Pakistan J. Zool., vol. 45(6), pp. 1579-1584, 2013.

Seropositivity and Active HCV Infection in Patients from Peshawar Division of Khyber Pakhtunkhwa Province, Pakistan

Abida Arshad,[1,2] Habib Ullah Khan,[3] Farzana,[3] Mehwish Saba Aslam,[4] Tayyab Rehman,[4] Muhammad Arshad,[5,*] Muhammad Saad Ahmed,[3,5] Ijaz Ali[3] and Ahmad Ur Rehman Saljoqi[3]

[1]*School of Life Sciences, Beijing Institute of Technology, Beijing, China*
[2]*Department of Zoology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan*
[3]*Department of Plant Protection, The University of Agriculture, Peshawar, Pakistan*
[4]*Khyber Medical University, Peshawar, Pakistan*
[5]*Department of Bioinformatics & Biotechnology, International Islamic University, Islamabad, Pakistan*
Abida Arshad and Habib Ullah Khan contributed equally to the work

Abstract.- Hepatitis C is one of the most common blood-borne diseases causing significant morbidity and mortality globally. This study was designed to evaluate any correlation between 3rd generations ELISA positivity for anti-HCV antibody plus elevated ALT levels with Real-time PCR based detection among various categories of patients attending Khyber Teaching Hospital Peshawar. Total 160 serum samples were collected from clinically HCV positive individuals. All samples were tested for anti-HCV antibodies and HCV RNA using 3rd generation ELISA and real time PCR. Our analysis of all 160 positive samples indicated that 158 (98.75%) had anti-HCV antibodies in their sera (Male, 82; Female, 76), while 2 subjects (a male and a female) were negative for anti-HCV using third generation ELISA. Real-time PCR analysis further revealed that 100 subjects (62.5%) had active HCV infection while the remaining 60 (37.5%) subjects did not have HCV RNA in their sera. Our study indicates that seropositivity does not reveal the presence of active HCV infection as it may be due to the self-limiting nature of the disease or due to false positivity, which is often experienced in case of antibodies based detection.

Key Words: Active hepatitis-C infection, HCV, 3rd generation ELISA, alamine aminotransferase.

INTRODUCTION

Identification of hepatitis C virus (HCV) was carried out in 1989 using advance molecular biology techniques (Choo *et al.,* 1989). The Taxonomy of the virus shows that it is an enveloped virus, hence grouped in a separate genus in the family Flaviviridae. The genetic material of hepatitis C virus consists of a single stranded RNA molecule comprising of 9.5-kilo bases (kb) in length. HCV genome shows considerable heterogeneity like other RNA viruses. There are six genotypes of HCV worldwide while the mutation in its genetic materials has resulted in the emergence of a large number of closely resembled subtypes. The six known genotypes have been assigned numbers 1 to 6 and the subtypes have been classified in the order of its discovery as a, b and c (Alter *et al*., 1994)

HCV cause hepatitis C, an infectious disease of the liver, which in most cases is asymptomatic initially, ultimately progressing to cirrhosis and hepatocellular carcinoma in a number of patients (Ali *et al*., 2011). Community barbershops are a common practice in Pakistan for face and armpit shaves. Among barbers, the level of awareness regarding HCV is low and they normally reuse razors (Janjua and Raja, 2008). One of the recent studies has shown that about 24% of the public in Internal Displacement Units (IDU's) in Pakistan is actively infected with HCV (Ali *et al*., 2011).

Regular procedures of blood screening reduce the risk of HCV transmission. However, in Pakistan, transfusion of contaminated blood is still a significant risk factor in the multiplication of hepatitis C owing to the lack of careful donor screening and extensive utilization of paid blood donors (Luby *et al*., 2000). Studies on HCV prevalence conducted in Pakistan from 1998 to 2002 indicated that 4.57% subjects were positive for anti-HCV antibodies with higher prevalence among the age group of 41-50 (Akhtar *et al*., 2004). Recently

* Corresponding author, m.arshad@iiu.edu.pk
0030-9923/2013/0006-1579 $ 8.00/0

several studies have been undertaken to investigate the prevalence of HCV amongst the general population with different risk group indicate that HCV infection is on rise in Pakistan (Amjad *et al*., 2011). An estimated 6% of the total population (10 million) carrying HCV in their blood and are unaware of the active infection nationwide. Conversely, one study revealed low prevalence of active HCV infection in KPK and FATA regions of Pakistan and reported the prevalence as 1.65%, relatively less than previous estimates (Ali *et al*., 2011).

The HCV in its initial stages cannot not be detected through the current serological procedures due to the reason that the anti-HCV antibodies develop after 45 days of infection (Kotwal *et al*., 1992). Considering the above-mentioned fact, a study conducted in Pakistan laid stress upon the use of ELISA for screening Anti-HCV antibodies in public sector hospitals and health care units (Ali *et al*., 2011). Overall, the HCV RNA detection by molecular techniques like polymerase chain reaction (RT PCR) is highly sensitive and is the most consistent method for the diagnosis of HCV infection in its earlier stages (Reddy *et al*., 2006). According to World Health Organization (WHO), active HCV infection is a pre requisite for undertaking therapy regimes. Hence, determination of the anti-HCV antibodies status of patients is not sufficient to start antiviral therapy. Current study aimed at finding correlation between seropositivity and active HCV infection in HCV positive individuals.

MATERIALS AND METHODS

Study design

This study was carried out at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar and Molecular Biology Laboratory, Khyber Teaching Hospital (KTH) Peshawar, Pakistan. Total 160 serum samples from clinically diagnosed patients were screened for Anti-HCV Antibodies by Enzyme Linked Immunosorbent Assay (Semi automated ELISA Human Humareader 3rd generation; Human GmbH Wiesbaden Germany) and HCV-RNA by Real Time PCR (Rotor-Gene 3000). All serum samples from HCV suspected patients from Peshawar Division of Khyber Pakhtunkhwa were collected at Department of Pathology, KTH for further processing.

ELISA

HCV positive samples were re-confirmed for anti-HCV antibodies using 3rd generation ELISA according to the instructions provided by the manufacturer.

Detection of HCV RNA by PCR

All the reagents for extraction were prepared and RNA was extracted from serum samples as stated by QIAamp viral RNA purification protocol (QIAGEN GmbH). Real-time PCR was carried out according to standard protocol described earlier in the literature (Ali *et al*., 2011).

Table I.- Anti HCV positive samples as determined by 3rd generation ELISA.

ELISA results	No. of samples	Percent	Males	Females
Normal (≤ 1)	2	1.2	1	1
Positive (>1)	158	98.8	81	77
Total	160	100	82	78

Cutoff value of anti HCV antibodies ≤ 1was Normal while >1 was Positive.

RESULTS

Of the total 160 samples, 82 (51.2%) were male and 78 (48.7%) were female. Our analysis of all the ELISA positive samples indicated that 158 (98.75%) had anti-HCV antibodies in their sera (male, 81; female, 77), while 2 subjects (a male and a female) were negative for anti-HCV antibodies (Table I). The samples with mean elevated alanine aminotransferase (ALT) were subjected to RNA extraction and subsequent real time amplification by PCR in order to determine active HCV infection. The results indicated that 100 (62.5%) subjects had active HCV infection while 60 (37.5%) turned out to be negative for HCV RNA (Table II).

Table II.- Real-time PCR results for the anti HCV positive samples.

PCR result	No. of samples	Males	Females
HCV RNA not detected	60 (37.5%)	25	35
HCV RNA detected	100 (62.5%)	57	43
Total cases	160 (100.0%)	82	78

Table III.- Categorization of patients based on subject's history, seropositivity and active HCV infection.

Category	Total cases	ELISA Ab positive	PCR detected
Outdoor cases	118	116 (98.3%)	74 (62.7%
Indoor cases	11	11 (100%)	09 (81.8%)
Unaware cases*	13	13 (100%)	12 (92.2%)
Therapy cases	18	18 (100%)	05 (27.7)
Total No. of cases	160	158 (98.7%)	100 (62.5)

*Unaware cases: volunteer blood donors, dental subjects who often visit dentists, patients which are diagnosed before surgery who were unaware of HCV infection.

Various categories of the subjects under investigation are given in Table III. The highest frequency of anti-HCV positivity (100%) and active HCV infection (92.2%) was found in the case of unaware category of subjects (volunteer blood donors, dental subjects who often visit dentists) followed by Indoor subjects (100% & 81.8%) and OPD subjects. Subjects who had been on IFN therapy also turned out to be positive for anti-HCV and HCV RNA. Correlation of HCV seropositivity and presence of HCV RNA in the plasma of both genders under study is discussed in Table IV. Of 81 seropositive male subjects, 57 had active HCV RNA in their plasma whereas out of 77 seropositive female subjects, 43 positive for HCV RNA. Comparison of Alanine transaminase (ALT) levels and seropositivity is given in Table V. When compared with anti-HCV seropositivity, Normal as well as elevated Mean ALT levels in case of different categories of patients did not have any importance with respect to predictability of HCV infection.

Table IV.- Correlation between gender, seropositivity and HCV RNA.

Gender	Seropositive	HCV RNA positive	Correlation (%)
Male	81	57	70
Female	77	43	55

Comparative analysis of the ALT levels, seropositivity and active HCV infection in the case of various age groups revealed that the youngest age group (1-20 years) had the highest prevalence of anti-HCV antibodies although their mean ALT level (50.3) was lower as compared to older age groups. However, the highest active HCV infection (66.6%) was found in age group (21-40 years). Mean enzyme levels, seropositivity and active HCV infection in the case of various age groups is given in Table VI. Furthermore, based on the total results, a non-significant correlation was found between the ELISA and PCR positive patients that indicate that seropositivity is least informative about active HCV infection (Table VII).

Table V.- Alamine aminotransferase (ALT) vs. ELISA among the various categories of the study groups.

Category	Total cases (No.)	Mean ALT (U/L)	Mean ALT(U/L)		ELISA positive	
			Males	Females	Males	Females
Outdoor (OPD) cases	118	74.4	76.6	61.5	56	60
Indoor (Admit) cases	11	62.6	65.1	56	08	03
Unaware cases	13	58.2	50.5	67.1	07	06
Therapy cases	18	44.6	45.5	43.3	10	08
Total No. of cases	160	64.5	68.7	53.3	81	77

Table VI.- Comparison of ALT levels, ELISA and PCR in case of various age groups in both sexes.

Age groups (Years)	Total cases (No.)	Males	Females	ALT mean (U/L)	ELISA Positive	PCR positive
1-20	16 (9.4%)	7	9	50.3	16 (100%)	10 (62%)
21-40	111 (69.4%)	54	57	72	110 (99%)	74 (66.6%)
41-60	32 (20%)	20	12	58.7	31 (96.8%)	16 (50%)
61 & above	01 (0.6%)	00	01	50	01 (100%)	0

Table VII.- Correlation of PCR and ELISA.

Correlations			ELISA	PCR
Spearman's Rho	ELISA	Corr. coefficient	1.000	.087
		Sig. (2-tailed)	.	.273
		N	160	160
	PCR	Corr. coefficient	.087	1.000
		Sig. (2-tailed)	.273	.
		N	160	160

DISCUSSION

Hepatitis C is a key health issue in the least developed as well as in developing states of the world. The epidemiology of the virus is less understood (Saha *et al*., 2000) and the signs and symptoms of the disease are very mild at the onset of infection. Most of the patients come to know about their status when the disease is in advance stages. In developing countries, the diagnostic facilities are not proper enough to overcome this problem. Anti-HCV is often screened by ICT and ELISA (Ji-Su *et al*., 1995). The usual serological tests are not so sensitive and reliable, therefore molecular techniques (PCR); which is comparatively accurate, highly sensitive and more specific technique than the serological methods, is used for the detection of viral RNA in the sera of the affected individuals. PCR is the gold standard for the correct diagnosis of HCV because it offers definitive identification of HCV replication in anti-HCV positive patients for the diagnosis of infection in immune-compromised patients (Albadalejo *et al*., 1998).

Earlier studies have documented high prevalence of anti-HCV and active HCV infection from various parts of KPK and elsewhere (Ahmed, 2004; Akhtar *et al*., 2013; Swellam *et al*., 2011). In this study, we collected blood samples from various categories of patients including outdoor subjects, indoor, unaware (volunteer blood donors, dental subjects who often visit dentists, patients diagnosed before surgery) and those on anti-viral therapy. All the subjects included in this study had a positive history of anti-HCV by ICT and most of them had elevated ALT levels. Some of the patients had experienced clinical symptoms associated with hepatitis like fatigue, Jaundice, lack of appetite, nausea and abnormal pain. The samples were first analyzed by 3rd generation ELISA which indicated that 98.75% patients were anti-HCV positive while only 2 subjects were negative revealing the fact that anti-HCV determination by ICT strips could be subjected to false positivity as has been reported by other investigators as well (Candai *et al*., 1998; Ali *et al*., 2011).

All the samples (160) were further subjected to RNA extraction followed by real time PCR. The results indicated that 100 (62.5%) patients had active HCV infection and the rest of the subjects were not actively infected. Either variation between seropositivity and active HCV infection could be attributed to the self-limiting nature of the disease in some individuals (Cohen, 1999) false positivity in the case of antibody-based tests (Ali *et al*., 2011) or the detection limit of the assay performed. Statistical analysis indicated that there was no significant correlation between ELISA positivity and PCR positivity indicating the fact that subjects with a positive ELISA for anti-HCV and having elevated ALT levels may not be actively infected and must be examined for HCV RNA prior to initiation of therapy. A simple correlation between seropositivity and active HCV infection in both genders indicate that 70% of seropositive male subjects had HCV RNA in their plasma whereas findings in female subjects exhibited that only 55% of seropositive subjects were positive for HCV RNA. This study is similar to the findings of Ali *et al*. (2011) that also exhibited high frequency of active HCV infection in

male individuals.

A non-significant correlation was observed between the mean ALT levels of the ELISA positive subjects and those who were actively infected, re-enforcing the same claim that the presence of anti-HCV along with elevated ALT level is least informative about active HCV infection (Candai *et al*., 1998), as ALT levels may be raised in the case of a number of other pathologies. The findings relate with the observed findings that HCV infected patients might have either irregular episodes of disease, characterized by periods of elevated ALT levels (Petrelli *et al*., 1994). Several studies have demonstrated that ALT level, one and a half times more than the normal value, could be a sign of histological advanced liver diseases, while others have reported that HCV infected patients had normal ALT levels (Jamal *et al*., 2000). However, It has been indicated earlier that anti-HCV positive patient who is immuno competent, who is not having detectable serum HCV RNA and with normal ALT values and samples showing decrease in the level of HCV antibodies could be considered as a resolved infection (Lanotte *et al*., 1998).

Disagreement between ELISA and PCR results has also been reported from Pakistan (Ahmed, 2004). Our study is not in conformity with Candai *et al*., 1998 who established a positive correlation between anti-HCV ELISA and RT-PCR in the case of subjects who were seropositive for anti-HCV and negative for plasma HCV RNA. However, this positive correlation was established only when PMBCs of the subjects were used for detection of viral RNA. Among the various age groups, active HCV infection was highly prevalent among the juvenile age group (21-40 years). Other regional and international studies have also confirmed earlier that people in the said age group are comparatively more affected with HCV infection (Ali *et al*., 2011) possibly due to increased exposure to a variety of HCV risk factors related to our cultural attributes *i.e.* barbers, surgeries, dentists and sexual aspects etc.

CONCLUSION

Hepatitis C is highly prevalent in Peshawar division of Khyber Pakhtunkhwa Province of Pakistan. Determination of active infection by RT-PCR must be carried out before deciding about treatment options as only the clinical picture, elevated ALT and a positive 3rd generation ELISA test does not tell about active HCV infection.

ACKNOWLEDGEMENTS

We are thankful to Dr. Sami Siraj, Khyber Medical University in the preparation of manuscript and members of Dr. Ijaz Ali lab for their help and cooperation in the research work.

REFERENCES

AHMED, N., 2004. A study of hepatitis C virus and its molecular epidemiology in the local population of Faisalabad region. *Pakistan Res. Reposit.,* p-121.

AKHTAR, A.M., KHAN, M.A., IJAZ, T., IQBAL, Z., RANA, M.Y., MAQBOOL, A. AND REHMAN A., 2013. Seroprevalence and determinants of hepatitis-C Virus infection in blood donors of Lahore, Pakistan. *Pakistan J. Zool.,* **45**: 1-7.

AKHTAR, S., YOUNUS, M., ADIL, S., JAFRI, S.H. AND HASSAN, F., 2004. Hepatitis C virus infection in asymptomatic male volunteer blood donors in Karachi, Pakistan. *J. Viral. Hepat.,* **11**: 527-535.

ALBADALEJO, J., ALONSO, R., ANTINOZZI, R., BOGARD, M., BOURGAULT, A.M., COLUCCI, G., FENNER, T., PETERSEN, H., SALA, E., VINCELETTE, J. AND YOUNG, C., 1998. Multicellular evaluation of the COBAS amplicor HCV essay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic lab. *J. clin. Microbiol.,* **36**: 862-865.

ALI, I., KHAN, N.U., AHMAD, N.U., IQBAL, A., REHMAN, L.U., MUNIR, I., REHMAN, M.U., KHAN, S., ALI, S., SIDDIQUE, L. AND SWATI, Z.A., 2011. Prevalence of active HCV infection among the blood donors of Khyber Pakhtunkwa and FATA region of Pakistan and evaluation of the screening tests for anti-HCV. *Virol J.,* **8**: 15.

ALTER, M.J., KRUSZON, M.D., NAINAN, O.V., MCQUILLAN, G.M., GAO, F., MOYER, L.A., KASLOW, R.A. AND MARGOLIS, H.S., 1994. The prevalence of HCV infection in the United States, 1988 through 1994. *N. Engl. J. Med.,* **341**: 556-562.

AMJAD, A., HABIB, A., IJAZ, A., SHEEMA, K., GULSHAN, Z. AND IDREES, M., 2011. Prevalence of active hepatitis C virus infection in district Mansehra Pakistan. *Virol. J.,* **7**: 334.

CANDAI, C., PANDULA, M.G., BASTIANORI, I., VALENSIN, P.E., SHYAMALA, V., HAN, J.,

BOGGIANO, C.A. AND ALMI, P., 1998. Antibody testing and RT-PCR results in hepatitis C viral infection; HCV RNA detection in PBMC of plasma viremia negative HCV seropositive persons. *Infection*, **3**: 151-4.

CHOO, Q.L., KUO, G., WEINER, A.J., OVERBY, L.R., BRADLEY, D.W. AND HOUGHTON, M., 1989. Isolation of a cDNA clone derived from a blood-borne non A, non-B viral hepatitis genome. *Science*, **244**: 359-362.

COHEN, J., 1999. Scientific challenge of hepatitis C. *Science*, **285**: 26-33.

JAMAL, M.M., SONI, A., QUINN, P.G., WHEELER, D.E., ARORA, S. AND JOHNSTON D.E., 1999. Clinical features of hepatitis C- infected patients with persistently normal Alanine Transaminase level in South West United States. *Hepatology*, **31**: 1307-1311.

JANJUA, K.A. AND RAJA, N.S., 2008. Epidemiology of hepatitis C virus infection in Pakistan. *J. Microbiol. Immunol. Infect*., **41**: 4-8.

JI-SU, LI., TORG, S.P., VITVITISKI, L. AND TREPO, C., 1995. Single step nested PCR for detection of different genotypes of hepatitis C virus. *J. med. Virol.*, **45**: 151-5.

KOTWAL, G.J., BAROUDY, B.M., KURAMOTOT, I.K., MCDONALD, F.F., SCHIFF, G.M., HOLLANDT, P.V. AND ZELDIS, J.B., 1992. Detection of acute Hepatitis C Virus infection by ELISA using a synthetic peptide comprising a structural epitope. *Proc. natl. Acad. Sci.*, **89**: 4486-4489.

LANOTTE, P., DUBOIS, F., LI, P.S., GUEROIS, C., FIMBEL, B., BACQ, Y. AND GRUEL, Y., 1998. The kinetics of antibodies against haptitis C virus may predict viral clearance in exposed hemophiliacs. *J. Infect. Dis.*, **178**: 556-569.

LUBY, S., KHANANI, R., ZIA, M., VELLANI, Z., ALI, M. AND QURESHI, A.H., 2000. Evaluation of blood bank practices in Karachi, Pakistan, and the government's response. *Hlth. Policy Plan*, **15**: 217-222.

PETRELLI, E., MANZIN, A., PAOLUCCI, S., CIOPP, A., BRUGIA, M., MURETTO, P. AND CLEMENTI, M., 1994. CLD and active hepatitis C virus infection in patients with antibodies to this virus. *J. clin. Pathol.*, **47**: 148-151.

REDDY, A.K., DAKSHINAMURTY, K.V. AND LAKSHMI, V., 2006. Utility of HCV core antigen ELISA in the screening for HCV infection in patients on hemodialysis. *Ind. J. med. Microbiol.*, **24**: 55-57.

SAHA, M.K., CHAKRABARTI, S., PANDA, S., NAIK, T.N., MANNA, B., CHATTERJEE, A., DETELS, R. AND BHATTACHARYA, S.K., 2000. Prevalence of HCV and HBV infection amongst HIV seropositive intravenous drug-user and their non-injecting wives in Mirpur India. *Indian J. med. Res.*, **111**: 37-39.

SWELLAM, M., MAHMOUD, M.S. AND ALI, A.A., 2011. Diagnosis of hepatitis C virus infection by enzyme-linked immunosorbent assay and reverse transcriptase-nested polymerase chain reaction: A comparative evaluation. *IUBMB Life*, **63**: 430-434.

(Received 13 June 2013, revised 12 September 2013)