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Health Care Waste Management Workers in Pakistan: A Population Presenting Highest Burden of Hepatitis B and Hepatitis C

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Abstract: *In Pakistan health care waste management workers (HWMW) are directly exposed to contaminated hospital waste materials. Most of them are unaware of personal safety measures and depth of danger they are dealing with. Previously conducted studies show that HWMW acquire more infections compared with other groups. In present study we used enzyme linked immuno sorbent assay, polymerase chain reaction and genotyping techniques to evaluate burden of hepatitis B and hepatitis C and various genotypes of hepatitis B and hepatitis C among HWMW. We tested four hundred and twenty six subjects for hepatitis B and hepatitis C and found H.W.M.W, a population with heavy burden of the hepatitis B and hepatitis C. Genotyping analysis showed that H.W.M.W have significant differences with the control population and they acquire more mixed infections of hepatitis then other populations. We concluded that H.W.M.W are a high risk population and they have highest infection rates of hepatitis with various genotypes of hepatitis B and hepatitis C.*

I. Introduction

Hepatitis, characterized by inflammation of the liver is among the most prevalent and life threatening diseases to mankind. There are many causes of Hepatitis disease including bacterial, parasitic and physiological disorders however infection with Hepatitis viruses is responsible for the majority of cases [1,2]. Viral Hepatitis is a growing concern of public health especially among developing countries, where prevention and hygiene is compromised by a lack of resources. Currently, six types of the viruses are known as causative agents of viral Hepatitis. These are types A, B, C, D, E and G. Among these Hepatitis B &Hepatitis C are well known for the chronic infections and causing considerable morbidity and mortality [1].

Globally, 2.2 % of the human population is infected with Hepatitis C virus (HCV), while Pakistan stands second in the list of world's most prevalent nations [2].Prevalence of Hepatitis B virus (HBV) infection in Pakistan is between 2.3 to 2.5 %, whereas HCV is more prevalent, ranging from 2.6 to 5.3 % [1] making both viruses crucial public health issues in Pakistan. HBV has been found to infect about 350 million people globally [3], HBsAg prevalence among the general population ranges from 2-8%, which places India in an intermediate HBV endemicity zone and India with 50 million cases, is also second largest global pool of chronic HBV infections [4,5] HBV has been classified into eight genotypes (A-H) that have distinct geographical distributions. These include a number of new HBV sub-genotypes (A1-2, B1-4, C1-4, D1-4 and F1-2) [6]that have received a lot of attention regarding their epidemiology, clinical implications and response to antiviral therapy [7, 1, 4] .The 8HBV genotypes show an intergroup divergence of 8% or more across the complete genome sequence [7].

Genotyping is very important for prognosis of the disease caused by HBV. Type A and D of HBV are responsible for liver cirrhosis and type C is responsible for cancer of the liver [7, 36]. In the Sindh region of Pakistan, the type D genotype is more prevalent while in Punjab, type C is more prevalent. Type C is less sensitive to interferon therapy, while type B shows a better response to analog therapy [8].

Phylogenetic analysis of HCV sequences has revealed six major genotypes (1, 2,3,4,5 and 6) and more than 50 subtypes[9]. HCV genotypes differ from each other in their nucleotide sequence by 31-34% [10, 11]. Genotypes 1, 2 and 3 are distributed almost worldwide. Half of the HCV patients from southern Brazil are infected by genotypes 2 and 3 [13]. Types 4, 5 and 6 have been found in distinct geographical areas [14]. Genotype 5 is dominant in South Africa but less prevalent in other African countries [15]. While, genotype 6 is common in South East Asia and Australians [16]. In Romania, HCV 1b is most common[17].

In Pakistan, genotype 3 is most common and treatment with interferon therapy of six month results in 50-70 percent sustained viral response[18, 19, 20]. A few small-scale studies show that there are different genotypes among different small groups in Pakistan but no national data is available which can explain geographical or high-risk groups, distribution of various genotypes of HBV and HCV within the country. Various studies show that genotype 3 of HCV is most easy to treat with a cure rate of up to 80 %[18,20].

In Pakistan sanitary workers are exposed to the hazardous and fatal occupational injury putting them at risk of great burden of viral hepatitis because of high rates of illiteracy and poverty. Limited access to health care systems, lack of immunization, poor standards of waste disposal, limited acceptance of preventive measures by waste management departments and non-availability of health coverage [21].Sanitary workers often handle garbage, hospital waste, sewage drainage and highly pathogenic materials of the medical laboratories and hospitals without any personal protective equipment (PPE). Most of them are never provided with the safety equipment nor are they taught for safety and health concerns. Studies showed that workers of waste management and sanitation are at greater risk of acquiring hepatitis infection as they are in direct and unprotected contact of deadly pathogens [21,22, 23]. Studies also showed that increasing age increases the risk of viral hepatitis [24,21, 1].

Hospital waste management workers represent a possible at-risk group for contracting HBV and HCV since it is a blood borne disease. This study had three major aims:

(i) To assess whether hospital waste management workers are at high risk of HBV and HCV.
(ii) To determine whether hospital waste management workers have epidemiologically distinct profiles of HBV and HCV infection, by comparing virus genotypes with the control population (say who they are).
(iii) To predict if hospital waste management workers require special attention for treatment and prevention.

II. Materials and Methods

Study population and controls

In this study we took hospital waste management workers (H.W.M.W)as high risk group and hospital blood bank visitors as control population because in Pakistan HWMW work without PPE, are not vaccinated nor they are educated about handling infectious materials. Age range of the study group was 16 -59 years and mean monthly income was 65 U.S dollars. All of the H.W.M.W were associated with this trade at least for five years. All candidates in study group were living at slum houses and average number of people living with them in a small room of ten by ten feet were six to ten. In the control group age range was eighteen to fifty five years. Their mean monthly income was ±250 U.S dollars. All the controls were living in different areas of the Lahore, Gujranwala, Faisalabad, Kasure, Gujarat and Raiwind (Cities of Punjab) while none of them was living in slum houses. Controls were sharing their living with two to fourteen people (two to five bed room houses) Standard ELISA-based techniques were used as the screening tool for HBV and HCV according to standard CDC procedures [25]. Further confirmation was attained by real time polymerase chain reaction (PCR).All the PCR positive samples were further processed for genotyping of hepatitis B and hepatitis C.

A three millilitre blood sample was collected from each of HCV positive patients. Plasma was separated from each sample using the recommended standard procedure given by Qiagen extraction kit.

HCV genotyping

Extraction of HCV RNA from each sample was done using QIAamp Viral mini kit by Qiagen. Amplification was achieved by using Atrus HCV RT-PCR kit by Qiagen from Germany. HCV genotyping of amplified samples was detected using Invader HCV Reagents on Palm Cycler for amplification and Cytoflour for detection of genotype. The Invader® chemistry is composed of two simultaneous isothermal reactions. A primary reaction specifically and accurately detects single-base changes, insertions, deletions and changes in gene and chromosome number for genetic, pharmacogenetic and infectious diseases. A second reaction was used for signal amplification. Genotypes of all samples were determined using Third Wave Technology.

Invader Third Wave Technology Principle: The third wave invader assays uses cleavase enzymes to recognize and cleave specific structures formed by the addition of two oligonucleotides (an invader oligo and a primary probe) to a nucleic acid target. The reaction conditions and sequences of the primary probe and invader oligo probe are selected so that the primary probe has a transient association with the target template to form an overlapping structure. The primary probe also contains a 5 prime flap sequence that is non complementary to the target nucleic acid. The 3 prime nucleotide of the invader oligo overlaps the primary probe, but need not hybridize to the target DNA. The cleavase enzyme recognizes this overlapping structure and cleaves off the unpaired 5 prime flap of the primary probe, releasing it as a target specific product. Thus, under the assay conditions, multiple primary probes associate with the target DNA isothermally. This allows for multiple rounds of primary probe cleavage for each target DNA, and amplification of the number of released 5 prime flaps. In the secondary reaction, each released 5 prime flap can serve as an invader oligo on a Fluorescence Resonance Energy Transfer (FRET) cassette to create another overlapping structure that is recognized and cleaved by the flurophore (F) and quencher (Q). The released 5 prime flap and the FRET cassette cycle, result in amplified fluorescence signal. The primary and secondary reactions run in the same well simultaneously. The bi-plex format of the invader DNA assay enables simultaneous detection of two reactions in a single well [26]

HBV genotyping

Serum HBsAg was assayed by Abbott-IMX HBsAg V2 Assay. DNA was extracted from 100 µl serum sample of all HBsAg positive patients using Biospin Blood Genomic DNA Mini-Prep Kit (Bioer Technology Co., Germany) according to the manufacturer's protocol, eluted in 70 µl buffer and stored at -20°C. Genotyping was performed using methodology as reported by Naito et al [27]. The first PCR was carried out in 40 ul of a reaction mixture containing 100 ng of each outer primer, a 200 mM concentration of each of the four deoxynucleotides, 2.5 U of Taq DNA polymerase (Promega, France) 1× PCR buffer containing containing (50 mM KCl, 10 mM Tris pH 8.3) and 1.5 mM MgCl2. The thermocycler was programmed to first incubate the samples for 5 min at 95°C, followed by 40 cycles consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. Two second-round PCRs were performed for each sample, with the common universal sense primer (B2) and mix A for types A through C and the common universal antisense primer (B2R) and mix B for types D through F. A 1 ml aliquot of the first PCR product was added to two tubes containing the second sets of each of the inner primer pairs, each of the deoxynucleotides, Taq DNA polymerase, and PCR buffer, as in the first reaction. These were amplified for 40 cycles with the following parameters: preheating at 95°C for 5 min, 30 cycles of amplification at 94°C for 1 min, 58°C for 1 min, and 72°C for 1.30 min Genotypes of HBV for each sample were determined by identifying the genotype-specific DNA bands. The two different second-round PCR products from one sample were visualized on an ethidium bromide-stained 3% agarose gel[28]. The sizes of PCR products were estimated according to the migration pattern of a 50 lane PCR marker. In this study, we considered cases as the high-risk population, who are more likely to encounter HBV and HCV as a result of occupational hazard. These are the subjects working in the hospital who have direct contact with the hospital wastage. Whereas controls are the visitors in the hospital blood bank

III. Results

Prevalence of HBV and HCV in the study populations

Data were obtained from 426 subjects, including 107 (25.1%) cases (hospital waste management workers) and 319 (74.9%) controls. We applied ELISA on all 426 subjects and found 60 individuals (14.1%) to be positive for HBV antigen indicating active infection (Table 1.1). Of these 24 (7.52%) controls tested positive for HBV whereas 36 (33.64%) hospital waste management workers tested positive demonstrating a much greater proportion of infection in the case population (Table 1.1).

ELISA test for HCV antibodies identified 73 (17.1%) of the 426 subjects positive for HCV antibodies indicating the either past or present exposure to the tested virus (Table 1.2)Of these, 39 (36.55%) were cases. Amongst the controls, 34 (10.66%) tested positive (Table 1.2). It is clearly evident that target population has 26.11% higher infectivity rates compared with control population.

Table 1.1: Hepatitis - B, ELISA

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	366	85.9	85.9	85.9
	Positive	60	14.1	14.1	100.0
	Total	426	100.0	100.0	

Table 1.2: Hepatitis - C ELISA

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	353	82.9	82.9	82.9
	Positive	73	17.1	17.1	100.0
	Total	426	100.0	100.0	

Prevalence of HBV and HCV genotypes in the study populations

We used PCR and genotyping methods to determine various genotypes of HCV and HBV in study and control groups. From the genotyping data, percentages of the isolated genotypes of hepatitis viruses among controls and target group were then determined. For HBV, genotype D has the highest frequency in the overall population i.e. 7.7 % followed by genotype A, which has prevalence of 3.3 %. Genotype B is least frequent in the population with 1.2 % only. Mixed infection was seen only with D+A genotypes, which represents 0.7 % of the total infections (Table 1.3).

Table 1.3: Genotype Status of a patient with/without Hepatitis B

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Not Detected	371	87.1	87.1	87.1
	A	14	3.3	3.3	90.4

	B	5	1.2	1.2	91.5
	D	33	7.7	7.7	99.3
	D+A	3	.7	.7	100.0
	Total	426	100.0	100.0	

When we investigated the distribution of the genotypes amongst the two populations, we found genotype D was highest among controls and target group population (Figure 1.1). Genotype B was missing from the control population and genotype A follows the genotype D in the control group. Mixed infections with genotypes D+A are fewer in number in the control population. It is also noticeable that mixed infections are significantly lower in control population compared with the target population. Target population shows an equal burden of infection with genotype A and B while mixed infections with genotype A+D is about 50 % higher than the control population.

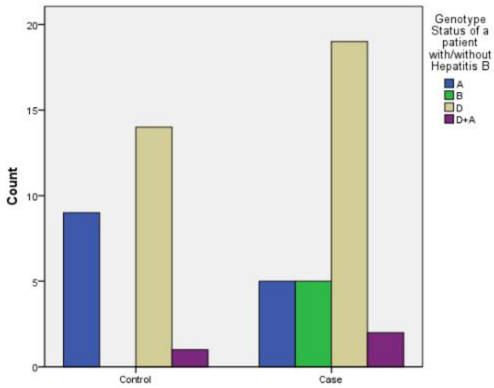

Figure- 1: Genotype status of hepatitis B

A total of 11 samples were positive for genotype 1, out of which 7 were from target group while 4 were from the controls group. Genotype 3a showed highest positivity rate, a total of 49 samples were positive for this genotype from which 23 were controls and 26 were from the target population. A single case only was tested positive for genotype 3b which came from the target population. Genotype 3h was more prevalent than the genotype 3b but less prevalent than the genotype 3a. A total of 7 cases were positive which share 5 controls and 2 from target population for tested positive results. Genotype 4 showed 2 positive cases with equal percentage from both groups. A single tested positive genotype h was from the controls and none was positive for this genotype from the target population (Table 1.4).

Table: 1.4		Genotype Status of patient with/without Hepatitis C				
		1	3a	3b	3h	4
Indicator for case and Control	Control	4	23	0	5	1
	Case	7	26	1	2	1
Total		11	49	1	7	2

When we investigated the distribution of the genotypes amongst the two populations, we found genotype 3a has highest prevalence in both controls and target group although target group shows greater number of tested positive compared with controls. Genotype 1 was only second to the genotype 3a. A comparison between the groups show that it is more common among the target group but still it shows second highest prevalence rate in the overall population. Genotype 3h is third most common genotype in the population, in contrast with the genotype 1 and 3a it is more prevalent in the control group compared to the target group. Genotype 4 shares an equal burden in both groups. Genotype h was found exclusive to the control group whereas genotype 3b was present in the target population only. Overall it could be concluded that genotype 3a is most prevalent in studied populations but there are distribution differences in controls and target population in this study.

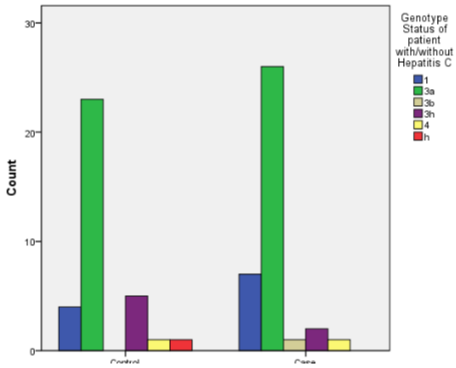

Figure- 1: Genotype status of hepatitis C

IV. Discussion

Serotyping is very important for prognosis of the disease. Type A and D of HBV are responsible for liver cirrhosis and type C is responsible for cancer of the liver. In region of the Sindh type D genotype is more prevalent while in type C is more prevalent in Punjab. Type C is known for lower response to the interferon therapy while type B show better response to analog therapy [8].

Many studies have been done on high-risk groups such as injecting drug users[29], commercial sex workers[30], transvestites [31]and patients receiving blood products [32]. All of these studies revealed a higher prevalence of HBs Ag and Anti-HCV in these groups compared to the general population. HBsAg infection was found in 12% of the commercial sex workers (women) in Lahore while it was only 3.4% among transvestites in Karachi who acknowledged commercial sex with men. The mean prevalence of HBV and HCV in these groups was 15.5% and 12.3% respectively. The risk of contracting HBV by healthcare personnel is four times greater than that of the general adult population, among those who do not work in healthcare institutions [33].

A study from Karachi showed that prevalence of Hepatitis B & C among the garbage scavengers was 8.5% and 18.5% respectively [34]. The present study shows that people associated with the hospital waste management in Pakistan, present the highest prevalence of Hepatitis B & C among the previously studied high risk groups. Vijaya et al showed that people who are involved in direct contact with hospital disposal are at greater risk of acquiring **hepatitis B** and **hepatitis C** because these viruses can survive on dried blood products. She also showed that ward attendants and hospital sweepers have higher infection rate compared to nurses and doctors [35].

The majority of the studies from Sindh province of Pakistan showed a high prevalence of genotype D while a study from Punjab showed high prevalence of genotype C. Many studies on HCV genotypes have been carried out and all showed that in over 80% the cases, genotype 3 was detected, followed by genotype 1, 2 and 4. Genotypes are important to determine the treatment response and disease transmission epidemiology. Better response to interferon and short-term treatment (6 months) is reported in hepatitis C patients infected with serotype 3 while for all other genotypes a longer duration of therapy is suggested[36, 37, 38].Sexual transmission of HCV was checked in few studies to see inter spousal transmission of the disease. Studies from Sindh show a higher frequency of disease in the spouses while those from Punjab show a low frequency[39].According to Altaf et al there is no national data on the leading causes of admissions to hospitals in Pakistan and the contribution of liver disease to overall mortality[1]. In this present study we found that genotype D is most prevalent for hepatitis B followed by genotype A & B. in case of hepatitis C genotype 3a is most common followed by genotype 1 and 3h. Our data of genotyping is in totally in line with the past studies. Apart from the fact that overall picture is similar we found that there could be valuable difference among populations under question. These differences shell be addressed properly for better treatment and prevention policies. This data favours the results of Ahmad et al who reported that the most commonly detected genotype in their study was genotype 3 (59.1%), with predominant subtype 3a (55.9%) and 3b (3.2%) [20](Ahmad et al., 2010). Another study by Butt et al showed prevalence of 3a (62%), 3b (9%), 1a (3%), 2a (2.144%), mixed (4.718%) and un-type able (17.16%) (Butt et al., 2010). Another study reported that genotype 1 was the most frequently genotype found in all regions of Brazil [40].

Interest of HCV genotyping by group screening is increased many fold as it is useful for the solution of epidemiological questions and development of vaccines against HCV. It has also been shown to be valuable to facilitate therapeutic decisions and strategies[1, 20].

No data is available regarding the health care waste generated in the country. According to an estimate, in Karachi, out of 8000 tons of refuse generated every day, 0.5 percent is health care waste, generated by well over 400 hospitals, clinics and laboratories. According to the Environment Protection Agency (EPA) study on hospital waste, 20% of health care waste generated every day in the city is infectious waste [41]. In Pakistan disposal of healthcare waste is not safe. It was observed that most of the healthcare waste generated by health facilities was not properly disposed of, either dumped at community waste sites or sold directly to the dealers of the junk. These junk dealers are also a high risk group. Scavengers driven by extreme poverty and ignorant of risks were, involved in sorting and handling the infected materials at community sites [41]. Unsafe collection and throwing health care medical waste at community waste site is a threat to the healthcare workers, community and the environment. Any person exposed to this kind of waste, can acquire infections, not just HBV and HCV but HAV, gastrointestinal infections, fungal infections, Leptospirosis and skin infections. Housekeeping staffs (sweepers) of healthcare establishments, doctors, nurses, hospital maintenance personnel, patients and visitors of healthcare centres are at risk of acquiring infections through this waste [41, 44].

In a cross-sectional study the employees of a Sewage Company were tested for hepatitis B virus (HBV) markers--HBsAg, anti-HBs, anti-HBc to determine the prevalence of HBV infection and assess the risk of exposed sewage workers becoming infected. The overall prevalence of HBV markers was 43.9% and 6.6% of the employees were HBsAg carriers. In the univariate analysis the prevalence of past and current infection was significantly associated with exposure to sewage. Workers exposed to sewage should therefore be considered for vaccination against hepatitis B virus [42].

A study from Copenhagen showed that hepatitis B infection rate is similar among general population and sanitary workers although sanitary workers show slightly high rates of gastrointestinal infections[43].

Unsafe disposal of hospital waste may be one of the reasons that we have high prevalence of hepatitis B and C infection in the country, particularly among health care workers, and anyone exposed to this waste outside the hospital. A study conducted in Karachi among health care workers reported high prevalence of hepatitis B infection, reaching 20% among sweepers of a medical centre [44]. We found no studies which can define genotypes of hepatitis B and hepatitis C among health care workers while related studies only revealed about overall prevalence of hepatitis among closely related groups. Our study however demonstrates that the cases were not exposed to different genotypes than the general population, which is in line with them acquiring infection from hospital waste, rather than other potential sources like drug abuse, blood transfusion and sexual transmission of the disease.

V. Conclusion

We concluded that H.W.M.W working in Pakistani hospitals are carrying highest burden of hepatitis B and hepatitis C in the world and they show genotypic differences compared with the control population. It is important that these individuals are provided with training to deal the bio-hazard materials of the hospitals. They shell be provided with proper personal safety equipment. Education of H.W.M.W in personal safety can reduce spread of infections. Hospitals need to adhere to safe disposal practices which includes autoclaving and incineration of hospital waste materials.

Future guidelines

National level studies from multiple participating cities shell be conducted and ministry of health should organize educational programs with hospital waste management staff so that they t can handle it in a safe way.

Conflict of interest:

We declare no competing interests

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References

[1]. Altaf Bosan, Irtaza Ahmad, Rehan Hafiz , Huma Qureshi , Khalif Mohamud Bile JPMA, A review of Hepatitis viral infections in Pakistan 2010
[2]. Alter MJ. Epidemiology of Hepatitis C virus infection. World J Gastroenterol. 2007; 13:2436-2441.
[3]. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004; 11: 97-107.
[4]. Gupta S, Gupta R, Joshi YK, Singh S. Role of horizontal transmission in hepatitis B virus spread among household contacts in north India. Intervirology 2008; 51:7-13.
WHO. Hepatitis B: WHO / CDS / CSR / LYO / 2002.2: Hepatitis B. 2009.
[5]. Toumy Guettouche and H James Hnatyszyn; Chronic hepatitis B and viral genotype: the clinical significance of determining HBV genotypes; Antiviral Therapy ; 2005; 10:593–604
[6]. Kidd-Ljunggren K, Miyakawa Y and Kidd AH. Genetic variability in hepatitis Bviruses. J. Gen. Virol 2002; 83: 1267 - 1280.
[7]. Idrees M, Khan S, Riazuddin S. Common genotypes of hepatitis B virus. J Coll Physicians Surg Pak 2004; 14: 344-7. 183.Baig S, Siddiqui AA, Ahmed W, Qureshi H, Arif A. The association of complex liver disorders with HBV genotypes prevalent in Pakistan. Virol J 2007; 4: 128

[8]. Yap P, Kolberg J, Urdea M. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. Journal of General Virology, (1993); 742391-2399
[9]. Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology, (2005); 42(4): 962-973.
[10]. Pawlotsky J-M. Mechanisms of antiviral treatment efficacy and failure in chronic hepatitis C. Antiviral research, (2003); 59(1): 1-11.
[11]. Chan S, McOmish F, Holmes E, Dow B, Peutherer J, et al. Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. The Journal of general virology, (1992); 731131-1141.
[12]. Silva CMDd, Costi C, Krug LP, Ramos AB, Grandi T, et al. High proportion of hepatitis C virus genotypes 1 and 3 in a large cohort of patients from Southern Brazil. Memórias do Instituto Oswaldo Cruz, (2007)
[13]. Bukh J, Purcell RH, Miller RH. At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. Proceedings of the National Academy of Sciences, (1993); 90(17): 8234-8238
[14]. Bonny C, Fontaine H, Poynard T, Hézode C, Larrey D, et al. Effectiveness of interferon plus ribavirin combination in the treatment of naive patients with hepatitis C virus type 5. A French multicenter retrospective study. Alimentary pharmacology & therapeutics, (2006); 24(4): 593-600.
[15]. Pybus OG, Markov PV, Wu A, Tatem AJ. Investigating the endemic transmission of the hepatitis C virus. International journal for parasitology, (2007); 37(8): 839-849.
[16]. Sultanat C, Oprisan G, Szmal C, Vagu C, Temereanca A, et al. Molecular epidemiology of hepatitis C virus strains from Romania. Journal of Gastrointestinal & Liver Diseases, (2011); 20(3).
[17]. Hussain AB, Hussain T, Anwar M, Hussain S, Kazmi Y, Tariq WU, et al. Treatment response in HCV related chronic hepatitis. J Coll Physicians Surg Pak 2004; 14: 466-9.
[18]. Muhammad N, Jan MA, Rahman N. Treatment response of hepatitis C patients to combination therapy of interferon plus ribavirin. J Postgrad Med Inst 2004; 18: 563-8.
[19]. Ahmed SI, Mahmud MR, Khan NY, Naseemullah M, Hanif M. Pegylated interferon and ribavirin in HCV genotype 3 detectable patients after 12 weeks of conventional interferon - ribavirin treatment. Pak J Gastroenterol 2006; 20: 58-62.
[20]. Shakeel Waqqar, Shahid Hussain Sheikh , Salman Aziz , Godfrey Paul William Farheen Ansari, Saima Bano, Hepatitis Prevalence of Epidemic Magnitude among Sanitary Workers in Pakistan ,2014 , IOSR-JDMS;13:6 ; I: 58-61
[21]. Brautbar N, Navizadeh N. Sewer workers: Occupational risk for hepatitis C-report of two cases and review of literature. Arch Environ Health 1999; 54: 328-330.
[22]. Murrill CS, Weeks H, Castrucci BC, et al. Age-specific seroprevalence of HIV, hepatitis B virus, and hepatitis C virus infection among injection drug users admitted to drug treatment in 6 US cities. Am J Public Health 2002; 92: 385-387.
[23]. Alter MJ. Hepatitis C virus infection in the United States. J Hepatol 1999; 31: 88-91
[24]. http://www.cdc.gov/hepatitis/HCV/GuidelinesC.htm
[25]. Arif. H, MuhammadI.N, Afzaland Aqeel Prevalence of HCV genotypes in patients reporting in tertiary health care hospitals of Karachi Pakistan Journal of Pharmacology Vol.28, No.2, July 2011, pp.23-29 D*
[26]. Naito H, Havashi S, Abe K: Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. J Clin Microbiol 2001, 39(4):1686.
[27]. Abdel-Rahman N Zekri, Mohamed M Hafez, Nahed I Mohamed, Zeinab K Hassan, Manal H El-Sayed, Mohsen M Khaled and Tarek Mansour Hepatitis B virus (HBV) genotypes in Egyptian pediatric cancer patients with acute and chronic active HBV infection; Virology Journal 2007, 4:74
[28]. Thomas DL, Vlahov D, Solomon L, Cohn S, Taylor E, Garfein R, Nelson KE Correlates of hepatitis C virus infections among injection drug users. Medicine (Baltimore). 1995 Jul;74(4):212-20
[29]. Chen Y, Shen Z, Morano JP, Khoshnood K, Wu Z, Lan G, Zhu Q, Zhou Y, Tang S, Liu W, Chen J, Tang Z..Bridging the Epidemic: A Comprehensive Analysis of Prevalence and Correlates of HIV, Hepatitis C, and Syphilis, and Infection among Female Sex Workers in Guangxi Province, China. PLoS One. 2015 Feb 27;10(2):e0115311
[30]. Carobene M, Bolcic F, Farías MS, Quarleri J, Avila MM.HIV, HBV, and HCV molecular epidemiology among trans (transvestites, transsexuals, and transgender) sex workers in Argentina. J Med Virol. 2014 Jan;86(1):64-70
[31]. D A Kelly. Hepatitis C infection after blood product transfusion. Arch Dis Child. 1996; 75(5): 363–365.
[32]. Byrne EB. Viral hepatitis: on occupational hazard of medical personnel. Experience of the Yale-New Haven Hospital, 1952-1965.JAMA 1996; 195: 362-4.
[33]. Vijaya Doddaiah, Katta Janakiram, Sathish Javagal, Seroprevalence of Hepatitis B virus and Hepatitis C virus in healthcare workers - AIMS, B G Nagara,American Journal of Life Sciences, 2013; 1(4): 145-149
[34]. Rajab Ali Khawaja, Asad Ali Khawaja, Hepatitis B virus Genotypes: “Clinical & Therapeutic Implications” PJMA,Vol. 59, No. 2, February 2009.
[35]. Kumar N, Sattar RA, Ara J. Frequency of hepatitis-C virus in the spouses of HCV positive patients and risk factors of the two groups. J Surg Pak 2004; 9: 36-9.
[36]. Irfan A, Arfeen. Hepatitis C virus infection in spouses. Pak J Med Res 2004; 43: 113-6.
[37]. Khokhar N, Gill ML, Alam AY. Interspousal transmission of Hepatitis C virus. J Coll Physicians Surg Pak 2005; 15: 587-9.
[38]. Campiotto S, Pinho J, Carrilho F, Da Silva L, Souto F, et al. Geographic distribution of hepatitis C virus genotypes in Brazil. Brazilian Journal of Medical and Biological Research, (2005); 38(1): 41-49.
[39]. Mukhtar A. Hospital waste poses grave threat to life: environment. Dawn July 12, 2001. p. 19.
[40]. Arvanitidou M, Constantinidis TC, Doutsos J, Mandraveli K, Katsouyannopoulos V.Occupational hepatitis B virus infection in sewage workers; Med Lav. 1998 Sep-Oct;89(5):437-44
[41]. Skinhøj P, Hollinger FB, Hovind-Hougen K, Lous P.Infectious liver diseases in three groups of Copenhagen workers: correlation of hepatitis A infection to sewage exposure. Arch Environ Health. 1981 May-Jun;36(3):139-43
[42]. Mujeeb SA, Zuberi SJ. Lodi Tz. Prevalence of HBV infection in health care workers. J. Pak. Med. Assoc., 1994;44: 265.