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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.05.007

Seroprevalence of HDV among non-hospitalized HBsAg positive patients from KPK-region of Pakistan

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ARTICLE INFO

Article history:
Received 25 Jan 2016
Received in revised form 15 Feb, 2nd revised form 24 Feb 2016
Accepted 28 Mar 2016
Available online 31 May 2016

Keywords:
HBV
HCV
HDV
Risk factors
Co-infection

ABSTRACT

Objective: To study the seroprevalence of hepatitis B virus (HBV) and hepatitis delta virus (HDV) infections in patients visiting outpatient department of a major tertiary care hospital in Khyber Pakhtunkhwa region of Pakistan.

Methods: Blood samples were collected from non-hospitalized patients. Serological analysis was done by ELISA and viral DNA was amplified by PCR. The amplified DNA was analyzed by agarose gel electrophoresis.

Results: Altogether, 946 blood samples were screened, overall percentage of HBsAg-positive patients remained 22.41% (prevalence: 224.10/1 000; *CI*: 0.197 5 ± 0.250 7) with the highest incidence rates among relatively younger age groups (20–29 years). The prevalence of HBV–HDV co-infection was found to be 46.75/1 000; *CI*: 0.031 8 ± 0.061 7. In HBsAg-positive patients, anti-HBc-total was detected in 86.79% while 25.00% were positive for anti-HBc-immunoglobulin M. Similarly, among these patients, HBV DNA was detected in 64.13% and 10.85% were co-infected with HDV. Different symptoms were associated with the prevailing infection, including malaise (62%), anorexia (66%) and fatigue (73%). The most commonly associated symptom was abdominal discomfort. Among these patients, certain risk factors, including surgery, visit to dentist and intravenus infusions were frequently associated with the infection ($\chi^2 = 95.23$; $df = 11$; $P < 0.000\,1$).

Conclusions: Overall, this study confirmed higher prevalence of active HBV/HDV infection, among young patients from Khyber Pakhtunkhwa region having no prior history of viral hepatitis.

1. Introduction

Hepatitis delta virus (HDV) is a defective negative-sense RNA virus that requires hepatitis B virus (HBV) for its assembly [1]. The genome of HDV is circular and consists of 1 700 nucleotides in length [2]. Both HBV and HDV have the same route of transmission [3]. It is estimated that out of 350 million HBV carriers, 18 million people are co-infected with HDV [4]. Co/super-infections due to HDV leads to severe acute liver disease and increases the risk of developing fulminant hepatitis [5]. Different studies conducted around the globe reported varying prevalence rates of HDV. For example, in Middle-East prevalence rate was reported to be 3.3% among the general population, while particularly in Saudi-Arabia, it was 8.6% in the hospitalized patients. In Oman, up to 7.7% of the registered dialysis patients were infected with HDV [6,7]. Similarly, from China prevalence rates were reported to be 7.2% in Shandong and 3.5% in Henan provinces [8].

From Pakistan different prevalence rates for HDV infection have been reported, ranging from 16.5% to 58.6% [9,10]. However, with the exception of few reports, most of these studies focused on stratified groups like hepatitis patients or patients at higher risk of HDV infection. Hence, little is known about the prevalence of

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The study protocol was performed according to the Helsinki declaration and approved by institutional research and ethics board of Hayatabad Medical Complex. A formal consent was obtained from each patient or his/her guardian/parent.

Foundation Project: Partly supported by Higher Education Commission, Pakistan (IPFP. 3782) and internal research funds of Qauid-i-Azam University Islamabad.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

HBV/HDV infection in general population of Pakistan. This study was carried out in the Khyber Pakhtunkhwa (KPK) Province of Pakistan and highlights the current prevalence rates of HBV/HDV infection and associated risk factors among patients having no prior history of viral hepatitis.

2. Materials and methods

2.1. Study population and sampling site

This cross-sectional study was carried out from August 2011 to August 2013. Sampling was conducted at outpatient department of Hayatabad Medical Complex (HMC) Peshawar, located in KPK region of Pakistan. Peshawar city is the provincial capital with a multi-ethnic assemblage and comprises a representative population originating from all areas of KPK. The study protocol was designed according to the Helsinki declaration. Ethical approval was granted by institutional research and ethics board of HMC. Prior to the collection of samples, a formal consent was obtained from each patient or his/her guardian/parent. A detailed questionnaire was designed to collect demographic profile, including age, gender, socioeconomic status, anthropometric data, vaccination history, and information related to various risk behaviors. HMC is a major tertiary care hospital that receives a large influx of patients from all corners of KPK region.

2.2. Inclusion criteria and sampling procedure

Patients visiting outpatient department having no prior history of viral hepatitis were included in this study while previously vaccinated individuals against HBV were excluded. Peripheral blood samples (4.0 mL) were collected by using 5.0 mL sterile BD syringe. A brief centrifugation step was performed for serum separation.

2.3. Serological analysis

Patients with elevated liver function tests were included for further evaluation based on relevant serological and molecular parameters. Samples were screened for the detection of HBsAg. Positive samples were evaluated for anti-HBc-IgM, anti-HBc-IgG, anti-HDV and anti-HCV. For this purpose, commercially available ELISA kits (MBS-SRL, Milano, Italy) were used according to the instructions provided by the manufacturer.

2.4. DNA extraction and amplification

Viral DNA was extracted by using commercially available kits (Sacace Biotechnologies S.r.l, Italy). For the amplification of DNA, previously reported sets of primers were used, amplification conditions were essentially the same as previously reported [11]. DNA fragments were resolved on 1% agarose gels and size of each amplified product was estimated by comparing it with 1 kb DNA ladder (Solis BioDyne).

2.5. Statistical analysis

The data were recorded in MS-excel and the standard descriptive analysis was done by using MS-excel and GraphPad Prism software (ver. 5). The distributions of various attributes of infections were explored across different demographic variables. Distributions were separately established for the male and female samples. Co-infections, clinical details, and risk factors were analyzed accordingly. *Chi*-test was performed to check the significance of distributions among different variables.

3. Results

3.1. Sample characteristics and prevalence of HBV

A total of 946 non-hospitalized patients were screened out of which 53% (n = 501) were males and 47% (n = 445) females. Overall the prevalence rate of HBsAg was 22.41% (prevalence: 224.10/1 000; *CI*: 0.1975 ± 0.2507, shown in Table 1). Relatively higher prevalence of HBV infection was observed in the male patients (prevalence: 227.54/1 000) in comparison to the female patients, prevalence: 220.22/1 000; OR: 1.04 (Tables 1 and 2). However, differences between the distributions of gender specific samples were not significant statistically ($P > 0.05$). To estimate the prevalence of HBV infection among different age groups, data were divided into six age categories. The prevalence of HBV infection was highest among younger age groups (Table 1). Although, a declining trend of HBV prevalence was evident between different age-intervals, differences in overall distribution of HBV infection across different age-groups were not significant statistically ($\chi^2 = 4.57$, $df = 5$; $P = 0.47$). No significant differences were evident when infected individuals were cross-tabulated in different age categories against gender (shown in Table 2).

3.2. Prevalence of co-infection with HDV and HCV

The prevalence of co-infection, *i.e.*, HBV with either HCV or HDV was witnessed in 36 patients (Table 2). Among the co-infected patients, 19 were males and 17 females. Overall, 10.85% (23/212) of the samples were reactive against HDV and 7.55% (16/212) against HCV. Out of all HDV infected patients, 43.48% (10/23) were males while 56.52% (13/23) were females ($\chi^2 = 0.851$; $df = 1$; $P = 0.356$) (Table 2). Unlike HDV infected individuals, higher percentage of HCV positive individuals 62.5% (10/16) were males in comparison to 37.5% (6/16) females ($\chi^2 = 0.595$; $df = 1$; $P = 0.441$; not significant).

3.3. Detection of anti-HBc-total antibodies and viral DNA

Among the 212 HBsAg-positive patients, anti-HBc-total was detected in 86.79%. Likewise, anti-HBc-IgM was detected in 25% of the patients out of which four (7.53%) were positive for anti-HDV and three (5.66%) for anti-HCV (Table 3). Viral DNA was detected in 64.13% (n = 136) of the HBsAg-positive patients and 11.51% (n = 16) of these were co-infected with HDV. Similarly, 5.14% (n = 7) of these patients were co-infected with HCV. Majority of the patients positive for HBV DNA were young males. Among co-infected (with either HDV or HCV),

Table 1
Prevalence of HBV in total, gender-wise and age-wise samples of scrutinized population.

Prevalence of hepatitis		Infected	Uninfected	Total	Prevalence/1 000	Proportion	95% *CI*	Odd ratios*
	HBV	212	734	946	224.10	0.224 1	0.197 5 ± 0.250 7	16.788 2
	HDV	23	923	946	24.31	0.024 3	0.014 5 ± 0.034 1	1.448 4
	HCV	16	930	946	16.91	0.016 9	0.008 7 ± 0.025 1	Reference
	HBV + HCV/HDV	36	734	770	46.75	0.046 8	0.031 8 ± 0.061 7	2.850 8
Gender-wise	HBV (male)	114	387	501	227.54	0.227 5	0.190 8 ± 0.264 3	1.043 0
	HBV (female)	98	347	445	220.22	0.220 2	0.181 7 ± 0.258 7	Reference
Age-categories (years)	Up to 9	19	57	76	250.00	0.250 0	0.152 6 ± 0.347 4	1.518 5
	10–19	62	189	251	247.01	0.247 0	0.193 7 ± 0.300 4	1.494 4
	20–29	65	202	267	243.45	0.243 4	0.192 0 ± 0.294 9	1.465 8
	30–39	32	143	175	182.86	0.182 9	0.125 6 ± 0.240 1	1.019 4
	40–49	18	82	100	180.00	0.180 0	0.104 7 ± 0.255 3	Reference
	50 and above	16	61	77	207.79	0.207 8	0.117 2 ± 0.298 4	1.194 9

*: The least value in each category was taken as reference.

Table 2
Gender differentials in the distribution of infections and age-specific samples.

Infection type/variable		Male	Female	Total
Hepatitis B	HBV	114	98	212
	Non-HBV	387	347	734
	Total	501	445	946
		$\chi^2 = 0.073$, $df = 1$, $P = 0.788$, NS		
Hepatitis D	HDV	10	13	23
	Non-HDV	491	432	923
		$\chi^2 = 0.851$, $df = 1$, $P = 0.356$, NS		
Hepatitis C	HCV	10	6	16
	Non-HCV	491	439	930
		$\chi^2 = 0.595$, $df = 1$, $P = 0.441$, NS		
Co-infection	HBV + HCV/HDV	19	17	36
	HBV only	95	81	176
	Un-infected	387	347	734
		$\chi^2 = 0.089$, $df = 2$, $P = 0.956$, NS		
HBV in age-categories (years)	Up to 9	12	7	19
	10–19	28	34	62
	20–29	39	26	65
	30–39	15	17	32
	40–49	9	9	18
	50 and above	11	5	16
		$\chi^2 = 5.696$, $df = 5$, $P = 0.337$, NS		
	Total	501	445	946

anti-HBc was detected in 9.24% ($n = 17$) and 6.6% ($n = 14$) of the patients, respectively (Table 3).

3.4. Symptoms and associated risk factors

Different clinical symptoms were observed among infected patients, including abdominal discomfort, fatigue, anorexia, malaise, fever, dark urine, jaundice, and splenomegaly (Table 4). Most of the symptoms were significantly associated with infections ($\chi^2 = 103.9$; $df = 7$; $P < 0.000\,1$). Abdominal discomfort was one of the most frequently reported symptoms associated with HBV infection (79%) that was followed by fatigue (73%), anorexia (66%) and malaise (62%). Similarly, fever, dark urine and jaundice were higher among HBV infected patients (Table 4). Different risk factors were found to be associated with the infections. Particularly, the association of certain factors like injection, intravenus infusion, hospitalization, dentist visit, and surgery, were considerably higher among the infected groups of patients (Table 5). All other enlisted risk factors showed significant association with the infections ($\chi^2 = 95.23$; $df = 11$; $P < 0.000\,1$).

Table 3
Detection of anti-HBc-IgM, anti-HBc-IgG and viral DNA among the HBsAg-positive patients.

Type of infection		No. of subjects	No. of HBc-IgM	No. of HBc-IgG	No. of DNA detected
Infection type	HBV + ve (total)	212	53	184	136
	HBV + ve (male)	114	37	104	72
	HBV + ve (female)	98	16	80	64
	HDV	23	4	17	16
	HCV	16	3	14	7
	HBV + HCV/HDV	36	7	31	22
	Only HBV	176	46	153	114
Age categories (years)	Up to 9	19	4	19	12
	10–19	62	20	53	38
	20–29	65	13	55	45
	30–39	32	10	27	19
	40–49	18	3	17	15
	50 and above	16	3	13	7

+ ve: Positive.

Table 4

Clinical symptoms and complications associated with hepatitis infection among the scrutinized patients.

Clinical symptoms*	Type of infection					Total (n = 946)
	HBV (n = 212)	HDV (n = 23)	HCV (n = 16)	HBV + HCV/HDV (n = 36)	Un-infected (n = 734)	
Abdominal discomfort	79.25	78.26	81.25	80.56	22.48	333
Fatigue	72.64	56.52	81.25	66.67	11.72	240
Anorexia	66.04	65.22	62.50	66.67	21.66	299
Malaise	61.79	65.22	68.75	66.67	11.44	215
Fever	53.77	47.83	50.00	50.00	22.75	281
Dark urine	44.34	43.48	31.25	38.89	12.26	184
Jaundice	26.42	26.09	37.50	30.56	11.85	143
Splenomegaly	0.94	0.00	0.00	0.00	8.58	65
	$\chi^2 = 103.9$, $df = 7$, $P < 0.0001$**					

*: Distributed in the descending order of prevalence and shown as percent occurrence; **: Statistically highly significant; *Chi*-test was carried out through the values in HBV and un-infected subjects.

Table 5

Risk factors associated with hepatitis infections among the scrutinized patients.

Clinical symptoms*	Prevalent hepatitis types and co-infection				Un-infected (n = 734)	Total (n = 46)
	HBV (n = 212)	HDV (n = 23)	HCV (n = 16)	HBV + HCV/HDV (n = 36)		
Injection	87.74	73.91	81.25	80.56	37.19	459
Intravenus infusion	83.96	86.96	87.50	88.89	10.76	257
Hospitalization	46.70	47.83	50.00	50.00	20.84	252
Skin piercing	43.87	47.83	50.00	47.22	35.83	356
Visit to barber (male)	69.30	40.00	70.00	57.89	47.70	318
Dentist visit	39.62	47.83	37.50	44.44	6.13	129
Surgery	25.47	13.04	31.25	19.44	12.40	145
Blood transfusion	8.02	13.04	12.50	8.33	6.13	62
Contact with jaundice person	6.60	4.35	12.50	8.33	10.22	89
Tattoos/acupuncture	5.19	13.04	0.00	5.56	6.13	56
Visit to beauty parlor (female)	6.12	7.69	0.00	11.76	5.84	32
Illegal injection use	2.36	4.35	6.25	5.56	1.50	16
	$\chi^2 = 95.23$, $df = 11$, $P < 0.0001$**					

*: Distributed in the descending order of prevalence and shown as percent occurrence; **: Statistically highly significant; *Chi*-test was carried out through the values in HBV and un-infected subjects.

4. Discussion

Viral hepatitis remains one of the most formidable challenges in Pakistan. National vaccination program against hepatitis B, as a part of global effort to reduce the burden of the disease has already been launched in the country. However, lack of national surveillance program and large scale community based studies may undermine the outcome of immunization program in the long run, particularly because there is a scarcity of the data about prevalence of HBV/HDV infections among general population of Pakistan across different regions. With a particular focus on outpatient department patients with no prior history of any kind of hepatitis altogether 946 patients were screened for HBV infection. Out of these 22.45% were found positive for HBsAg. Previously, different prevalence rates of HBV infection were reported from Pakistan ranging from minimal 5% to maximal 41.26% [9,12,13].

In this study, serological scrutiny confirmed that 86.79% of the HBV infected patients were positive for anti-HBc-total. Detection of both HBsAg and anti-HBc-total has been documented in chronic patients while HBeAg may or may not be detected in such patients [14]. Hence, detection of anti-HBc-total, alone may not suffice an evidence for chronic HBV infection in these patients. However, detection of anti-HBc-total in the presence of viral DNA in such patients may reflect active presence of HBV infection that is likely to proceed towards chronic stage. In this study, viral DNA was detected in 53.77% of the anti-HBc-total positive patients which are at the higher risk of developing chronic disease. Moreover, due to the active state of their infection these individual pose a risk for further dissemination of HBV infection. While majority of the HBsAg positive patients were also positive for anti-HBc-total, 25% showed presence of anti-HBc-IgM, which is considered as an important serological marker to diagnose recent HBV infections, in so-called "window period".

Among HBsAg positive samples, screening for co-infection of HDV was one the most important aspect of this study. Seroprevalence of HDV varies greatly across different countries, for example, in Turkey, Saudi Arabia and Bangladesh prevalence rates were observed 5.2%, 3.3% and 24.4%, respectively [15,16]. Previously, only few studies have been conducted to assess the seroprevalence of HDV in Pakistan and demonstrated some inconsistencies [9,17]. Observed differences in seroprevalence of HDV in these studies were attributed to several factors, including different geographical locations, population differences and detection methods used (RNA or anti-HDV). Based on anti-HDV detection prevalence rate for HDV infection in this study appeared to be 10.84%. Notably, it is the first report from Pakistan which highlights the prevalence of HBV/HDV co-infection in a population with no prior history of viral hepatitis.

Prevalence of co-infection with HCV varies among infected HBV patients ranging from 10% to 20% [18]. HCV infections are reported among different groups of patients having organ

transplantation, injection drug users and patients with beta-thalassemia [19–24]. Co-infection with HDV/HCV leads to deleterious clinical outcomes including cirrhosis and hepatocellular carcinoma [25]. In our study 7.4% of HBsAg positive patients were co-infected with HCV.

Generally, a wide range of clinical manifestations are associated with HBV infections including asymptomatic seroconversion, non-specific symptoms (anorexia, nausea, *etc*.) and extra hepatic symptoms. We observed significantly higher association of HBV infection with various symptoms, including abdominal discomfort, fatigue, anorexia, malaise, fever, dark urine, jaundice and splenomegaly. Abdominal discomfort was one of the most prominent symptoms associated with HBV infection in this study. Similarly, significant association of different risk factors and HBV infection was also observed. Overall use of injection was the major risk factor that followed intravenus infusion.

Conclusively, from KPK region, it is the first report which highlights HBV/HDV infection in outpatient department patients which indicates higher prevalence of HBV/HDV infection in patients with no prior history of any kind of hepatitis and majority of these patients belong to younger age group. In addition, serological and viral DNA based evidence suggest active prevalence of HBV infection among majority of the patients which indicates higher risk of further HBV transmission by these patients.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

A part of this work has been supported by the research grant provided by Higher Education Commission, Pakistan (IPFP. 3782) and internal research funds of Qauid-i-Azam University Islamabad.

References

[1] Lin CC, Lee CC, Lin SH, Huang PJ, Li HP, Chang YS, et al. RNA recombination in hepatitis delta virus: identification of a novel naturally occurring recombinant. *J Microbiol Immunol Infect* 2015; http://dx.doi.org/10.1016/j.jmii.2015.10.013.

[2] Chao M, Lin CC, Lin FM, Li HP, Iang SB. Whole-genome analysis of genetic recombination of hepatitis delta virus: molecular domain in delta antigen determining trans-activating efficiency. *J Gen Virol* 2015; **96**: 3460-9.

[3] François-Souquière S, Makuwa M, Bisvigou U, Kazanji M. Epidemiological and molecular features of hepatitis B and hepatitis delta virus transmission in a remote rural community in central Africa. *Infect Genet Evol* 2016; **39**: 12-21.

[4] Abbas Z, Jafri W, Raza S. Hepatitis D: scenario in the Asia-Pacific region. *World J Gastroenterol* 2010; **16**(5): 554-62.

[5] Ghamari S, Alavian SM, Rizzetto M, Olivero A, Smedile A, Khedive A, et al. Prevalence of hepatitis delta virus (HDV) infection in chronic hepatitis B patients with unusual clinical pictures. *Hepat Mon* 2013; **13**(8): e6731.

[6] Amini N, Alavian SM, Kabir A, Aalaei-Andabili SH, Hosseini SYS, Rizzetto M. Prevalence of hepatitis D in the eastern mediterranean region: systematic review and meta analysis. *Hepat Mon* 2013; **13**(1): e8210.

[7] Aghanashinikar PN, al-Dhahry SH, al-Marhuby HA, Buhl MR, Daar AS, Al-Hasani MK. Prevalence of hepatitis B, hepatitis delta, and human immunodeficiency virus infections in Omani patients with renal diseases. *Transpl Proc* 1992; **24**: 1913-4.

[8] Goyal A, Murray JM. The impact of vaccination and antiviral therapy on hepatitis B and hepatitis D epidemiology. *PLoS One* 2014; **9**(10): e110143.

[9] Mumtaz K, Hamid SS, Adil S, Afaq A, Islam M, Abid S, et al. Epidemiology and clinical pattern of hepatitis delta virus infection in Pakistan. *J Gastroenterol Hepatol* 2005; **20**: 1503-7.

[10] Abbasi A, Bhutto AR, Butt N, Mahmood K. HDV seroprevalence in HBsAg positive patients. *J Coll Physicians Surg Pak* 2014; **24**(9): 624-7.

[11] Zhang QR, Wu GH, Richards E, Jia SG, Zeng CQ. Universal primers for HBV genome DNA amplification across subtypes: a case study for designing more effective viral primers. *Virol J* 2007; **4**: 92.

[12] Khan J, Shafiq M, Mushtaq S, Ayaz S, Ullah R, AbdEI-Salam NM, et al. Seropositivity and coinfection of hepatitis B and C among patients seeking hospital care in Islamabad, Pakistan. *Biomed Res Int* 2014; **2014**: 516859.

[13] Ali A, Nisar M, Idrees M, Ahmad H, Hussain A, Rafique S, et al. Prevalence of HBV infection in suspected population of conflict-affected area of war against terrorism in North Waziristan FATA Pakistan. *Infect Genet Evol* 2012; **12**(8): 1865-9.

[14] Horvat RT, Tegtmeier GE. Hepatitis B and D viruses. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. *Manual of clinical microbiology*. Washington: ASM Press; 2006, p. 1641-59.

[15] Din G, Malik S, Ali I, Ahmed S, Dasti JI. Prevalence of hepatitis C virus infection among thalassemia patients: a perspective from a multi-ethnic population of Pakistan. *Asian Pac J Trop Biomed* 2014; **7**: S127-33.

[16] Alavian SM, Alavian SH. Hepatitis D virus infection; Iran, Middle East and Central Asia. *Hepat Mon* 2005; **5**(4): 137-43.

[17] Shaikh MA, Shaikh WM, Solangi GA, Shaikh BA, Soomro MA. Frequency of hepatitis D virus infection in hepatitis B surface antigen-positive liver diseases. *J Coll Physicians Surg Pak* 2011; **21**(1): 23-5.

[18] Kvitko DT, Bastos GA, Pinto ME. Prevalence of risk factors for hepatitis C and associated factors: a population-based study in southern Brazil. *Arq Gastroenterol* 2013; **50**(2): 117-22.

[19] Reker C, Islam KM. Risk factors associated with high prevalence rates of hepatitis C infection in Egypt. *Int J Infect Dis* 2014; **25**: 104-6.

[20] Pereira LM, Martelli CM, Moreira RC, Merchan-Hamman E, Stein AT, Cardoso RMA, et al. Prevalence and risk factors of hepatitis C virus infection in Brazil, 2005 through 2009: a cross-sectional study. *BMC Infect Dis* 2013; **13**: 60.

[21] Kandeel AM, Talaat M, Afifi SA, El-Sayed NM, Fadeel MAA, Hajjeh RA, et al. Case control study to identify risk factors for acute hepatitis C virus infection in Egypt. *BMC Infect Dis* 2012; **12**: 294.

[22] Denniston MM, Jiles RB, Drobeniuc J, Klevens RM, Ward JW, McQuillan GM, et al. Chronic hepatitis C virus infection in the United States, National Health and Nutrition Examination Survey 2003 to 2010. *Ann Intern Med* 2014; **160**(5): 293-300.

[23] Mahboobi N, Porter SR, Karayiannis P, Alavian SM. Dental treatment as a risk factor for hepatitis B and C viral infection. A review of the recent literature. *J Gastrointestin Liver Dis* 2013; **22**(1): 79-86.

[24] Kershenobich D, Razavi HA, Sanchez-Avila JF, Bessone F, Coelho HS, Dagher L, et al. Trends and projections of hepatitis C virus epidemiology in Latin America. *Liver Int* 2011; **31**: 18-29.

[25] Centers for Disease Control and Prevention (CDC). Establishment of a viral hepatitis surveillance system, Pakistan, 2009-2011. *Morb Mortal Wkly Rep* 2011; **60**(40): 1385-90.