

Tuberculosis and Hepatitis Infections among the Underprivileged Orphan Children of Northern Pakistan

Abdul Rauf,^{1,2} Muhammad Shahid Nadeem,^{1,3} Haroon Riaz,^{1,4} Muhammad Muzammal Latif,⁴ Muhammad Zahid Latif,⁴ Nisar Ahmed⁵ and Abdul Rauf Shakoori^{1,*}

¹School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan.

²Department of Zoology, University of Azad Jammu and Kashmir Muzaffarabad. Pakistan.

³Department of Genetics, Hazara University, Mansehra 21300. Pakistan.

⁴Healthcare Diagnostics and Research Centre, Allama Iqbal Town, Lahore, Pakistan

⁵Human Appeal International (HAI), Sector G-9/2, Islamabad, Pakistan.

Abstract.- The hepatitis B, C and tuberculosis infections were not investigated in orphan children with low socioeconomic status living in the northern Pakistan. A total of 542 (292 male and 250 female), randomly selected orphan children with an age limit between 5 to 17 years were included in this study with informed consent. The subjects were screened for Anti HBsAg, Anti HCV and anti tuberculosis antibodies using immune-chromatography kits. Those found positive for initial screening were further confirmed by PCR amplification of RNA and DNA of related pathogens. HCV virus was also investigated for its genotypes. Among the total investigated subjects, 9.6% were found positive against HCV, 8.3% were found positive against TB and only one positive case against HBV was found. The genotype 3a was detected in majority PCR positive samples followed by genotype 2a> an unknown genotype >1a>1b and 2b. The higher frequency of viral hepatitis C and TB among the orphan children refers to an alarming situation about these infections and health care conditions of underprivileged orphan children in northern Pakistan.

Keywords: Hepatitis C, hepatitis B, tuberculosis, poor health conditions, orphan children, Northern Pakistan.

INTRODUCTION

Tuberculosis is one of those infectious diseases that have caused severe threats to human health since the times olden civilizations (Duraiswami and Tuli, 1991; Dhillon and Tuli, 2001). According to the recent estimates about 32 percent of the world's population is infected with *Mycobacterium tuberculosis* and it kills 1.7 million people every year world-wide (Dye *et al.*, 1999; Asif *et al.*, 2011). The association of tuberculosis with poverty is well documented (Link and Phelan 1995) and the disease has received considerable attention in the developing countries in the recent years. According to the estimates about 95% cases and 98% deaths from TB are reported from developing countries (Pio *et al.*, 1999; Rajeswari *et al.*, 1999). Similarly, hepatitis B and C infections are also a serious threat to human life world-wide. Worldwide, 53% of HCC cases are attributed to

chronic HBV infection (Perz *et al.*, 2006). It has been estimated that about 350 million people worldwide have been infected with hepatitis B virus (Ganem *et al.*, 2006). Hepatitis C infections are a rapidly increasing health problem of today. According to the recent estimates of World Health Organization (WHO), about 170 million people (some 3% of the world's population) are infected with hepatitis C virus (HCV) (George *et al.*, 2001; Higuchi *et al.*, 2001). There are several genotypes of HCV reported in the literature with nine well studied genotypes (Ohno *et al.*, 1997).

Collecting and comparing health data from all over the country can describe health problems and help decision-makers to set priorities. The global epidemiology of most of the infectious diseases is well established. However, in developing countries, like Pakistan, there is no solid mechanism to estimate the prevalence of such diseases among the different social groups of population accurately. Most of the data is obtained from the hospital-based studies with patients, because there is a dearth of community based studies (Khan 2005; Alavian *et al.*, 2007; Sultan *et al.*, 2007). The studies with normal population of children in Pakistan have

* Corresponding author: arshaksbs@yahoo.com,
arshakoori@sbs.pu.edu.pk

0030-9923/2013/0006-1765 \$ 8.00/0

Copyright 2013 Zoological Society of Pakistan

reported a serofrequency ranging from 0.4% to 4.1% HCV (Akram *et al.*, 1995; Khan, 1996; Hyder *et al.*, 2001; Jafri *et al.*, 2006). However, there is a severe need to collect data from the population groups having very poor living conditions, food and health facilities.

In developing countries where the health facilities are very limited for the common man, the condition of deprived and neglected orphan children is extremely miserable that constitute a high risk group for acquisition of TB and other infectious diseases because of their living conditions, poor dietary conditions and generally low socio-economic status (Saiman *et al.*, 2001; Francis *et al.*, 2002; Braitstein *et al.*, 2009). Our ongoing research project is aimed to study the prevalence of infectious diseases among the healthy populations in different areas (Rauf *et al.*, 2011) Present study describes the epidemiology of Tuberculosis, hepatitis B and C among the orphan children residing in northern Pakistan.

METHODS

Subjects and methodology

The study was conducted on apparently healthy 542 male and female orphan children residing in northern areas of Pakistan.

A hepatitis B,C and tuberculosis screening study was conducted among the orphan population of male and female children in the Northern Areas of Pakistan. These orphans were randomly selected and were being financially supported by Human Appeal International (HAI) for their education and living. Besides screening for the antigen and anti body for these diseases, other symptoms such as pain in upper right part of abdomen, anorexia, nausea, dyspepsia, vomiting, fever and jaundice were also recorded for hepatitis B and C, while poor health, coughing, loss of body weight were recorded in tuberculosis antibody positive cases. The samples found positive in the screening were further confirmed by Polymerase Chain Reaction (PCR).

For primary screening, the blood samples were obtained by veno-punctures, and the sera were separated from the coagulated blood by centrifugation at 5,000 rpm for 10 minutes at room temperature. The HBV screening was based on the

detection of HBsAg while HCV and TB screening was based on the detection of antibodies against the related virus and bacteria in the sera. For primary screening of anti HCV and anti HBsAg, screening kits (ICT: ACON®, ACON Laboratories Inc., San Diego, CA 92121, USA) were used. Distinct®, USA was used for identification of anti MTB.

The serum sample from antigen positive individual was used for DNA isolation with DNAzol® BD using the procedure described by the manufacturer. Isolated DNA was used for PCR reaction using following primers in the first round:

5'-catcctgctgctatgccctatct-3' and
5'-cgaaccactgaacaaatggcact-3'.

The PCR product of the first round was then used as template for the nested PCR based amplification using the following primers:

5'-ggtatgttgcctctct-3' and
5'-ggcactagtaaactgagcca-3'.

The second round primers were selected after the inner regions of the DNA fragment amplified in the first PCR reaction. The reaction mixture for the first and second round contained 1.5 mM MgCl₂, 0.2 mM dNTPs, 40 pmol of both primers, and 2.5 U of Taq polymerase. The thermocycler was adjusted at 94°C for denaturation for 2 min, followed by 30 cycles, each of 30 seconds of denaturation at 94°C, annealing for 40 sec at 53°C and 30 sec at 72°C with final extension at 72°C for 1 min. Both rounds of PCR were carried out under the same PCR conditions.

For confirmation of TB in the above screened positive samples, 250µL of blood sample was mixed with 500 µL of DNAzol®BD. Cat. No. DN129 (a Product of Molecular Research Centre, Inc., Cincinnati, OH, USA) for DNA isolation. The protocol was followed as described by the manufacturer. The isolated DNA was dissolved in 50 µL of nuclease free water and used as template in the Polymerase Chain Reaction (PCR) for the detection of *Mycobacterium tuberculosis*. Following set of reverse and forward primers was used in the PCR amplification of *Mycobacterium* DNA:

TB-1	5' gtg cgg atg gtc gca gag at	3'
TB-2	5' ctc gtc cag cgc cgc ttc g	3'
TB-3	5' ctc gat gcc ctc acg gtt ca	3'
TB-4	5' cct gcg agc gta ggc gtc gg	3'

The PCR reaction mixture (20 μ L) contained 1mM dNTPs, 2mM MgCl₂, 1 μ l each of 40 pM of each of the forward and reverse primers, 1X Taq buffer, 1U of Taq polymerase. The reaction was prepared in nuclease free water and 10 ng of DNA template was used in the reaction. The PCR-thermocycler was adjusted at 94°C (denaturation) for five minutes, followed by 35 cycles, each of 30 seconds at 94°C (denaturation), 40 seconds at 45°C (annealing) and 30 seconds at 72°C (extension) with final extension at 72°C for 5 min.

For detection of HCV RNA among the positive subjects in the antibody screen test, total RNA was isolated from blood samples with Trizol-reagent kit (Invitrogen). The complementary DNA (cDNA) was generated by gene-specific reverse primers with reverse transcriptase polymerase chain reaction (RT-PCR) involving Moloney Murine Leukemia Virus (MuLV) reverse transcriptase at 37°C for 50 min. The viral RNA was detected with nested PCR. The primers used for the first PCR-amplification were as follows:

HCVF1: 5'-ccctgtgaggaactactgtcttcacgc-3'

HCVR1: 5'-actcgcaagcaccctatcaggcagctac-3',

Whereas, the primers used for amplification of the internal region were:

HCVF2: 5'-gaaagcgtctagccatggcg-3'

HCVR2: 5'-cacaaggccttcgcgacc-3'

The 20 μ L reaction mixture for the first and the second round of PCR was prepared with 1mM dNTPs, 2mM MgCl₂, 40pM of each of the primers, 1unit of Taq polymerase, 4 μ L cDNA sample prepared by RT-PCR was used as the template in the first reaction whereas 1 μ L of PCR product after the completion of reaction one was used as the template in the second reaction to amplify the internal region of DNA fragment obtained from viral cDNA. The second reaction is basically a confirmation of the nature of viral cDNA. The PCR-thermocycler was adjusted at 94°C for denaturation for five minutes, followed by 35 cycles, each of 30 seconds at 94°C (denaturation), 40 seconds at 64°C (annealing) and 30 seconds at 72°C (extension) with final extension at 72°C for 5 min. The nested PCR was carried out under the same PCR conditions with an annealing temperature at 53°C. The double

stranded DNA obtained from single stranded cDNA was visualized on 1.5 % agarose gel for determining the presence or absence of hepatitis C virus in the blood serum.

Fresh serum samples were used for isolation of RNA as described above for HCV PCR. Viral cDNA was generated with RT-PCR using reverse transcription PCR method with core-gene specific reverse primer. The genotype was identified with multiplex-PCR. Two reaction mixtures containing two sets of primers each were processed in parallel in the nested PCR. PCR-thermocycler was adjusted at 94°C for denaturation for five minutes, followed by 30 cycles, each of 30 seconds at 94°C (denaturation), 40 seconds at 64°C (annealing) and 30 seconds at 72°C (extension) with final extension at 72°C for 5 min. The nested PCR was carried out under the same PCR conditions as above, except for the annealing temperature which was 53°C. The details of primers and the expected sizes of the PCR products are shown in Table I.

RESULTS

Prevalence of tuberculosis, HCV and HBV

In the present study, 52 out of the 542 investigated normal orphan children (292 males and 250 females) of 5 to 17 years of age were found positive for anti-hepatitis C (anti-HCV), thereby indicating a 9.6% infected subjects. Among the total anti-HCV positive samples 32 were males (5.5% of total) and 20 were females (4.1% of total). A total of 45 subjects (8.3% of total subjects) were found positive for TB as analysed by antibody based and culture based diagnosis. The positive subjects for TB in the first screening included 26 males (4.8% of total) and 19 females (3.5% of total). Only one male person was found positive for HBV.

PCR based HCV genotyping

The anti-HCV positive samples were further investigated for the presence and types of hepatitis C related RNA using nested PCR method. Of the 52 HCV antibody test positive subjects, 32 (5.5%) were found positive for the presence of hepatitis C RNA in the serum, 23 (4.24%) of which were males and 9 (1.66%) were females. The PCR-positive samples were further investigated for the HCV genotypes. The genotype 3a was detected in 11 samples (34.3%

Table I.- The details of primers for genotyping as described by Ohno *et al.* (1997).

Primer*	Nucleotide Sequence (5'–3')	Expected position	Band size (bp)
Sc2	GGGAGGTCTCGTAGACCGTGCACCATG	-24–3	441
Ac2 1	GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC	417–391	
Mix 1			
S7	AGACCGTGCACCATGAGCAC	-12–8	
S2a	AACACTAACCGTCGCCACAA	40–60	
G1b	CCTGCCCTCGGGTTGGCTA(AG)	222–203	234
G2a	CACGTGGCTGGGATCGCTCC	178–159	139&190
G2b	GGCCCAATTAGGACGAGAC	325–306	337
G3b	CGCTCGGAAGTCTTACGTAC	164–145	176
Mix 2			
S7	AGACCGTGCACCATGAGCAC	-12–8	
G1a	GGATAGGCTGACGTCTACCT	196–177	208
G3a	GCCCAGGACCGGCCTTCGCT	220–211	232
G4	CCCGGAACTTAACGTCCAT	87–58	99
G5a	GAACCTCGGGGGGAGAGCAA	308–289	320
G6a	GGTCATTGGGGCCCAATGT	334–315	346

*Abbreviations used in the names of primers: S, sense; A or G, antisense; c, core region. The notations 1a to 6a are in accordance with the HCV genotype nomenclature proposed by Simmonds *et al.* (1994).

of the PCR positive samples) followed by genotype 2a in 8 samples (25%), an unknown genotype detected in 6 samples (18.8%), genotype 1a in 3 sample (9.4%), 1b in 3 samples (9.4%) and genotype 2b was found in only 1 sample (3.1%). No other genotype was detected in the samples (Figures of gels are not being included). Out of 52 HCV positive samples 13 were also positive for TB.

PCR based diagnosis of TB

DNA of *Mycobacterium tuberculosis* was detected in the serum of 27 (5.9%) subjects out of 45 found positive for TB by antibody and culture tests. Among the PCR positive subjects 18 (3.3%) were males and 9 (1.7%) were females. The results are shown in Table II.

Table II.- prevalence of HCV and TB in male and female orphan children.

	Overall	Males	Females
Anti HCV	52 (9.6%)	30 (5.5%)	22 (4.1%)
HCV-PCR	32 (5.9%)	23 (4.24%)	9 (1.66%)
Anti HBsAg	1 (0.0018 %)	1 (0.0034%)	0
HBV-PCR	1 (0.0018%)	1 (0.0034%)	0
Anti TB	45 (8.3%)	26 (4.8 %)	19 (3.5%)
TB PCR	27 (5.0%)	18 (3.3%)	9 (1.7%)

DISCUSSION

Hepatitis B, C and Tuberculosis are widespread infectious diseases representing major health problems. Several factors, such as the social status of the specific group or population, living and nutritional conditions influence the prevalence of these infections in a particular community (Sherlock, 1997). The orphan and neglected children, especially in the developing countries often have to live under the conditions that facilitate the spread of infectious diseases. In the present study, a total of 542 orphan children of 5 to 17 years of age were investigated for hepatitis B, C and tuberculosis infections. Overall 9.6% of the total subjects were found infected with HCV and 8.3% were found positive for tuberculosis. The results of our study are different from other studies carried out in Pakistan with the general population (Hussain *et al.*, 2003; Ali *et al.*, 2009). Waheed *et al.* (2009) have reported 1.72±0.24% prevalence of HCV in paediatric population of Pakistan. In a population however, a substantial number of patients remain asymptomatic and are never clinically identified. Studies conducted in healthy general populations in Turkey, Zimbabwe and Pakistan revealed the hepatitis C sero-prevalence of 2.2, 7.7 and 5.3%,

respectively (Demirturk *et al.*, 2006; Gangaidzo *et al.*, 1997; Khokhar *et al.*, 2004).

The main cause of spread of tuberculosis among the subjects of the present study are poor sanitary, hygienic and socio-economic conditions of the community and orphan houses. The reuse of syringes and needles is major factor contributing towards increased HCV prevalence (Simonsen *et al.*, 1999; Khan *et al.*, 2000). Although the main route of transmission of HCV is via contaminated blood, but in many cases no recognizable transmission factor/route could be identified. A number of other routes of transmission such as sexual or household exposure to infected contacts have been investigated with conflicting results (Alavi and Hajiani, 2011)

In the present study hepatitis B infection is found to be very low and only one case of HBV was found. Ali *et al.* (2011) have reported 4.3318±1.644% prevalence of HBV in the general population of Pakistan which is higher than our reported prevalence of HBV in orphan children. The low prevalence of HBV in the studied group may be due to minimal exposure to HBV transmission incidents because of young age of the subjects as well as the HBV vaccination carried out by the government and non government organization in those areas.

ACKNOWLEDGEMENTS

Ethical Committee of School of Biological Sciences, University of the Punjab, Lahore. This work was supported by Higher Education Commission, Islamabad, Pakistan through its "Indigenous Ph.D. Fellowship Programme" under which a fellowship was awarded to A.R., School of Biological Sciences, University of the Punjab, Lahore, and Human Appeal International (HAI), Sector I, Islamabad, Pakistan.

Conflict of interests statement

None of the authors has any conflict of interest regarding any financial and personal relationships with other people or organizations that could inappropriately influence this work. One of the authors (N.A.) who is working as an employee in a non-governmental organization, Human Appeal International (HAI) based in Sector I of Islamabad,

managed logistic support, courtesy HAI, for getting access to the orphan children in difficult areas of northern Pakistan.

REFERENCES

- AKRAM, D.S., ISOMARA, S., AGBOATWALA, M., 1995. The prevalence of antibodies to hepatitis A, B and C virus in children of Karachi. *J. Coll. Physic. Surg. Pak.*, **5**: 17-18.
- ALAVI, S.M. AND HAJIANI, E., 2011. Hepatic C infection: a review on epidemiology and management of occupational exposure in health care workers for general physicians working in Iranian health network setting. *Jundishapur J. Microbiol.*, **4**: 1-9.
- ALAVIAN, S.M., FALLAHIAN, F. AND BAGHERI-LANKARANI, K.B., 2007. Comparison of sero-epidemiology and transmission modes of viral hepatitis B in Iran and Pakistan. *Hepatit. Month.*, **7**: 233-238.
- ALI, M., IDREES, M., ALI, L., HUSSAIN, A., REHMAN, I.U., SALEEM, S., AFZAL, S. AND BUTT, S., 2001. Hepatitis B virus in Pakistan: A systematic review of prevalence, risk factors, awareness status and genotypes. *Virol. J.* **8**: 102
- ALI, S.A., DONAHUE, R.M.J., QURESHI, H. AND VERMUND, S.H., 2009. Hepatitis B and hepatitis C in Pakistan: prevalence and risk factors. *Int. J. Infect. Dis.*, **13**: 9-19.
- ASIF, H.M., AKRAM, M., RAO, S.A., AHMAD, I., AWAN, A., SHAMSHAD, N., SHABBIR, A. AND SALEEM, Q.E., 2011. Tuberculosis: A case study of Pakistan. *Afr. J. Microbiol. Res.*, **5**: 4029-4032.
- BRAITSTEIN, P, NYANDIKO W, VREEMAN, R., WOOLSKALOUSTIAN, K., SANG, E., MUSICK, B., SIDLE, J., YIANNOUTSOS, C., AYAYA, S. AND CARTER, E.J., 2009. The clinical burden of tuberculosis among human immunodeficiency virus-infected children in Western Kenya and the impact of combination anti-retroviral treatment. *Pediatr. Infect. Dis. J.*, **28**:626-632.
- DEMIRTURK, N., DEMIRDAL, T., TOPRAK, D., ALTINDIS, M. AND AKTEPE, O.C., 2006. Hepatitis B and C in west central Turkey: Seroprevalence in healthy individuals admitted to a university hospital for routine health checks. *Turk. J. Gastroenterol.*, **17**: 267-272.
- DHILLON, M.S. AND TULI, S.M., 2001. Osteoarticular tuberculosis of the foot and ankle. *Foot, Ankle Int.* **22**: 679-686.
- DURAIKWAMI, P.K. AND TULI, S.M., 1991. Five thousand years of orthopaedics in India. *Clin. Orthop.*, **75**:269-280.
- DYE, C., SCHEELE, S., DOLIN, P., PATHANIA, V. AND RAVIGLIONE, M.C., 1999. Global burden of tuberculosis. *J. Am. med. Assoc.*, **282**: 677-686.
- FRANCIS, J., REED, A., YOHANNES, F., DODARD, M. AND FOURNIER, A.M., 2002. Screening for

- tuberculosis among orphans in a developing country. *Am. J. Prev. Med.*, **22**:117-119
- GANEM, D. AND SCHNEIDER, R.J., 2006. The molecular biology of the hepatitis B viruses. In: *Fields virology* (eds. D. M. Knipe, P. M. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B. Roizman and S.E. Straus). 4th ed., Lippincott Williams & Wilkins, Philadelphia, Pa, pp. vol. 2. 2923-2970.
- GANGAIDZO, I.T., MOYO, V.M., KHUMALO, H., SAUNGWEME, T., GOMO, Z.A.R., RONALT, T, GORDFENK, V.R., 1997. Hepatitis C virus in Zimbabwe. *Cent. Afr. J. Med.*, **43**: 122-125.
- GEORGE, M., LAUER, M.D., BRUCE, D. AND WALKER, M.D., 2001. Hepatitis C virus infection. *N. Engl. J. Med.*, **345**: 41-52
- HIGUCHI, M., TANAKA, E. AND KIYOSAWA, K., 2002. Epidemiology and clinical aspects on hepatitis C. *Jpn. J. Infect. Dis.*, **55**: 69-77.
- HUSSAIN, H., AKHTAR, S. AND NANAN, D., 2003. Prevalence of and risk factors associated with *Mycobacterium tuberculosis* infection in prisoners, North West Frontier Province, Pakistan. *Int. J. Epidemiol.*, **32**: 794-799.
- HYDER, S.N., HUSSAIN, W., ASLAM, M. AND MAQBOOL, S., 2001. Seroprevalence of anti-HCV in asymptomatic children. *Pak. J. Pathol.*, **12**: 89-93.
- JAFRI, W., JAFRI, N., YAKOOB, J., ISLAM, M., TIRMIZI, S.F.A., JAFAR, T., AKHTAR, S., HAMID, S., SHAH, H.A. AND NIZAMI, S.Q., 2006. Hepatitis B and C: prevalence and risk factors associated with seropositivity among children in Karachi, Pakistan. *BMC Infect. Dis.*, **6**:101.
- KHAN, H., 2005. Threat of hepatitis B and C: who will take the action. *Gomal J. med. Sci.*, **3**:2-5
- KHAN, H.I., 1996. A study of seroprevalence of hepatitis B and C in mothers and children in Lahore. *Pak. Pediatr. J.*, **20**:163-166
- KHAN, A.J., LUBY, S.P., FIKREE, F., KARIM, A., OBAID, S., DELLAWALA, S., MIRZA, S., MALIK, T., FISHER-HOCH, S. AND McCORMICK, J.B., 2000. Unsafe injections and the transmission of hepatitis B and C in a periurban community in Pakistan. *Bull. Wld. Hlth. Org.*, **78**: 956-963.
- KHOKHAR, N., GILL, M.L. AND MALIK, G.J., 2004. General seroprevalence of hepatitis C and hepatitis B virus infections in population. *J. Coll. Physic. Surg. Pak.*, **14**: 534-536.
- LINK, B.G. AND PHELAN, J., 1995. Social conditions as fundamental causes of diseases. *J. Hlth. Soc. Behav.*, **42**: 80-84
- OHNO, T., MIZOKAMI, M., WU, R.R., SALEH, M.G., OHBA, K.I., ORITO, E., MUKAIDE, M., WILLIAMS, R. AND LAU, J.Y.N., 1997. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J. clin. Microbiol.*, **35**: 201-207.
- PERZ, J.F., ARMSTRONG, G.L., FARRINGTON, L.A., HUTIN, Y.J. AND BELL, B.P., 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J. Hepatol.*, **45**:529-538.
- PIO, A., LUELMO, F., KUMARESAN, J. AND SPINACI, S., 1999. National tuberculosis program review: experience over the period 1990-1995. *Bull. Wld. Hlth. Org.*, **75**: 569-581
- RAJESWARI, R, BALASUBRAMANIAN, R, MUNIYANDI, M., GEETHARAMANI, S., THRESA, X. AND VENKATESAN, P., 1999. Socioeconomic impact of tuberculosis on patients and family in India. *Int. J. Tuberc. Lung Dis.*, **3**: 869-877.
- RAUF, A., NADEEM, M.S., ALI, A., IQBAL, M., MUSTAFA, M., LATIF, M.M., AHMED, N. AND SHAKOORI, A.R., 2001. Prevalence of hepatitis B and C in internally displaced persons of war against terrorism in Swat, Pakistan. *Eur. J. Publ. Hlth.*, **21**: 638-642
- SAIMAN, L., ARONSON, J., ZHOU, J., GOMEZ-DURATE, C., GABRIEL, P.S., ALONSO, M., MALONEY, S. AND SCHULTE, J., 2001. Prevalence of infectious diseases among internationally adopted children. *Pediatrics*, **108**: 608-612
- SHERLOCK S., 1997. Chronic hepatitis. In: *Diseases of the liver and biliary system* (eds. S. Sherlock and J. Dooley). 10thed. Blackwell Science Ltd, London, pp. 303-336.
- SIMMONDS, P., ALBERTI, A., ALTER, H.J., BONINO, F., BRADLEY, D.W., BRECHOT, C., BROUWER, J.T., CHAN, S.W., CHAYAMA, K., CHEN, D.S., CHOO, Q.L., COLOMBO, M., CUYPERS, H.T.N., DATE, T., DUSKEIKO, G.M., ESTEBAN, J.I., FAY, O., HADZIJANNIS, S.J., HAN, J., HATZAKIS, A., HOLMES, E.C., HOTTA, H., HOUGHTON, M., IRVINE, D., KOHARA, M., KOLBERG, J.A., KUO, G., LAU, J.Y.N., LELIE, P.N., MAERTENS, G., MCOMISH, F., MIYAMURA, T., MIZOKAMI, M., NOMOTO, A., PRINCE, A.M., REESINK, H.W., RICE, C., ROGGENDORF, M., SCHALM, S.W., SHIKATA, T., SHIMOTOHNO, K., STUYVER, L., TREPO, C., WEINER, A., YAP, L.P. AND URDEA, M.S., 1994. A proposed system for the nomenclature of hepatitis C viral genotypes. *Hepatology*, **19**: 1321-1324.
- SIMONSEN, L., KANE, A., LLOYD, J. ZAFFRAN, M. AND KANE, M., 1999. Unsafe injections in the developing world and transmission of blood borne pathogens: a review. *Bull. Wld. Hlth. Org.*, **77**: 789-800.
- SULTAN, F., MEHMOOD, T. AND MAHMOOD, M.T., 2007. Infectious pathogens in volunteer and replacement blood donors in Pakistan: a ten-year experience. *Int. J. Infect. Dis.*, **11**: 407-412
- WAHEED, Y., SHAFI, T., SAFI, S.Z. AND QADRI, I., 2009. Hepatitis C virus in Pakistan: A systematic review of prevalence, genotypes and risk factors. *Wld. J. Gastroenterol.*, **15**: 5647-5653.

(Received 3 January 2013, revised 7 December 2013)