodies by ELISA (table 1). The seroprevalence did not differ significantly by gender, and area (P>0.05) using ELISA but prevalence of anti-HCV antibodies was found increase in males (7.5%) than females (5.9%) (table 1). The highly significant association between age groups (P<0.05) was found in age group 41-50 years (8.8%) than age group 18-30 (4.2%), 31-40 (5.5%) and 51-60 (7.3%) respectively. It was also noted that seroprevalence with age group 4 (51-60 years) 8.8% was higher than age group 1 (18-30 years), 2 (31-40 years) and 4 (51-60) respectively (table 1). There was statistically no significant difference found within areas (P> 0.05), but prevalence percentage (%age) of anti-HCV antibodies was higher in Sibi and Jaffarabad regions than Quetta, Kalat, Killah Saifullah and Panjgur. All positive samples were analyzed for ALT level. A raised ALT level (>40 IU/L) was observed (table 2).

Table 1 Seroprevalence of anti-HCV antibodies by gender, age among the target area of general population of Balochistan

Characteristics	=n	Anti-HCV+ve cases by ELISA (%)
Gender		
Male	1872 (66.8%)	141 (7.5%)
Female	928 (33.2%)	55 (5.9%)
Age		
18-30 years	432 (15.43%)	18 (4.2%)
31-40 years	581 (20.75%)	32 (5.5%)
41-50 years	1059 (37.82%)	93 (8.8%)*
51-60 years	728 (26.00%)	53 (7.3%)
Area		
Quetta	700 (25.00%)	49 (7.0%)
Kalat	450 (16.07%)	29 (6.4%)
Sibi	500 (17.86%)	38 (7.6%)
Jaffarabad	525 (18.75%)	41 (7.8%)
Killah Saifullah	350 (14.06%)	24 (6.9%)
Panjgur	275 (11.94%)	15 (5.5%)
Total	2800	196 (7.0%)

P Value=0.006

Table 2 Age wise distribution of mean ALT level (IU/L) of the anti-HCV antibodies positive subjects by ELISA

Age Group	=n	Mean ALT IU/L (STD)
18-30 years	18	57.50±7.73
31-40 years	32	64.81±17.94
41-50 years	93	73.80±16.15
51-60 years	53	68.96±16.64
Total	196	69.5±16.7

DISCUSSION

Hepatitis C virus infection is endemic in Pakistan[23] but it varies from 1.18 - 4.8%[31]. The present study was carried out in general population of Balochistan province of Pakistan and prevalence of anti-HCV antibody infection was found (7%) by ELISA in the general population which is high and alarming and comparable with studies reported from other countries and different regions of Pakistan. Previous studies on anti-HCV antibodies are conducted among the blood donors in various regions of Pakistan. The cross sectional study was conducted among volunteer blood donors at Quetta Balochistan, seroprevalence rate of anti-HCV antibodies was 1.85% but blood donor rejection rate on the basis of interview was 8.2%[24]. The prevalence of anti-HCV antibodies did not differ by gender and area but found increased significantly in age group 3 and people above 50 years of age. Among the subjects younger than 50 years of age, prevalence of anti-HCV antibodies was observed 4.2% in 18 to 30 years of

age, 5.5% in 31-40 years of age to a peak of 8.8% in 41 to 50 years of subjects and 7.3% in 51 to 60 years of age people (table 1). Our results are in agreement with the result reported by some workers among middle age group 40 to 50 years of age[25]. 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 instrument, unsafe blood transfusion or poor socio economics[26,27]. Other several studies reported highest prevalence rate of HCV infection in people more than 40 years of age[17-25]. The findings of present study regarding prevalence of anti-HCV antibodies was 8.8% among 41-50 years of age which is lower than the prevalence rate of HCV infection reported 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 subject[15,19,28].

In present study the prevalence of anti-HCV antibodies has been observed higher in male population than female. Our findings are comparable with results reported by some other workers[29]. Similarly, the other studies have reported higher percentage among males as compared to females[30]. There is a wide variation in the prevalence of anti-HCV anti-bodies world wide. It is estimated that 10 million people are living with chronic HCV infection in Pakistan[31]. The global prevalence of HCV is 3% and there may be more than 170 million patients with chronic HCV infection in the world, and 3 to 4 million individuals are diagnosed as new cases each year[8,9,22]. The prevalence rate of anti-HCV antibodies is observed 7% in this study and is comparable with the studies reported with in country that is 17.77% in Faisalabad, 10% in Islamabad and from other countries such as Egypt which is 20%[17,32,33].

In present study the overall prevalence of anti-HCV antibodies was recorded 7.0% by ELISA [table 1] in patients from different regions of Balochistan. These findings corroborate with various studies conducted in different regions of Pakistan, such as Mardan, D I Khan, Faisalabad, Karachi, Rawalpindi, and Islamabad (24.6%)[15,16,17,18,34,35]. Some studies have shown that HCV seropositivity is strongly associated with male sex and greater age[36,37]. In the present study strong association with age group was found. Anti-HCV antibodies (8.8%) in age group 3 differed significantly (P<0.05) by ELISA, and in males subjects anti-HCV antibodies percentage was higher than females (7.5% and 5.9%) respectively (table 1). ELISA tests for the detection of anti-HCV antibodies has been evolved from 1990 and third and fourth generation of ELISA assay is now used for the detection anti-HCV antibodies, which is more than 80% sensitive[22]. The ICT and ELISA 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 kind of tests does not tell us about the disease presence in patients. The characteristic feature of ALT pattern is fluctuating with course of acute hepatitis. Normalization of ALT values may occur and suggest that HCV is eliminated or patient has developed full recovery. ALT elevation indicates chronic HCV infection, and is a significant medical threat and requires urgent need for therapy[38,39]. Predictive positive cases are largely affected by the disease prevalence. In this study out of total number of subjects, 196 ELISA positive subjects had upper limit or increased ALT level. Mean ALT serum levels were higher with age group 3 in anti-HCV positive subjects. Many studies have reported a high HCV infection in developing countries where resources are very limited or unavailable for the diagnosis and disease prevention and treatment[40]. 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[41]. It is well known that serological tests in immuno-compromised patients may 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[42].

The ELISA kits are quite suitable for underdeveloped countries like Pakistan due to lack of suitable facilities such as infrastructure and limited resources[22]. However, no diagnostic test regarding HCV gives 100% accuracy. Consequently, supplement test may be used to confirm anti-HCV antibodies test result such as PCR[22].

MATERIALS & METHODS

This study was conducted with the approval of ethics Committee University of Balochistan Quetta at Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan Quetta from August 2010 to November 2011. A total of 2800 subjects were selected from both gender (male and female) with age of 18-60 years from six different districts/areas and were tested for anti-HCV antibodies by the use of ELISA kit (ACON Laboratories, Inc. USA). The kit was used to measure alanine aminotransferase (ALT) level by enzymatic method (DiaSys Diagnostic System).

All 2800 subjects were grouped according to sex, age and area. The study population was divided into four different age groups, group 1 (18-30 years), group 2 (31-40 years), group 3 (41-50 years)

and group 4 (51-60 years). Male subjects were 1872 (66.8%) and female 928(33.2%) respectively. However, subjects below 18 years of age and above 60 years of age were not included in this study. The geographical and ethnically areas included in this study were Quetta, Kalat, Sibi, Jaffarabad, Killah Saifullah and Panjgur. The ALT level >40 IU/L was considered as abnormal and upto 40 IU/L value was considered as normal level. Mean values of ALT were observed for ELISA positive subjects and age groups.

Consented questionnaire was developed to collect the demographic data including age, sex and area. Disposable sterile syringes were used to collect blood samples of 2800 subjects from Bolan Medical Complex Hospital, Civil Sandeman Provincial Hospital, Tariq Hospital and A-one Laboratory Quetta Balochistan. The blood samples were centrifuged at 3000 rpm and serum/plasma was separated.

All the samples were tested for anti-HCV antibodies by enzyme-linked immunosorbant assay (ELISA) according to the instructions of manufacture.

The positive samples of anti-HCV antibodies by ELISA were tested for alanine aminotranferase (ALT) level and ALT assessments were carried out according to the instructions of manufacture.

Statistical analysis: The results were statistically analyzed by using SPSS version 16.0 Chi-square. Fishers exact test was also used to find relationship between the various variables such as age group, gender and area. In this study P value of less than 0.05 (P<0.05) was accepted statistically significant and more than 0.05 (P>0.05) was considered as insignificant.

CONCLUSION

This study concluded that the occurrence of HCV infection persists in the general population of Balochistan and is also comparable to other provinces of Pakistan and developing countries. There is lack of routine serological screening in our provincial hospitals prior to invasive procedures which is one of the major risk factors responsible for spreading of viral hepatitis C. Furthermore, we suggest that screening facilities should be provided to district hospitals to reach the majority of the target population, across the province. Also preventive measures must be adopted such as awareness about screening of anti-HCV antibodies, risk factors and treatment to prevent the spread of disease.

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