Global Advanced Research Journal of Biotechnology (GARJB) Vol. 1(2) pp. 017-022, September, 2012
Available online http://garj.org/garjb/index.htm

Full Length Research Paper

Hepatitis C Virus infection in IDPs of war against terrorism in South Waziristan Agency, Pakistan

Zia Ur Rahman Awan[1, 2*], Abdul Haleem Shah[1], Hafiz Munib Ur Rahman[1], and Sanaullah Khan[3]

[1]Department of Biological Sciences, Gomal University Dera Ismail Khan, Pakistan
[2]Department of Zoology, Govt. Postgraduate College Bannu, Pakistan
[3]Department of Zoology, Kohat University of Sciences and Technology Kohat, Pakistan

Accepted 22 August, 2012

Hepatitis C virus (HCV) is a major public health problem which causing chronic liver disease and affected an estimated 180 million people throughout the world. The main aim of the current study was to investigate the HCV infection and the various associated risk factors in IDPs of South Waziristan Agency. A total of 250 individuals of the age groups 01-60 years were screened for the detection of anti-HCV antibodies using 3rd generation assay. All the subjects were also analyzed for ALT and ALP level. Among the total individuals, 28.8% were found positive for the presence of anti-HCV anti bodies. These were further processed for Polymerase Chain Reaction (PCR), for the presence of HCV RNA confirmation and active infection. A total of 21.6% patients were found to have HCV RNA and were confirmed for active infection of HCV. Prevalence of HCV was found to be higher in males than females. Similarly higher active infection of HCV was found in the old aged peoples and illiterate peoples were more infected than educated peoples. The risk factors observed were dental surgery, skin tattooing, reuse of blades, general surgery, reuse of syringes, drugs addicts, blood transfusion and intrafamilial prevalence. Awareness programs and timed screening needs to prevent the transmission of this dreadful disease in the study area.

Keywords: HCV infection, IDPs, PCR, Risk factors, War against terrorism

INTRODUCTION

HCV is the main cause of severe liver disease, including hepatocellular carcinoma, cirrhosis and end stage liver disease (Wahid et al., 2011). Approximately 200 million people are infected with this virus which covers about 3.3% of world's population (Wands, 2004; Lausanne, 1996). HCV infection leads to chronic infection in 50-80 percent of individuals (Wahid et al., 2011). It was estimated by World Health Organization in 2004 that the annual deaths due to liver cancer caused by HCV and cirrhosis were 308,000 and 785,000 respectively (WHO, 2004). In Pakistan the prevalence of HCV is 5% in general population (Wahid et al., 2011).Hepatitis C is comparable to a "viral time bomb". The worldwide prevalence is estimated to an average of 3.0% (ranging from 1.0% 5.0% in different countries) (WHO, 1999). There are 150 million (3.0%) chronic carriers worldwide. Four million individuals are diagnosed as new cases every year (Lo Re, 2005; Ray Kim, 2002). About 4 million infected people are in the United States and 5 million in

*Corresponding author Email: ziabiotech78@yahoo.com

Western Europe. The prevalence seems to be higher in Eastern Europe than in Western Europe. The industrialized countries are more affected than non-industrialized countries (EASL Consensus Conference, 1999). The prevalence of HCV is said to be 5 times greater as compared to human immunodeficiency virus type-1 (HIV-1), thus representing a viral pandemic worldwide (Georg, 2001). In the United States alone, nearly 30,000 persons are newly detected each year. Similarly, 8 to 10 thousands death are attributed to HCV annually (NIH, 1997; Miriam, 1998). General population estimates are difficult to obtain. Prevalence varies between different countries and between ethnic groups within one country (Boxall, 1994). In Pakistan currently, approximately 10 million people are suffering from this tremendous disease which cover 6% of the overall population. A high prevalence of HCV Ab (38% weighted average) was described in the studies of patients undergoing chronic dialysis in Pakistan (Khan et al., 2011a). The spread of HCV in Pakistan is fuelled due to lack of education and awareness of disease, shortage of medically qualified and scientifically trained health care workers especially dentists, lack of health infrastructure such as use unsterilized instruments, use of high numbers of therapeutic injections and practice of daily face and armpit shaving in community barber shops (Raja and Janjua, 2008; Khan et al., 2011b). A study was conducted on "Internally displaced persons" (IDPs) by Rauf et al. (2010), at Swat, Pakistan and the HCV infection found was 40%. Similarly, Tahir et al. (2000) performed a study in Hazara division and found HCV positivity to be 40.8%. Majority of studies about HCV prevalence in Pakistan are generally confined to central and northern parts, capital areas, and other big cities. The present study was designed with the main aim to determine the HCV infection in internally displaced persons of South Waziristan in a camp of D.I. Khan, due to war against terrorism.

MATERIALS AND METHODS

Study Area

The study was conducted in a camp for internally displaced persons in D.I.Khan District of Khyber Pakhtunkhwa. This camp was temporarily organized by the Government of Pakistan for the internally displaced persons of South Waziristan Agency during war against terrorism. Majority of the IDPs belonged to mountainous areas of South Waziristan Agency, the areas where Afghan refugees were firstly migrated and settled. Afghan refugees are the carriers of HCV and the active source of HCV transmission from one area to the other. Keeping in view all the facts, present study was designed.

Study Samples

A total of 250 blood samples were taken from those individuals having one or more health problems such as fatigue, anorexia, fever, joint pain, malaise, abdominal pain, nausea, burden on stomach, dark yellow eye, dark yellow urine, jaundice etc. As all these problems up to some extent are associated with liver infection, these persons were asked to fill a questionnaire printed both in English and Urdu languages. This questionnaire contained all the appropriate information such as patients' personal profile (name, age, sex, profession, education, economic status, address etc.), past history, problems facing and biochemical diagnosis.

A 3 ml blood sample was collected in a vacutainer from each patient; serum was separated and stored at -20°C in the Molecular Parasitology and Virology Laboratory, Department of Zoology, Kohat University of Science and Technology Kohat, Pakistan for further processing.

HCV Screening

HCV screening was carried out with 3rd generation Test (Accurate Diagnostics USA), for anti HCV anti bodies, according to the manufacturer instructions.

Biochemical Analysis

The liver function tests (LFTs) especially Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP), were performed using Microlab 300 (Merck USA) using their related kits (Diasys Diagnostic System Germany) as described in manufacturer's manual.

Extraction of RNA

HCV RNA was extracted from 100 µL serum, using 0 µl of HBsAg positive serum, using GF1 nucleic acid extraction kit (Vivantas USA) according to manufacturer's instructions.

PCR amplification and Detection

HCV RNA was reverse transcribed with 200 U of Maloney murine leukemia virus reverse transcriptase (Fermentas USA) and amplified with nested primers specific for 5' untranslated region of HCV genome. PCR

Table 1 Sex-wise Prevalence of Hepatitis C in IDPs of South Waziristan Agency

Gender	Total No. of samples	Results (%)			
		Anti-HCV +ve	Anti-HCV -ve	HCV RNA +ve	HCV RNA -ve
Male	150	47 (31.33)	103 (68.67)	39 (26)	08 (5.33)
Female	100	25 (25)	75 (75)	15 (15)	10 (10)
Total	250	72 (28.8)	178 (71.2)	54 (21.6)	18 (7.2)

Table 2 Age-wise prevalence of HCV RNA +ve patients in IDPs of South Waziristan Agency

Age Group Years	HCV RNA +ve	%	Total No. of samples
01 – 20 Years	12	17.4	69
21 – 40 Years	17	21.8	78
41 – 60 Years	25	24.3	103

reactions were carried out in a thermal cycler (Nyxtechnik USA) Amplified product was subjected in 2% agarose gel for electrophoresis.

For contamination control RNA extraction, cDNA amplification and electrophoresis were carried in separate areas. Both negative and positive controls were run parallel to the patient samples in each batch.

Statistical Analysis

The data was analyzed by using SPSS version 14.0 for windows. The results were obtained in percentage.

RESULTS

A total of 250 individuals (150 males and 100 females) of the age group 1 to 60 years were screened for the presence of anti HCV antibodies. Of the total individuals, 72 (28.8%) were found positive for anti HCV antibodies and these were further processed for the detection of HCV RNA. After PCR, 54 (21.6%) individuals including 39 males and 15 females were confirmed for the presence of HCV RNA and the remaining 18 (7.2%) individuals including 08 males and 10 females were found negative. Sex-wise distribution of HCV infection was assessed, which shows that male population was more infected 39 (26%) as compared to female population (15%). It shows that HCV prevalence with reference to gender is higher in males than females (Table 1).

HCV prevalence was found to be directly proportional to age factor i.e. higher the age higher was the HCV prevalence. The HCV infection in HCV RNA positive patients was found to be higher in the age group of 41-60 years (24.3%) and lower in the age group of 1-20 years (17.4%). In the age group of 21-40 years it was 21.8% (Table 2).

Similarly literacy rate was also taken in consideration. The HCV RNA positive patients were grouped into illiterate people 44 (81.48%), school level 06 (11.11%), college level 03 (5.55%), and university level 01 (1.85%). Maximum prevalence of Hepatitis C was found in illiterate people, followed by school level and then college level. Statistical analysis shows that education was significantly associated with HCV prevalence (Figure 1).

Association of HCV prevalence with marital status was also considered. Higher prevalence of HCV RNA positive individuals was found in married people 30 (55.55%) as compared to unmarried people 24 (44.44%.). It shows significant association of HCV infection with marital status (Figure 2).

Association of various risk factors with HCV infection was also studied during the current research work. Among the HCV RNA positive patients, the highest number of risk factors was observed in patients with history of dental surgery (53.7%). Other risk factors observed were exposure to tattoos and body piercing (37.04), reuse of shaving blades (14.81%), reuse of syringe (11.11%), drug addiction (7.40%), blood transfusion (38.9%), general surgery and intrafamilial prevalence 5.55% each (Table 3).

DISCUSSION

Hepatitis C is rapidly emerging as a major health problem in developing countries including Pakistan. The World Health Organization estimates that approximately 3% of the world populations have been infected with HCV (Ali et al., 2010). There are about 170 million patients with HCV

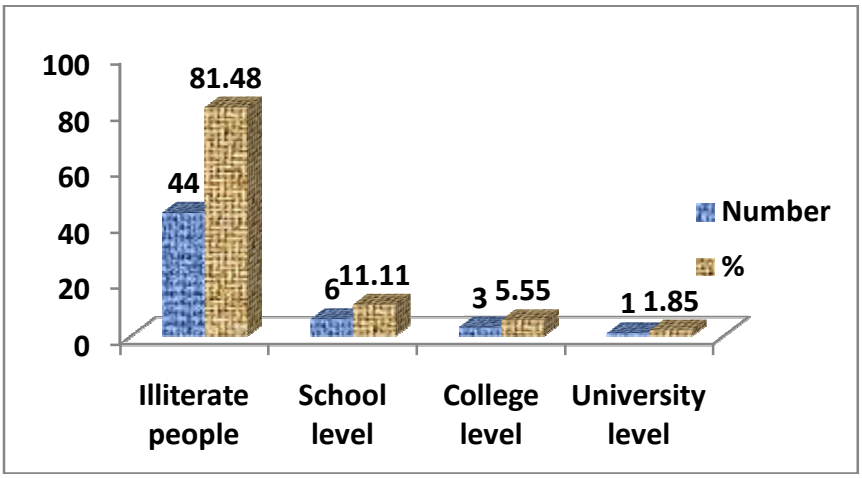

Figure 1 Education-wise prevalence of HCV RNA +ve patients in IDPs of South Waziristan Agency (n = 54)

Figure 2 Prevalence of HCV RNA +ve individuals with reference to marital state In IDPs of South Waziristan Agency (n = 54)

in the world and 3-4 million individuals are diagnosed as new cases every year (Lo RV and Kostman, 2005; Colombo et al., 2003). The first description of HCV in Pakistan was recorded in 1992 and about 6%, i.e. at least 10 million persons are carriers among a population of 140 million, this showed that there is no proper review of HCV and it is becoming a Herculean challenge. With the current disease burden, Pakistan has left behind the surrounding countries like India, Nepal, Myanmar, Iran and Afghanistan (Khan et al., 2011).

The current study match with the fact that male population of IDPs is more affected by HCV as is indicated by HCV prevalence of 26% in the male population. The female population of IDPs is least affected with 15% active prevalence as compared to male population. High prevalence in male and lower prevalence in female again could be attributed to their exposure status to various HCV risk factors which was quite evident from the life style and history of the individuals sampled for this study. Higher HCV prevalence in males is thought to be due to their social responsibilities such as their jobs and business, barber contact, blood and organ donation, drug abuse. All these are HCV transmitting factors. High prevalence of HCV infection in male population has earlier been recorded by other studies from Pakistan and around the world (Ali et al., 2009).

The study revealed that young people (in the age groups of 01-20 years) have less active (17.4%) HCV infection. This may be due to their least exposure to

Table 3 Prevalence of HCV infection with reference to different risk Factors in IDPs of South Waziristan Agency (*n = 54)

Risk Factors	Level/Observed		Total (%)
	Male	Female	
Tattoos/Piercing	09	11	20 (37.04)
Dental Surgery	15	14	29 (53.7)
Re-use of Blades	03	05	08 (14.81)
General Surgery	01	02	03 (5.55)
Reuse of Syringe	04	02	06 (11.11)
Drug Addicts	03	01	04 (7.40)
Blood Transfusion	17	04	21 (38.9)
Intrafamilial prevalence	01	02	03 (5.55)

Per subject, more than one category is possible
*Total number of HCV RNA positive individuals

some of the high risk factors causing HCV such as exposure to barbers etc. The highest active HCV infection was 24.3% observed in age group 41-60. These people had variable history of exposure to HCV risk factors such as major/dental surgery or blood transfusion. Second highest active HCV infection was 21.8% observed in age group 21-40). Highest HCV prevalence in old ages may be due to weak immunity in these stages. Secondly it may be due insurgency in old days in proper blood screening prior to transfusion, reuse of shaving blades and injectable syringes.

Association of HCV prevalence with literacy was also significant. The result was inversely proportional to education level i.e. lower the education higher was the HCV prevalence. Lowest prevalence was reported in people with master level of education. Highest HCV infection was found in illiterate people followed by school level and then college level. Educated people adopt safe precautions. They try to live a healthy life. They follow universal preventive measures to avoid diseases. These observations are supported by Wang et al. (2002) who also showed an inverse HCV correlation with education level. Wang et al. (2002) conducted a study in Taiwan in Junior high school and reported 73.9% HCV infectivity in below junior high school while 39.7% in junior or above junior high school population.

HCV prevalence was higher in married people as compared to unmarried. Marital status was significantly associated with HCV prevalence. Although sex is not significantly correlated with HCV transmission, however married couple frequently exposed to each other, could easily transmit HCV.

This study also revealed the association of various risk factors with active HCV infection. The highly observed risk factor was dental surgery (53.7%), followed by blood transfusion, skin tattooing and body piercing, re-use of blades, re-use of syringes, drugs addicts, general surgery and interfamilial prevalence. Dental surgery is one of the major risk factors for HCV transmission in Pakistan. Some studies have reported that dental procedures were the major source of exposure (39.7%) followed by injections (16.6%) and surgical procedures (16.6%) (Ali et al., 2011). Also the contaminated dentist equipments were the source of HCV infection in 17.94% people (Mohammad et a., 2009). Some of the previous studies also revealed the association of various risk factors with HCV infection (Janjua and Nizamy, 2004; Sawayama et al., 2000; Waheed et al., 2009; Waheed et al., 2010; Wazir et al., 2008).

The Sero-prevalence of Hepatitis C virus in different parts of Pakistan, reported in the last 5 years, is from 2.2%-13.5% (Ahmad et al., 2012). The highest sero-prevalence of hepatitis C has been reported from Lahore (13.5%) (Amin et al., 2004) Jasmshoro (9%) and Mardan (9%) (Almani et al., 2002; Khan et al., 2004). Pakistan being a developing country the literacy rate is also low, due to which lack of information regarding the pathogenecity, routes of transmission and the proper procedures of diagnosis and treatment are rarely followed. Therefore, HCV infection has become an economic burden on the people of Pakistan and especially in Khyber Pakhtunkhwa province.

The previous research work has focused on the prevalence of anti-HCV antibodies which is least informative about the active HCV infection. Polymerase Chain Reaction (PCR) has emerged as a powerful molecular diagnostic tool for the detection of active infection which is manifested by the presence of HCV RNA in the blood of the infected person.

HCV infectivity for anti-HCV antibodies in internally displaced people (IDPs) was 28.8% and active infection of HCV RNA was 21.6%. Most of the IDPs belonged from mountainous areas of South Waziristan. Actually these are areas where Afghan refugees were firstly migrated. So it is possible that HCV may be emerged from Afghan refugees. A lot of relative of IDPs work abroad especially in gulf countries. Dubai has a high HCV prevalence. So this is also the possible route of HCV transmission. As no

study has earlier been conducted to figure out the prevalence of anti-HCV antibodies or HCV RNA among the IDPs of D.I.Khan, we for the first time conducted our study to find out prevalence of HCV infection and the associated risk factors in internally dispersed persons (IDPs) of South Waziristan Agency in a camp organized by the government in D.I.Khan Division of Khyber Pakhtunkhwa, Pakistan.

CONCLUSION

Hepatitis C is progressively transmitted from South Waziristan to Dera Ismail Khan and the neighboring areas. The progression rate is similar to other areas of the country. The disease is actively spreading in people belonging to low socioeconomic class. Similarly, prevalence also correlates with level of education. It is higher in illiterate people than educated people. Major factors responsible for HCV transmission are dental extraction, reuse of shaving blades, syringes, general surgery. Tattoos/body piercing, smoking & drug addiction and intra-familial transmission are less significantly associated. So proper training of barbers and health worker is necessary to control HCV progression. extensive awareness programs and other preventive measures and cure should be taken to stop the spread of this alarming disease in the study area.

REFERENCES

Ahmad B, Ali S, Ali I, Azam S, Bashir S (2012). Response rates of standard interferon therapy in chronic HCV patients of Khyber Pakhtunkhwa (KPK). Virol. J. 9:18.

Ali A, Ahmad H, Ali I, Khan S, Zaidi G, Idrees M (2010). Prevalence of active hepatitis c virus infection in district Mansehra, Pakistan. Virol. J. 7:334.

Ali I, Siddique L, Rehman LU, Khan NU, Iqbal A, Munir I, Rashid F, Khan SU, Attache S, Swati ZA, Aslam MS (2011). Prevalence of HCV among the high risk groups in Khyber Pakhtunkhwa. Virol. J. 8:296.

Ali SA, Rafe M, Donahue J, Qureshi H, Vermund SH (2009). Hepatitis B and hepatitis C in Pakistan: prevalence and risk factors. Int. J. Infect. Dis. 13:9-19.

Almani SA, Memon AS, Qureshi AF, Memon NM (2002). Hepatitis viral status in Sindh. Professional Med. J. 9(1):36-43.

Amin J, Yousf H, Mumtaz A, Iqbal M, Ahmed R, Adhami SZ (2004). Prevalence of Hepatitis B surface antigen and Anti Hepatitis C virus. Professional Med. J. 11(3):334-337.

Colombo M, Rumi MG, Ninno ED (2003). Treatment of chronic hepatitis C in Europe. J. Hepatobil. Pancreat. Surg. 10:168-1671.

EASL (1999). International Consensus Conference on Hepatitis C: Paris, 26-28, February 1999, consensus statement. J. Hepatol. 30:956-61.

Georg M, Lauer MD, Bruce D, Walker MD (2001). Hepatitis C virus infection; a Review Article, Published by Massachusetts Medical Society.

Janjua NZ, Nizamy MA (2004). Knowledge and Practices of Barbers about Hepatitis B and C Transmission in Rawalpindi and Islamabad. J Pak Med Assoc. 54, 116–119.

Khan MSA, Khalid M, Ayub N, Javed M (2004). Seroprevalence and risk factors of Hepatitis C virus (HCV) in Mardan: N.W.F.P. Rawal Med. 29:57-60.

Khan S, Attaullah S, Ali I, Ayaz S, Naseemullah, Khan SN, Siraj S, Khan J (2011a). Rising burden of Hepatitis C Virus in heamodialysis patients. Virol. J. 8:438.

Khan S, Attaullah S, Ayaz S, Khan SN, Shams S, Ali I, Bilal M, Siraj S (2011b). Molecular Epidemiology of HCV among the health care workers of Khyber Pakhtunkhwa. Virol. J. 8(1):105.

Lausanne, L Tomei, R DeFrancesco (2004). Identification and properties of the RNA- dependent RNA polymerase of hepatitis C virus. EMBO J. 15, 12–22 (1996).

Lo RV, Kostman JR (2005). Management of chronic hepatitis C. Postgrad. Med. J. 81:376-382.

Miriam J Alter HS, Margolis MD (1998). Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-Related Chronic Disease. *MMWR*. 47 (19): 1 – 2.

Mohammed AJ, Qudah AA, Shishi KF, Sarayreh AA, Quraan LA (2009). Hepatitis C virus (HCV) infection in hemodialysis patients in the south of Jordan. Renal data from the Arab world. 20, 488–492.

National Institutes of Health (1997) Consensus Development Conference Panel statement: management of hepatitis C. Hepatology. 26: Suppl 1:2S-10S.

Raja NS, Janjua KA (2008). Epidemiology of hepatitis C virus infection in Pakistan. J. Microbiol. Immunol. Infect. 41:4-8.

Rauf A, Nadeem MS, Ali A, Iqbal M, Mustafa M, Latif MM, Latif MZ, Ahmed A, Shakoori AR (2010). Prevalence of hepatitis B and C in internally disposed persons of war against terrorism in Swat, Pakistan. Eur. J. Public. Health, 1-5.

Ray Kim W (2002). Global epidemiology and burdon of hepatitis C.Microbs Infect. 4: 1219 – 1225.

Sawayama Y, Hayashi J, Kakuda K, Furusyo N, Ariyama I, Kawakami Y(2000). Hepatitis C virus infection in institutionalized psychiatric patient's possible role of transmission by razor sharing. Dig. Dis. sci. 45, 351–6.

Waheed Y, Saeed U, Safi SZ, Chaudhry WN, Qadri I (2010). Awareness and risk factors associated with barbers in transmission of hepatitis B and C from Pakistani population: barber's role in viral transmission. Asian Biomed. 4, 435–442.

Waheed Y, Shafi T, Safi SZ, Qadri I, HCV in Pakistan (2009). A systematic review of prevalence genotypes and risk factors. World J. Gastroenterol. 15, 5647–5653. doi:10.3748/wjg.15.5647.

Wands JR (2004). Prevention of hepatocellular carcinoma. N. Engl. J. Med. 351, 1567– 1570. doi: 10.1056/NEJMe048237.

Wang CS, Chang TT, Yao WJ Chou P (2002). Comparison of hepatitis B virus and hepatitis C virus prevalence and risk factors in a community based study. Am. J. Trop. Med. Hyg. 66 (4): 389 – 393.

Wazir MS, Mehmood S, Ahmed A, Jadoon HR (2008). Awareness among barbers about health hazards associated with their profession. J. Ayub Med. Coll. Abbottabad. 20, 35–38.

Waheed Y, Safi SZ, Qadri I (2011). Role of Potash Alum in Hepatitis C virus Transmission at Barber's Shop. Virol. J. 8: 211.

World Health Organization (1999). Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis prevention board, Antwerp, Belgium. J. Vir. Hepat. 6 (1): 35 – 47.

World Health Organization (2004). Department of Measurement and Health Information. http://www.who.int/healthinfo/statistics/bodgbddeathdalyestimates.xls.