

HEPATITIS-C IN HUMAN URBAN POPULATION OF BAHAWALPUR DISTRICT, PUNJAB-PAKISTAN: SEROPREVALENCE AND RISK FACTORS

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SUMMARY

Hepatitis C virus (HCV) infections have been reached epidemic proportions in many parts of the world. Present study was carried out to determine prevalence of Hepatitis C in relation to promotive factors among human urban population of Bahawalpur-district, Punjab-Pakistan for a period of 12 months (October 01, 2007 to September 30, 2008). Determination of serum anti-HCV activity and obtaining answers from a study-specific questionnaire was done. HCV-positive (Rapid immunochromatographic tests) participants were interviewed regarding past and present life style to determine promotive factors. Seroprevalence of Hepatitis C was 6.29% in the observed urban population (10-70 years) of Bahawalpur (n=1352) *i.e.* divided into three age groups; old (>50 years), mature (20≤50 years) and young (10≤20 years). Maximum prevalence of hepatitis C (6.55%: female, 6.63; male, 6.49) was found in old in comparison to mature (6.49%: female, 6.67; male, 6.31) and young (5.42%: female, 5.15; male, 5.67) population. Analysis of socioeconomic status indicated a higher HCV (7.86, 95% CI: 0.05760-0.10643) in the individuals with a low socioeconomic level and a high rate of overcrowding (8.02, 95% CI: 0.05546-0.11502). A high seroprevalence was found in parlor/barber-shop female and male users (7.24, 95% CI: 0.04840-0.10717; 6.25, 95% CI: 0.0424-0.09133). Virus was found maximum (13.01, 95% CI: 0.09960-0.168 and 7.30, 95% CI: 0.0567-0.0934) due to blood transfusion and non-sterile pricking respectively. Dentist visitors and surgically operated population was infected 7.10% (95% CI: 0.0541-0.09266) and 8.47% (95% CI: 0.06069-0.11710) respectively.

HCV was found spreading rapidly among population of Bahawalpur district which was linked to deprived socioeconomic conditions, poor sanitation, non-sterile pricking, blood transfusion, barber/parlor's shops, dentist's clinics and surgical operations.

Key words: Blood transfusion, hepatitis C, population, socioeconomic status.

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INTRODUCTION

Hepatitis C virus (HCV) is among the principal causes of severe liver disease, including hepatocellular carcinoma and cirrhosis-related end-stage liver disease (Joseph *et al.*, 2006). The World Health Organization (WHO) estimates that there are 170 million people with chronic HCV infection worldwide (WHO, 2000; Ali *et al.*, 2009). Hepatitis C is estimated to cause 366 000 deaths annually (Perz *et al.*, 2006). Given its large population (165 million) and intermediate to high rates of infection, Pakistan is among the worst afflicted nations (Ali *et al.*, 2009). Pakistan has one of the world's highest fertility rates, exceeding four children per woman (The world factbook, 2007). Its approximately 800 000 sq km are slightly less than twice the size of the state of California in the USA and Pakistan is larger than either Turkey or Chile. Considering Pakistan's size and large, growing population, there is a surprising dearth of information about hepatitis prevalence and about its risk factors (Shepard *et al.*, 2005; Ali *et al.*, 2009).

Hepatitis viruses can be transmitted by means of acupuncture, tattooing, and sharing razors. Needle stick injuries in the health care setting result in a 3% risk of HCV transmission. HCV prevalence among health care workers has also been reported similar to that of the general population (Russmann *et al.*, 2007). Nosocomial patient-to-patient transmissions may occur by means of a contaminated colonoscope, via dialysis, or during surgery, including organ transplantation (Ross *et al.*, 2009). The uncommon routes of transmission of hepatic viral infections, which affect less than 5% of the individuals at risk, include high-risk sexual activity and maternal-fetal transmission have been identified. Casual household contact and contact with the saliva of those infected are inefficient modes of transmission (de Waure *et al.*, 2009).

Hepatic viral infections have been found with shared routes of transmission, can cause serious morbidity and mortality globally (Smith *et al.*, 2006). Major routes of transmission for Hepatitis-C are more often associated with vertical transmission, sexual contact and both household and occupational contacts. Hepatitis C is primarily transmitted through percutaneous exposures, particularly in the context of injection drug use, though it appears that it can be transmitted sexually as well as vertically (Memish *et al.*, 2006). Co-infection with HIV type 1 appears to increase the risk of both sexual and maternal-fetal transmission of HCV and HBV (Smith *et al.*, 2006; Vincent *et al.*, 2007; Carrano *et al.*, 2009).

In an earlier report the prevalence of Hepatitis-C was determined in population of the urban area of Bahawalpur district, while presently aimed to examine the seroprevalence of Hepatitis-C infection in same population in relation to promotive factors. No comparable studies were done recently and no previous studies have looked at the prevalence in relation to promotive factors in such a population.

MATERIALS AND METHODS

The present study was carried out from October 01, 2007 to September 30, 2008. A randomly selected segment of population; 1352 peoples (both sex) of different age and profession of urban areas of Bahawalpur, Pakistan, was evaluated for Hepatitis C-infection and promoting factors.

Selection and division of population

Population of both sex, age, profession; 10-70 years (without any previous diagnosis) was divided into three groups *i.e.* young (10≤20 years), mature (20≤50 years) and old (>50 years). Same ages male and female were grouped separately. A willingness certificate signed by each individual/parents/guardians for co-operation in carrying out the purpose of present study was obtained before his/her inclusion in the study (Tassaduqe *et al.*, 2004).

HCV promoting factors

Hepatitis-C promoting factors, relating to the participants were age, sex, type of housing, use of barber/parlor's shop(s), home crowding, family income (in minimum wages), blood transfusions, non-sterile pricking, dental and surgical procedures. An interview with the participants and their parent(s)/guardian(s) was conducted to fill out the questioner (Nurgalieva *et al.*, 2002; Russmann *et al.*, 2007).

Blood sample collection and evaluation

3.0 mL of fresh blood sample was taken from each volunteer by vene-puncture arm vein. Specimen was allowed to clot without hemolysis which was centrifuged at 3500 rpm (Labofuge, Heraeus, Germany) for 20 minutes. Sample serum was tested immediately for HCV antibody by using rapid chromatographic immunoassay from Acon Laboratories USA (Tassaduqe *et al.*, 2004).

Rapid chromatographic immunoassay for Hepatitis C

The qualitative, solid phase, single test devices (Acon) were used to detect the infections with HCV. The Acon devices are two-site sandwich immunoassay for the detection of HCV antibodies in serum/plasma (Blumberg, 1971; Wilber, 1993).

Statistical analysis

The data was analyzed statistically by using tests of proportions, Chi-square test (χ^2) and confidence interval. A $P < 0.05$ was considered statistically significant. A 95% confidence interval was calculated as follows

$$P \pm (\sqrt{pq/n}) \times Z_{d/2}$$

Where

P = HCV-seropositive proportions of population

q = 1-p

n = total number of cases

Z = standard normal variate (1.96)

d = degree of freedom

RESULTS**HCV-seroprevalence**

A total of 1352 volunteers (277 young, 663 mature and 412 old of both sex) evaluated for the presence of anti-HCV in this study. Seroprevalence of infection was 6.29% in the observed population (Fig.1). Maximum prevalence was found in mature female group (6.67%, 95% CI: 0.0635-0.1411) in comparison to all other male and female groups (Table 1).

Effect of promoting factors on HCV-seroprevalence

The prevalence of infection was 2.46% (95% CI: 0.01215-0.04977), 6.88% (95% CI: 0.0511-0.09201) and 7.86% (95% CI: 0.05760-0.10643) among the subjects with the high, middle and low socioeconomic status. Home crowding indicated 4.79% (95% CI: 0.02842-0.08034) infection in those residing 1-2 person/room, 6.08% (95% CI: 0.04591-0.0801) and 8.02% (95% CI: 0.05546-0.11502) infection living 3-4 and more than 4 persons/room respectively. The virus was found maximum in the barber/parlor's shop users (6.69%, 95% CI: 0.05054-0.08805) and in those (13.01%, 95% CI: 0.09960-0.168) who were blood transfused. HCV infection was high due to non-sterile pricking (7.30%, 95% CI: 0.0567-0.0934), 7.10% (95% CI: 0.0541-0.09266) and

8.47% (95% CI: 0.06069-0.11710) were infected due to dental and surgical procedures respectively (Table 2).

Table 1. Prevalence of anti-HCV positive by age and gender

Group	Age (year)	Anti-HCV positive (%)		
		Total (N=1352) % [95% CI] (n)	Female (N _f =605) % [95%CI] (n)	Male (N _m =747) % [95%CI] (n)
Young	10≤20	5.42 [0.0324-0.0881] 15	5.15 [0.0233-0.1043] 7	5.67 [0.0274-0.1096] 8
Mature	20≤50	6.49 [0.0483-0.0864] 43	6.67 [0.0635-0.1411] 22	6.31 [0.0591-0.1344] 21
Old	>50	6.55 [0.0451-0.0940] 27	6.63 [0.0373-0.1134] 12	6.49 [0.0390-0.1051] 15

HCV, hepatitis C virus; CI, confidence interval

Table 2. HCV-seroprevalence distribution according to the promoting factors among female and male population

Promoting factors of HCV		Anti-HCV positive (%)		
		Total (N=1352) % [95% CI] (n)	Female (N _f =605) % [95%CI] (n)	Male (N _m =747) % [95%CI] (n)
Socioeconomic status	High	2.46 [0.01215-0.04977] 7	2.27 [0.00825-0.06450] 3	2.61 [0.01062-0.06516] 4
	Middle	6.88 [0.0511-0.09201] 41	6.87 [0.04405-0.10601] 18	6.89 [0.04643-0.1012] 23
	Lower	7.86 [0.05760-0.10643] 37	8.06 [0.05109-0.12528] 17	7.69 [0.05049-0.11586] 20
Crowding (No of person/room)	1-2	4.79 [0.02842-0.08034] 13	5.10 [0.02256-0.11393] 5	4.62 [0.02392-0.08858] 8
	3-4	6.08 [0.04591-0.0801] 46	5.85 [0.03869-0.08778] 21	6.28 [0.04300-0.09110] 25
	>4	8.02 [0.05546-0.11502] 26	7.43 [0.04230-0.12825] 11	8.52 [0.05255-0.13592] 15
Use of barber/parlor shops	No	5.87 [0.04331-0.07930] 39	5.98 [0.038-0.09256] 18	5.79 [0.03826-0.08683] 21
	Yes	6.69 [0.05054-0.08805] 46	7.24 [0.04840-0.10717] 22	6.25 [0.0424-0.09133] 24
Blood Transfusion	No	3.76 [0.02747-0.05145] 37	3.87 [0.02442-0.06114] 17	3.68 [0.02400-0.05610] 20
	Yes	13.01 [0.09960-0.168] 48	13.25 [0.08935-0.19263] 22	12.81 [0.08906-0.18114] 26
Injection/pricking	Sterile pricking	4.90 [0.0342-0.0699] 28	5.08 [0.03009-0.08494] 13	4.76 [0.02921-0.07708] 15
	Non-sterile pricking	7.30 [0.0567-0.0934] 57	7.74 [0.05381-0.11025] 27	6.94 [0.0491-0.09743] 30
Visit to dentist's clinic	No	5.44 [0.0395-0.0743] 36	5.32 [0.0331-0.08461] 16	5.54 [0.03626-0.0840] 20
	Yes	7.10 [0.0541-0.09266] 49	7.57 [0.05106-0.11100] 23	6.74 [0.04647-0.09689] 26
Surgical operation	No	5.44 [0.04187-0.07050] 53	5.53 [0.03783-0.08039] 25	5.36 [0.03744-0.07644] 28
	Yes	8.47 [0.06069-0.11710] 32	8.50 [0.05059-0.14002] 13	8.44 [0.05489-0.12817] 19

HCV, hepatitis C virus; CI, confidence interval

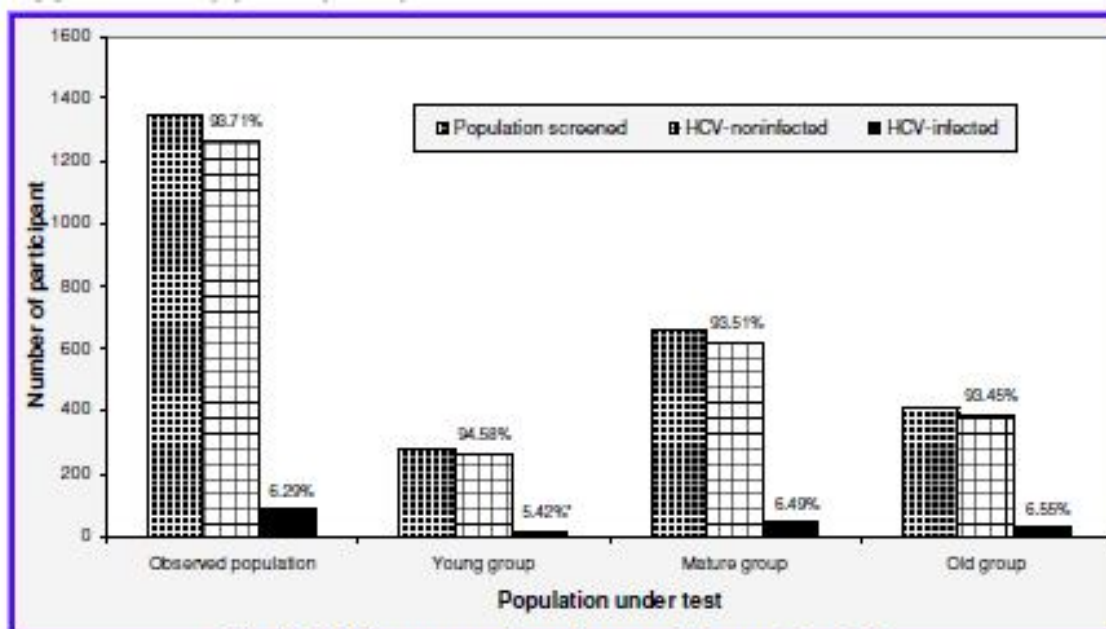


Fig. 1. HCV-seroprevalence in population under trial

HCV-infection % in young group: significant from HCV-infection % in mature and old groups * – $p < 0.05$
 HCV-infection % in mature and old groups are non-significant ($p > 0.05$) from each other

DISCUSSION

The prevalence of HCV infection in relation to promotive factors was investigated in a population-based sample of urban population of Bahawalpur district of Pakistan in the present study. A total of 1352 individuals were tested for the presence of infection out of which 85 were infected (Fig.1). The study showed an overall 6.29% HCV-seroprevalence (Fig.1). This figure is higher than the prevalence of infection detected earlier in the similar study (Rifat-uz-Zaman, 2006). However, observed high prevalence of HCV seropositivity was found inconsistent with other studies of adult populations worldwide (Habib *et al.*, 2001; McQuillan *et al.*, 2004; Ross *et al.*, 2009).

The analysis of data indicated a maximum prevalence of hepatitis C; 6.60% in old in comparison to mature (6.46%) and young (6.40%) population (Fig.1). Maximum prevalence, 6.67% (95% CI: 0.0635-0.1411) and 6.49% (95% CI: 0.0390-0.1051) was observed in mature female and old male groups respectively (Table 1). However, male and female group of same age remained only slightly different to each other. Minimum prevalence of infection was observed in young group in comparison to old and matures (Table 1) probably due to less exposure to the virus which is in agreement with the CDC, (2009). Findings are also in consistent with the previous study (Rifat-uz-Zaman, 2006). The increased prevalence in persons older than 12 years might associate with the initiation of sexual activity (Jacobs *et al.*, 1997). The drug abuse, divorced or separated marital status and low educational level remain some other means (Gunn *et al.*, 2003).

Socioeconomic status is often a surrogate marker for the level of sanitary and hygienic practices and it is a major factor that directly correlates with viral hepatic infections (Joshi *et al.*, 2008). Data showed less rate of infection in both female and male groups of the upper socioeconomic levelers and an inverse association between socioeconomic status verses presence of infections was observed. (Table 2) Crowding is another indirect measure of household hygiene and it has been reported to be an important risk factor for hepatic viral infection acquisition (Mastromatteo *et al.*, 2001). The findings demonstrated the direct relation of crowding with the spread of infection.

Data showed the barber/parlor-shops as the major source of viral particle and among the visitors of barber/parlor's shops, 7.24% (95% CI: 0.04840-0.10717) female and 6.25% (95% CI: 0.0424-

0.09133) male while 5.98% (95% CI: 0.038-0.09256) female and 5.79% (95% CI: 0.03826-0.08683) non-users got infection. This study further pointed out that 13.25% (95% CI: 0.08935-0.19263) female and 12.81% (95% CI: 0.08906-0.18114) male who blood transfused were infected compared with 3.87% (95% CI: 0.02442-0.06114) female and 3.68% (95% CI: 0.02400-0.05610) male who were not ever transfused. The similar findings were also reported by Deisenhammer *et al.*, (2006), Khaja *et al.*, (2006) and Mirza *et al.*, (2007).

Furthermore, non-sterile pricking and injection with re-used syringes presented high risk of viral infection. It has been noted previously that such practice is a risk factor for infection (Deisenhammer *et al.*, 2006; Xia *et al.*, 2008; Jauffret-Roustide *et al.*, 2009). Another major finding of the current study was the higher prevalence of infection among those who treated in the dentist clinic; among treated female, 7.57% (95% CI: 0.05106-0.11100) and male, 6.74% (95% CI: 0.04647-0.09689) were found infected and finding is inconsistent with Russmann *et al.*, (2007). Additional risk factor for transmission of infection was found surgical operations carried out by non-sterile and infected surgical equipments (Table 2) (Fry, 2007).

The living conditions or exposure to environmental factors, cultural practices, nutritional factors or sanitary circumstances are relevant factors in addition to percutaneous and surgical factors for increasing prevalence of HCV. As several other studies have shown, poor socioeconomic conditions associated with overcrowding and inadequate hygiene at home are important risk factors for infection (Chiquete *et al.*, 2006; Lock *et al.*, 2007). Indeed, in present study a significant correlation was found between socioeconomic conditions, household crowding, non-sterile pricking, blood transfusion, use of barber/parlor's shops, dental and surgical procedures, confirming the previously reported data (Quddus *et al.*, 2006; Rifat-uz-Zaman, 2006). This study indicated further, the marked increase in the prevalence of infection in same segment of population within three years.

REFERENCES

1. **Ali, S.A., M.J.D. Rafe, H. Qureshi, S.H. Vermund** 2009. Hepatitis B and hepatitis C in Pakistan: prevalence and risk factors. *Int. J. Infect. Dis.*, 13: 9-19.
2. **Blumberg, B.S.** 1971. The discovery of Australian antigen and its relation to viral hepatitis. *Vitro*, 7:223.
3. **Carrano, A., E.A. Silva, E.T. Leite, R. Patatas, A. Ribeiro, M. Polite, R. Pereira, J.M. Kutner, M. Castilho, A.N. Martins, O.C. Ferreira and A. Tanuri** 2006. Hepatitis C virus incident cases open the possibility to study transmission route and source of infection. *Transfusion*, 49(6):1270-3.
4. **CDC** 2009. Hepatitis C Virus Transmission at an Outpatient Hemodialysis Unit --- New York, 2001--2008. *MMWR*, 58(08);189-4.
5. **Chiquete, E., L.V. Sánchez and A. Panduro** 2006. Routes of infection and clinical outcome of Mexican women reactive to anti-hepatitis C virus antibodies. *Hepatology Research*, 36(2):100-6.
6. **de Waure, C., C. Cefalo, G. Chiaradia, A. Sferrazza, L. Miele, G. Gasbarrini, W. Ricciardi, A. Grieco and G. La Torre** 2009. Intrafamilial Transmission of Hepatitis C Virus in Italy: A Systematic Review. *J. Epidemiol. Community Health*, [Epub ahead of print].
7. **Deisenhammer, S., K. Radon, D. Nowak and J. Reichert** 2006. Needlestick injuries during medical training. *J. Hospital Infection*, 63(3):263-7.

8. **Fry, D.E.** 2007. Occupational risks of blood exposure in the operating room. *Am Surg.*, 73(7):637-46.
9. **Gunn, R.A., P.J. Murray, C.H. Brennan, D.B. Callahan, M.J. Alter and H.S. Margolis** 2003. Evaluation of screening criteria to identify persons with hepatitis C virus infection among sexually transmitted disease clinic clients: results from the San Diego Viral Hepatitis Integration Project. *Sex Transm. Dis.*, 30(4):340-4.
10. **Habib, M., M.K. Mohamed, F. Abdel-Aziz, L.S. Magder, M. Abdel-Hamid, F. Gamil, S. Madkour, N.N. Mikhail, W. Anwar, G.T. Strickland, A.D. Fix and i. Sallam** 2001. Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. *Hepatology*, 33(1):248-3.
11. **Jacobs, B., P. Mayaud, J. Chagalucha, J. Todd, G. Ka-Gina, H. Grosskurth, Z.A. Berege** 1997. Sexual transmission of hepatitis B in Mwanza, Tanzania. *Sex Transm. Dis.*, 24(3):121-6.
12. **Jauffret-Roustide, M., Y. Le Strat, E. Couturier, D. Thierry, M. Rondy, M. Quaglia, N. Razafandratsima, J. Emmanuelli, G. Guibert, F. Barin and J.C. Desenclos** 2009. A national cross-sectional study among drug-users in France: epidemiology of HCV and highlight on practical and statistical aspects of the design. *BMC Infect. Dis.*, 9:113.
13. **Joseph, F.P. and G.L. Armstrong, L.A. Farrington, Y.J.G. Hutin and B.P. Bell** 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J. Hepatology*, 45(4):529-38.
14. **Joshi, S., Y.M. Song, T.H. Kim, S.I. Cho** 2008. Socio-economic status and the risk of liver cancer mortality: a prospective study in Korean men. *Public Health*, 122(11):1144-51.
15. **Khaja, M.N., C. Madhavi, R. Thippavazzula, F.A. Nafeesa, M. Habib, M. Chittoor, Habibullah and R.V. Guntaka** 2006. High prevalence of hepatitis C virus infection and genotype distribution among general population, blood donors and risk groups. *Infection, Genetics and Evolution*, 6(3):198-4.
16. **Lock, G., M. Dirscherl, F. Obermeier, C.M. Gelbmann, C. Hellerbrand, A. Knoll, J. Scholmerich and W. Jilg** 2007. Hepatitis C-Contamination of toothbrushes: myth or reality? *Minerva. Stomatol.*, 56(1-2):81-2.
17. **Mastromatteo, A.M., G.L. Rapaccini, M. Pompili, S. Ursino, V. Romano-Spica, G. Gasbarrini, G. Vanini** 2001. Hepatitis C virus infection: other biological fluids than blood may be responsible for intrafamilial spread. *Hepatogastroenterology*, 48(37):193-6.
18. **McQuillan, G.M., D. Kruszon-Moran, B.J. Kottiri, L.R. Curtin, J.W. Lucas and R.S. Kington** 2004. Racial and ethnic differences in the seroprevalence of 6 infectious diseases in the United States: data from NHANES III, 1988–1994. *Am. J. Public Health*, 94:1952-8.
19. **Memish, Z.A., and O.A. Osoba** 2006. International travel and sexually transmitted diseases. *Travel Med. Infect. Dis.*, 4(2):86-3.
20. **Mirza, I.A., S.M. Kazmi and A.N. Janjua** 2007. Frequency of hepatitis B surface antigen and anti- HCV in young adults - experience in southern punjab. *J. Coll. Physicians Surg Pak.*, 17(2):114-5.

21. **Nurgalieva, Z.Z., H.M. Malaty, D.Y. Graham, R. Almuchambetova, A. Machmudova, D. Kapsultanova, M.S. Osato, F.B. Hollinger and A. Zhangabylov** 2002. *Helicobacter pylori* infection in Kazakhstan: Effect of water source and household hygiene. *Am. J. Trop. Med. Hyg.*, 67(2):201-6.
22. **Perz, J.F., G.L. Armstrong, L.A. Farrington, Y.J. Hutin and B.P. Bell** 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J. Hepatol.*, 45:529-38.
23. **Quddus, A., S.P. Luby, Z. Jamal and T. Jafar** 2006. Prevalence of hepatitis B among Afghan refugees living in Balochistan, Pakistan. *Int. J. Infec. Diseases*, 10(3):242-7.
24. **Rifat-uz-Zaman** 2006. Prevalence of Hepatitis B and Hepatitis C viruses in human urban population of Bahawalpur District, Pakistan. *J. Med. Sci.*, 6(3):429-35.
25. **Ross, R.S., S. Viazov, Y.E. Khudyakov, G.L. Xia, Y. Lin, H. Holzmann, C. Sebesta, M. Roggendorf and O. Janata** 2009. Transmission of hepatitis C virus in an orthopedic hospital ward. *J. Med. Virol.*, 81(2):249-57.
26. **Rusmann, S., E.A. Dowlatshahi, G. Printzen, S. Habicht, J. Reichen and H. Zimmermann** 2007. Prevalence and associated factors of viral hepatitis and transferrin elevations in 5036 patients admitted to the emergency room of a Swiss university hospital: cross-sectional study. *BMC Gastroenterol.*, 7:5.
27. **Shepard, C.W., L. Finelli and M.J. Alter** 2005. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.*, 5:558-67.
28. **Smith, J.O. and R.K. Sterling** 2006. HIV Co-infection with Hepatitis C and Hepatitis B. *Curr. Infect. Dis. Rep.*, 8(5):409-18.
29. **Tassaduqe, K., M. Ali, A. Salam, H. Kalsoom, A. Salam and S. Umar** 2004. Studies on the Prevalence of Hepatitis B Virus in Relation to Sex, Age, Promotive Factors, Associated Symptoms and Season Among Human Urban Population of Multan, Pakistan. *J. Med. Sci.*, 4(3):183-7.
30. **The world factbook. Pakistan** 2007. USA: Central Intelligence Agency. Available at: <https://www.cia.gov/library/publications/the-world-factbook/geos/pk.html> (accessed September 2009).
31. **Vincent, L.R., F.I. Frank, R. Gross, M. Synnestvedt, A.R. Localio, J.R. Kostman and B.L. Strom** 2007. Self-reported hepatitis B and C virus infections had low sensitivity among HIV-infected patients. *J. Clin. Epid.*, 60(3):294-9.
32. **Wilber, J.C.** 1993. Development and use of laboratory tests for hepatitis C infection; a review. *J. Clin. Immunology*, 16:204.
33. **World Health Organization fact sheets** 2000. Hepatitis C. Geneva: World Health Organization. Available at: <http://www.who.int/mediacentre/factsheets/fs164/en/> (accessed September 2009).
34. **Xia, X., J. Luo, J. Bai and R. Yu** 2008. Epidemiology of hepatitis C virus infection among injection drug users in China: systematic review and meta-analysis. *Public Health*, 122(10):990-03.