ISSN 1684-8403
Journal of Statistics
Volume 17, 2010, pp. 33-46

Risk Factors for Hepatitis C Virus among Urban/Rural Settings of Patients Visiting Tertiary Care Hospitals at Lahore, Pakistan

Muhammad Ghias[1], Muhammad Khalid Pervaiz[2] and Ayaz Aslam[3]

Abstract

The prevalence of hepatitis C is increasing day by day all over the world. Most particularly in Pakistan hundreds of thousands people are dying by acquisition of HCV virus. There is no vaccine available and its treatment is very expensive. Hence, we being the under-developed country, solely have a blatant option regarding accurate determination of risk factors in our community to minimize economic strain and scheming for better prevention strategies. The need of the day is to conduct such types of studies on immediate basis. Main objective of this study is to find out associated factors with hepatitis C through the parsimonious Logistic Regression Model for urban/rural patients separately. A cross sectional survey from 400 patients was conducted on self-designed questionnaire from three major hospitals of Lahore. Further cases and controls were classified for urban and rural populations separately to analyze comparison of risk factors. Urban Logistic Regression Model depicts that ever-married patients, patients having un-educated mother, family history of hepatitis, surgical operations and road accidents are independent risk factors with hepatitis C. While in rural model patients' age, patient history of jaundice, and shaving by barber has been pragmatically different. Ever-married patients and family history of hepatitis are the common factors in both populations just mentioned. Risk of hepatitis C is found to be highest in urban ever-married patients. It is concluded that some risk factors for the transmission of HCV are different in urban and rural areas. Moreover, in Pakistan, this study revealed that the road accident is newly found vital risk factor in urban areas. Finally, our Logit Models can be used for the prediction of hepatitis C in both urban/rural settings of patients.

[1] Department of Statistics, GC University, Lahore, Pakistan
Email: ghiasgcu@yahoo.com
[2] Department of Statistics, GC University, Lahore, Pakistan
[3] Research Internee, Inmol Hospital, Lahore, Pakistan

Keywords

Hepatitis C, Risk factors, Chi-Square, Logistic regression, Odds ratios

1. Introduction

Hepatitis C is a worldwide health problem with an estimated 170 million cases world over (Lavanchy, 1999). Seroprevalence of hepatitis C virus varies substantially in different geographical areas throughout the world (Libby et al., 2002). The risk factors for the transmission of HCV virus may vary substantially in Pakistan (Shazi and Abbas, 2006). Around 80% of patients infected with HCV develop chronic hepatitis which is often asymptomatic and about one third of these develop progressive liver injury, fibrosis and cirrhosis over a period of 20-30 years (Leone and Rizzetto, 2005). Chronic HCV infection accounts for 50% cases of hepatocellular cancer in United States (Yao et al., 2005). After its discovery in 1989, a wide variety of tests based on detection of Anti-Hepatitis C antibody have been developed.

The overall estimated prevalence of HCV in Pakistan is 3% (range 0.5-31.9%), whereas in Punjab the estimated risk is 4.3% (range 0.4-31.9%) with wide variations in different areas (Ali et al., 2009). Therapeutic injections, contaminated blood/blood products, dental surgeries and shaving by barbers have been shown to be the major risk factors associated with transmission of HCV in Pakistan in previous studies (Bari et al., 2001; Huma, et al., 2009; Idrees and Riazuddin, 2008; Raja and Janjua, 2008). Populations at risk for HCV infection are patients receiving organ transplants, health-care workers, hemodialysis patients, and infants born to HCV-infected mothers (Tillmann and Manns, 1996). Shared use of personal hygiene items like tooth brushes, razor blades, nail clippers and manicure pliers have also been shown to be the risk factors for intra-familial transmission of HCV (Cavalheiro et al., 2009). However, the exact mode of transmission remains elusive in a vast majority of cases (Hyder et al., 2009).

We have previously reported that blood transfusion, dental surgery, general surgery, family history of hepatitis, tattooing, body piercing and hospitalization were significantly associated risk factors with hepatitis C (Ghias and Pervaiz, 2009). These results were drawn from the overall data but in this present study we revised the analysis to compare the risk factors in urban/rural populations separately. This segregation of risk factors might help in designing and prevention of hepatitis C in a better way. Most of the researches have applied weak statistical descriptive analysis to find out the significantly associated risk factors, so their results cannot be generalized. We aimed to build a separate prediction model for urban/rural settings of patients based upon Logistic Regression technique. Over the last decades Logistic Regression has become the gold standard and popular statistical approach for the prediction of binary response variable. Hence, separate Logit Models have been defined for the urban/rural patients and these models definitely will facilitate the clinicians and researchers to predict the chances of hepatitis C under the presence or absence of certain significant risk factors evaluated in these models. Comparison of risk factors in both urban/rural populations had not been previously studied.

In the absence of a vaccine, the only valuable option is the development of a well-built prevention program which can address the increasing burden of HCV in developing countries like Pakistan. It can only be implemented if accurate risk factor assessment is done satisfactorily in these countries.

2. Material and Methods

A cross sectional survey of total 400 patients presenting to the medical outdoors of three tertiary care hospitals in Lahore, Pakistan; Jinnah, Sheikh Zayed, and Mayo hospitals was conducted on a self-designed questionnaire to gather the requisite information both from cases and controls. Out of 400 sample size there were 185 urban and 215 rural patients. Further categorization of cases and controls in each group was [Urban: cases=126 & controls=59; Rural: cases=154 and controls=61]. Cases were those patients who found positive of Anti-HCV antibodies on ELISA method and controls with negative evidence of HCV. "A rural area was defined as an area or community without electricity, industries, and/or other minor social amenities, while urban are those areas where these facilities are available" (Goldstein, 1980). All patients agreed to participate in the study and signed a proper informed consent form.

Questionnaire's reliability analysis was done by applying Cronbach's Alpha test. Its value was found to be about 0.736 elucidating a reliable questionnaire for data collection. After consent, a standardized questionnaire was filled and all data regarding the socioeconomic and demographic factors was recorded (age, sex, area of residence, marital status, level of education, occupation, geographical location). History of unsafe injections, parenteral exposure to blood or blood products, previous hospitalizations, dental surgeries, needle accidents, major or minor surgical/medical procedures, personal or family history of jaundice or hepatitis, body piercing, tattooing, sharing of house-hold items, history of mass vaccination and history of cuts by tools/instruments under common use were taken into account. Risk factors were determined in detail and patients with multiple risk factors were assigned a risk factor taking into account the most probable one. Controls were also selected from the outdoor of these hospitals specifically patients who did not have a proven evidence of either Anti-HCV antibodies on ELISA or HBs Ag and had currently not presented with jaundice. Great care was taken to correctly classify both the cases and the controls according to the urban or rural dwellers, so that risk factors could be properly identified among both of these different populations.

3. Statistical Analysis

All data collected was recorded and analyzed by the SPSS (Statistical Package for Social Sciences) version 16.0. Data was analyzed descriptively and analytically. In descriptive analysis mean ± SD was calculated for quantitative variables like age, household income, family size, while count and percentage comparison was given for all possible risks factors (see Table 1). In analytical section, Bivariate and Multivariate analysis was performed to get the significantly associated risk factors. In Bivariate Chi-Square test of independence was applied along with Cramer's V and Phi Statistics. Phi and Cramer's V Statistics give the degree or strength of association of categorical risk factors with hepatitis C (Carol, 2006). The variables selected through Bivariate analysis were further verified by the Multiple Logistic Regression technique. Odds ratios and 95% confidence interval with a significance level of 5% were calculated. Forward LR (Likelihood ratio) criterion was used for variable selection method. Hosmer and Lemeshow test was applied to check overall model significance and Negalkerke R Square for goodness of fit for model (David and Hosmer, 1989). Finally, ROC (Receiver Operating Characteristic) curve was plotted for both urban/rural models adequacy comparison.

4. Results

Among whole sample of size 400 subjects 185 (46.25%) and 215 (53.75%) were the urban and rural patients, respectively. Out of total urban 185 subjects, 126 (68.11%) were cases and 59 (31.89%) were controls. Similarly, among the rural 215 patients, representing 154 (71.63%) cases and 61 (28.37%) controls. Gender-wise percentage comparison has shown that about 60.3% cases were males of urban area and 69.5% from rural area. Hence, this elucidates that males are more prevalent in both areas as compared to females. Frequency and percentage of cases in both areas were found to be high in ever-married patients i.e. 88.9% and 90.9%. About 42.9% cases lived in rural based joint families and 27.0% in urban dwellings. This indicates that in rural areas majority of the people prefer to live in joint families and hence they increase the risk of spreading hepatitis C among the others. This situation becomes more alarming when patients are un-educated. Parents are the key members in our families and if they are well educated then definitly they will have better knowledge about the spread of this disease and can convey to their family members. In our sample, about 57.9% and 72.2% cases have illiterate fathers and mothers, respectively, in urban settings. This percentage is significantly high in rural patients. About 47.6% and 57.1% cases from urban and rural areas, respectively, have no education. Overall results depict that majority of the cases have poor socio-economic status and zero education. Percentage comparison of clinical risk factors in urban/rural patients is shown in Table 1.

Bivariate Chi-Square test of independence was applied and significantly associated factors were found both in urban and rural patients at 5% level of significance. Their Phi & Cramer's V Satistics give the type and degree of association between the two categorical variables (see Table 2).

Table 1: Percentage Comparison of Risk Factors In Urban/Rural Dwellings

Variable	Category	Urban				Rural			
		Control	%	Case	%	Control	%	Case	%
Patient history of jaundice	No	51	86.4%	102	81.0%	54	88.5%	103	66.9%
	Yes	8	13.6%	24	19.0%	7	11.5%	51	33.1%
Family history of hepatitis	No	50	84.7%	90	71.4%	56	91.8%	123	79.9%
	Yes	9	15.3%	36	28.6%	5	8.2%	31	20.1%
History of blood transfusion	No	49	83.1%	84	66.7%	48	78.7%	107	69.5%
	Yes	10	16.9%	42	33.3%	13	21.3%	47	30.5%
Dental surgery	No	47	79.7%	83	65.9%	44	72.1%	98	63.6%
	Yes	12	20.3%	43	34.1%	17	27.9%	56	36.4%
Kidney dialysis	No	58	98.3%	123	97.6%	60	98.4%	148	96.1%
	Yes	1	1.7%	3	2.4%	1	1.6%	6	3.9%
Tattooing	No	53	89.8%	105	83.3%	54	88.5%	143	92.9%
	Yes	6	10.2%	21	16.7%	7	11.5%	11	7.1%
Body piercing	No	44	74.6%	76	60.3%	41	67.2%	109	70.8%
	Yes	15	25.4%	50	39.7%	20	32.8%	45	29.2%
More than one marriages	No	59	100.0%	123	97.6%	60	98.4%	144	93.5%
	Yes	0	.0%	3	2.4%	1	1.6%	10	6.5%
Have injected drugs	No	56	94.9%	125	99.2%	58	95.1%	151	98.1%
	Yes	3	5.1%	1	.8%	3	4.9%	3	1.9%
Contact with blood and needle	No	53	89.8%	112	88.9%	54	88.5%	137	89.0%
	Yes	6	10.2%	14	11.1%	7	11.5%	17	11.0%
Sharing syringes	No	58	98.3%	121	96.0%	57	93.4%	148	96.1%
	Yes	1	1.7%	5	4.0%	4	6.6%	6	3.9%
Sharing tooth brush	No	58	98.3%	126	100.0%	61	100.0%	149	96.8%
	Yes	1	1.7%	0	.0%	0	.0%	5	3.2%
Sharing razor	No	58	98.3%	124	98.4%	56	91.8%	138	89.6%
	Yes	1	1.7%	2	1.6%	5	8.2%	16	10.4%
Barber shaves	Never	16	27.1%	55	43.7%	22	36.1%	47	30.5%
	Occasionally	38	64.4%	64	50.8%	36	59.0%	86	55.8%
	Daily	5	8.5%	7	5.6%	3	4.9%	21	13.6%
Loss of blood due to accident	No	50	84.7%	66	52.4%	39	63.9%	89	57.8%
	Yes	9	15.3%	60	47.6%	22	36.1%	65	42.2%

History of cuts	No	44	74.6%	81	64.3%	52	85.2%	123	79.9%
	Yes	15	25.4%	45	35.7%	9	14.8%	31	20.1%
Ever admitted in hospitals	No	24	40.7%	41	32.5%	20	32.8%	44	28.6%
	Yes	35	59.3%	85	67.5%	41	67.2%	110	71.4%
Family history of liver disease	No	31	52.5%	46	36.5%	37	60.7%	65	42.2%
	Yes	28	47.5%	80	63.5%	24	39.3%	89	57.8%
Have organ transplant	No	59	100.0%	125	99.2%	61	100.0%	154	100.0%
	Yes	0	.0%	1	.8%	0	.0%	0	.0%
History of injections	Yes	59	100.0%	126	100.0%	61	100.0%	154	100.0%
History of injections	Govt. hospitals	11	18.6%	17	13.5%	8	13.1%	31	20.1%
	Private hospitals	15	25.4%	31	24.6%	7	11.5%	20	13.0%
	General practitioners	21	35.6%	36	28.6%	13	21.3%	25	16.2%
	Dispenser	12	20.3%	42	33.3%	33	54.1%	78	50.6%
Ever imprisoned in past	No	56	94.9%	113	89.7%	55	90.2%	139	90.3%
	Yes	3	5.1%	13	10.3%	6	9.8%	15	9.7%

Table 2: Bivariate Analysis of Risk Factors for Urban/Rural Patients

Factors	Pearson Chi-Square	p-value	Phi & Cramer' V
Urban Factors			
Marital status	24.701	0.000	0.365
Family history of hepatitis	4.194	0.41	0.151
History of blood transfusion	5.285	0.022	0.169
Surgical operation	17.878	0.000	0.319
Ever admitted in hospitals	5.013	0.025	0.165
Family history of liver disease	4.043	0.045	0.147
Rural Factors			
Pt. History of jaundice	11.146	0.001	0.228
Family history of hepatitis	3.989	0.046	0.136
Ever admitted in hospitals	6.439	0.011	0.173

Table 3: Multiple Logistic Regression Outputs for Urban/Rural Patients

Variable	B	S.E.	Wald-Statistic	p-value	Exp(B)	95.0% C.I. for EXP(B)	
						Lower	Upper
Urban Model							
Ever-married	2.569	.543	22.364	.000	13.051	4.501	37.845
Illiterate mother	1.034	.515	4.034	.045	2.813	1.025	7.717
Family History of Hepatitis	1.255	.490	6.568	.010	3.507	1.343	9.153
Surgical Operations	1.597	.442	13.028	.000	4.938	2.075	11.752
Road Accidents	.839	.423	3.922	.048	2.313	1.009	5.305
Constant	-2.483	.599	17.168	.000	.083		
Rural Model							
Age (Years)	.044	.015	8.088	.004	1.045	1.014	1.076
Ever-married	1.222	.556	4.830	.028	3.395	1.141	10.100
Patient history of jaundice	1.816	.511	12.655	.000	6.149	2.260	16.725
Family history of hepatitis	1.569	.606	6.698	.010	4.802	1.463	15.755
Barber Shave: Occasionally	.008	.398	2.000	.984	1.008	.462	2.198
Barber Shave: Daily	1.968	.845	5.428	.020	7.159	1.367	37.500
Constant	-2.543	.623	16.675	.000	.079		

In this final Logistic Regression output five different risk factors have been evaluated (see Table 3) for urban patients. First factor is “ever-married” patients with positive value of coefficient, i.e., 2.569 and odds ratio 13.05; CI: 4.501-37.485 assuming that base category was never-married patients. Results suggest that there are about 13 times more chances of getting hepatitis C in those patients who are ever-married. Similarly, other risk factors like illiterate mother, family history of hepatitis, surgical operations, road accidents related positively with HCV acquisition and increase the risk for 2.81, 3.51, 4.93 and 2.31 times, respectively. Every 95% CI of corresponding odds ratio gives a significant results because none of the 95% CI includes the figure exactly 1.

In Table 3 a separate Binary Logistic Regression analysis was performed and results suggested that two factors, i.e., ever-married patients and family history of hepatitis were the same as in urban model while patients age, patients history of jaundice and barber shave were found additional factors in rural model. Mainly barber shave is the key risk factor of rural patients and increases the risk of disease up to 7.16 times in those patients who daily shave from the barbers.

Wald test checks the significance of Regression coefficients individually under the hypothesis that Logit effect assumed to be zero. For checking the adequacy of fitted models HL (Hosmer and Lemeshow) test was used. This test relatively gives better results when the sample size is small and few variables are continuous than the traditional χ^2. Both urban and rural models were found to be statistically adequate with χ^2=6.704, p=0.349 and χ^2=6.580, p=0.583, respectively. In order to see the goodness of the fit for both models Cox and Snell R^2 and Nagelkerke R^2 were applied and their estimated values for urban model [0.263 and 0.368] and for rural model [0.260 and 0.376] (David and Hosmer, 1989).

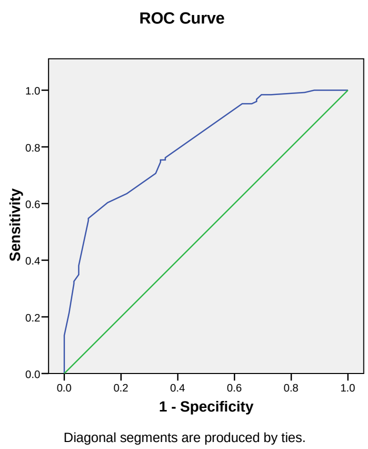
Logit Models:

Urban Model:

Y= -2.483 + 2.569(Ever-married) + 1.034(Illiterate mother)+1.255 (Family history of hepatitis) + 1.597 (Surgical operation) + 0.839 (Road Accident)

Rural Model:

Y= -2.543 + 0.044(AGE) +1.222 (Ever-married) + 1.816 (PAHOFJ) + 1.569 (FAHOFH) + 0.008 (Barber Shave (occasionally)) + 1.968 (Barber Shave (daily))



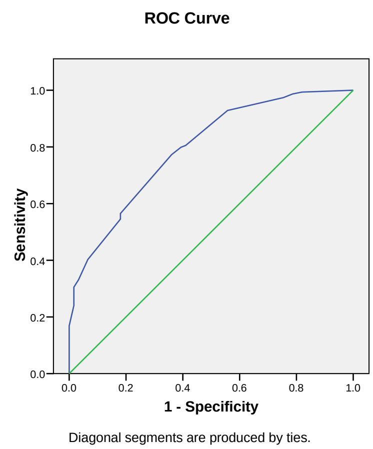


Figure. 1: ROC Curve for Overall Adequacy of the Fitted Models

ROC (Receiver operating characteristics) curve is a plot between the sensitivity and (1-specificity) showing that how much our Regression model gives the correct prediction of true positive and true negative outcomes. "The closer the ROC curve is to the upper left corner, the higher the overall accuracy of the fitted model"(Zweig & Campbell, 1993). In our case overall adequacy shown by ROC curve for urban and rural models is 80.4% and 79.4% respectively (Figure. 1).

5. Discussion

A risk factor is anything that increases a chance of developing a disease. While these risk factors are not direct causes, they do increase the chance of developing a disease. We studied the risk factors among urban and rural settings and separate models were constructed for measuring the predictive strength of significant risk factors in each model. It was done because of lifestyle differences and level of education varies not only among rural as well as in urban settings. Patients in the urban and rural settings have different occupations, level of education, and availability of medical facilities and hence have different occupational hazards and awareness of the risk factors for HCV. Similarly, environmental hazards, hospital facilities, level of awareness and education vary considerably in the urban and the rural circumstances. Hence, it was a need of such a separate study which identifies the risk factors of hepatitis C in urban/rural settings separately. According to our best information there is no article published where all possible demographic, socio-economic and clinical factors were discussed in different settings of patients like urban and rural areas. So, in this way effort has been made to segregate the risk factors at their right place.

Barber shaving was found to be a risk factor among the rural males only. In a previous study done in rural areas of Punjab, it was found that about 58% of barbers denied any health hazards associated with their profession (Wazir et al., 2008). It was also noted that 14% barbers were also performing minor surgeries like circumcision, ingrowing toe nail excision and abscess drainage (Luby et al., 2006). Both the customers and barber himself are at risk. In another cross-sectional study done in Rawalpindi-Islamabad, it was shown that razor blades were being reused in 46% of shaves (Janjua & Nizamy, 2004). It is clearly evident that barbers are totally unaware of the transmission risk factors of HCV and are still playing a role in the transmission of HCV in the community. In our study, shaving was not found to be a risk factor among the urban males. This may be a factor due to the campaigns generated by the government, mass media and the public health sectors in the previous few years regarding awareness among the general public concerning HCV risk factors. But these seem to have affected the urban areas only, which emphases the fact that the countryside areas are still prone and require particular attention.

Urban Logistic Model exposed that road accident leading to hospitalization was also significantly related. Road accidents are more common in the urban than the rural areas. Road accidents lead to major and minor surgeries thus exposing the patient to potential risk factors for the parenteral transmission of HCV. It was found that the level of the mother's education related negatively with HCV acquisition. As the level of mother's education was higher the chances of contracting HCV were lower and vice versa. It might be a possibility because with mother's education the family awareness regarding health increases, eventually leading to a decrease in the chances of acquiring HCV. Personal history of jaundice, surgical operations were found to be a risk factor for HCV. Surgical operation was also found to be more prevalent among the females, which points towards the unsafe medical practices both by the hospitals and the unqualified practitioners. A study conducted by Hepburn & Lawitz (2004) for evaluation of risk factors of hepatitis C among an urban population in Haiti revealed that intravenous drug use, and number of sexual partners were independently associated with HCV. These factors are quite different from our urban population shown by Multiple Logistic Regression. This augments the significance of our present study for enlightening the people about the spread of hepatitis C in urban/rural dwelling.

In Bivariate analysis some risk factors are comparatively different than selected by the Logistic Regression technique. It is not an astonishing thing, it may be possible that variables selected through Bivariate analysis may be different than Multivariate analysis because both statistical procedures have different criterion for the selection of significant factors. Multivariate analysis has relatively well-built criteria for the selection of significant variables than Bivariate analysis. This argument can be proved from the results of a study conducted by Heun and Hein (2005) on “Risk factors of major depression in the elderly”. Results from this study depict that some risk factors that were found significant in Bivariate analysis but stayed insignificant in case of Multivariate analysis.

Limitation: Our total sample was divided and subdivided into urban/rural patients and then into cases/controls for each group. This reduces power of the test so we need another study in this regard with larger sample size. At this moment due to unavailability of such sort of studies in Pakistan, we conducted this small sample study.

6. Conclusion

This study provides useful information that risk factors are different in the urban and the rural scenery. Taking prevention as the best option available yet for HCV, a differential approach is required by the government and the mass media in re-educating the healthcare providers and provision of more facilities to the medical community in the urban and rural societies.

References

1. 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. *International Journal of Infectious Diseases,* **13(1)**, 9-19.
2. Bari, A., Akhtar, S., Rahbar, M. H. and Luby, S. P. (2001). Risk factors for hepatitis C virus infection in male adults in Rawalpindi–Islamabad, Pakistan. *Tropical Medicine & International Health,* **6(9)**, 732-738.
3. Carol, E. O. (2006). *Statistical Application for Health Information Management*. 2nd Edition, Jones and Barlett, Canada.

4. Cavalheiro, N. P., De La Rosa, A., Elagin, S., Tengan, F. M., Araújo, E. S. A. and Barone, A. A. (2009). Hepatitis C: sexual or intrafamilial transmission? Epidemiological and phylogenetic analysis of hepatitis C virus in 24 infected couples. *Revista da Sociedade Brasileira de Medicina Tropical,* **42(3)**, 239-244.
5. David W. and Hosmer, S. L. (1989). *Applied Logistic Regression Analysis*. John Wiley & Sons, New York.
6. Ghias, M. and Pervaiz, M. K. (2009). Identification of epidemiological risk factors for hepatitis C in Punjab, Pakistan. *Journal of Ayub Medical College, Abbottabad,* **21(2)**, 156-161.
7. Goldstein, H. (1980). The reported demography and causes of blindness throughout the world. *Advanced Ophthalmology,* **40**, 1-99.
8. Hepburn, M. J. and Lawitz, E. J. (2004). Seroprevalence of hepatitis C and associated risk factors among an urban population in Haiti. *BMC Gastroenterology,* **4**, 1-4.
9. Heun, R. and Hein, S. (2005).Risk factors of major depression in the elderly. *Eur Psychiatry,* **20(3)**, 199-204.
10. Huma, Q., Ambreen, A., Kashif, R., Syed, A., Waquaruddin, A. and Syed, M. (2009). Determination of risk factors for hepatitis B and C in male patients suffering from chronic hepatitis. *BMC Research Notes,* ***2***, doi:10.1186/1756-0500-2-212.
11. Hyder, Q., Burhan-ul-Haq, M., Rashid, R., Qazi, S., Mehmood, S. and Hadi, S. F. (2009). Non-conventional Transmission of Hepatitis C: A true possibility ignored. *Journal of Pakistan Medical Association,* **59(7)**, 430-433.
12. Idrees, M. and Riazuddin, S. (2008). Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. *BMC Infectious Diseases,* **8(1)**, doi:10.1186/1471-2334-8-69.
13. Janjua, N. and Nizamy, M. (2004). Knowledge and practices of barbers about hepatitis B and C transmission in Rawalpindi and Islamabad. *Journal of Pakistan Medical Association,* ***54*****(3)**, 116-118.
14. Lavanchy, D. (1999). Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *Journal of Viral Hepatitis,* **6(1)**, 35-47.
15. Leone, N. and Rizzetto, M. (2005). Natural history of hepatitis C virus infection: from chronic hepatitis to cirrhosis, to hepatocellular carcinoma. *Minerva gastroenterologica e dietologica,* **51(1)**, 31-46.

16. Libby, P., Ridker, P. M. and Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation*, **105(9)**, 1135-1143.
17. Luby, S., Khanani, R., Zia, M., Vellani, Z., Ali, M., Qureshi, A., et al. (2006). Evaluation of blood bank practices in Karachi, Pakistan, and the government's response. *The Journal of the Pakistan Medical Association,* **56(1 Suppl 1)**, S25.
18. Raja, N. S. and Janjua, K. A. (2008). Epidemiology of hepatitis C virus infection in Pakistan. *Journal of Microbiology, Immunology, and Infection,* **41(1)**, 4-8.
19. Shazi, L. and Abbas, Z. (2006). Comparison of risk factors for hepatitis B and C in patients visiting a gastroenterology clinic. *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP,* **16(2)**, 104-111.
20. Tillmann, H. L. and Manns, M. P. (1996). Mode of hepatitis C virus infection, epidemiology, and chronicity rate in the general population and risk groups. *Digestive Diseases and Sciences,* **41**, 27-40.
21. Wazir, M. S., Mehmood, S., Ahmed, A. and Jadoon, H. R. (2008). Awareness among barbers about health hazards associated with their profession. *Journal of Ayub Medical College, Abbottabad,* **20(2)**, 35-38
22. Yao, F., Guo, J. M., Xu, C. F., Lou, Y. L., Xiao, B. X., Zhou, W. H., et al. (2005). Detecting AFP mRNA in peripheral blood of the patients with hepatocellular carcinoma, liver cirrhosis and hepatitis. *Clinica Chimica Acta*, **361(1-2)**, 119-127.
23. Zweig, M. H. and Campbell, G. (1993). Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin. Chem.*, **39(4)**, 561-577.