RESEARCH ARTICLE

Epidemiology of viral hepatitis B and C in Punjab, Pakistan: a multicenter cross-sectional study, 2017-18 [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Globally, approximately 240 million people are chronically infected with hepatitis B virus (HBV) and hepatitis C virus (HCV), which are responsible for 96% of all hepatitis-related mortality. Pakistan has the second highest prevalence of HCV in the world.

Methods: We conducted this study to ascertain the prevalence and potential risk factors associated with HBV and HCV infections in Punjab. A multi-center cross-sectional study was conducted, involving 24 Hepatitis Prevention and Treatment Clinics of the Pakistan Kidney and Liver Institute and Research Center clinics, Lahore. A total of 141,705 individuals who visited the clinics during 2017-18 were included for seroprevalence analysis of hepatitis B (HBsAg) and C (Anti-HCV). In addition, 12,427 individuals from the main group underwent detailed face-to-face interviews based on a predesigned questionnaire for risk factor assessment.

Results: The overall prevalence for HBV and HCV was 8.4% and 42.7%, respectively. Of those with HCV infection, 4.2% had a co-infection. The transgender population had a higher seroprevalence of HBV (11.8%) and HCV (58.8%). Higher HBV seroprevalence was found in a younger age group (16-30 years), while the older age group (≥ 61 years) had a higher HCV seroprevalence. Geographically, Muzaffargarh district had the highest HBV seroprevalence at 26%, followed by Rajanpur district (20.3%). HCV seroprevalence was significantly (P value < 0.05) higher in Shujabad district (66.4%), followed by Muzaffargarh (65.2%). Using multivariable logistic regression analysis, age, gender, intravenous injections, hijama therapy, dental procedure, circumcision by barbers, barber shaving, hospitalization and having had surgical procedures were all found to be significant risk factors ($OR > 1$; $p < 0.05$) for HBV and HCV.

Conclusions: There is an urgent need for proper implementation of

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preventive and control strategies, as well as formal evaluation and monitoring mechanisms. Sustainable and adequate funding of public-sector hepatitis programs is also an extremely important area that should not be neglected.

Keywords

Epidemiology, viral hepatitis, seroprevalence, risk factors, Pakistan

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Abbreviations

HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; GHSS, Global Health Sector Strategy; IDU, Injectable drug usage; PKLI-RC, Pakistan Kidney and Liver Institute and Research Center; HPTP, Hepatitis Prevention and Treatment Program; HPTC, Hepatitis Prevention and Treatment Clinic; EMR, Electronic Medical Record

Introduction

Hepatitis B (HBV) and hepatitis C (HCV) viral infections are major global health problems. Globally, approximately 240 million people are chronically infected with HBV and 130–150 million with HCV^{1,2}. Hepatitis B and C are responsible for 96% of all hepatitis-related mortality, leading to an estimated 1.45 million deaths annually (WHO, 2015). The consequences of chronic viral hepatitis and HIV infections are among the top ten lethal infections worldwide¹. Globally, 80% of the HCV burden is concentrated in low and middle-income countries (LMICs)³. However, published data suggest that a large proportion of HBV⁴ and HCV positive patients are not aware of their serostatus in developing countries, especially those belonging to low income households⁵. Furthermore, community-based studies indicate low screening uptake and considerable numbers of dropouts in both HBV and HCV care continuums⁶. The Global Health Sector Strategy (GHSS) on viral hepatitis stresses the need for aggressive targets for eliminating viral hepatitis as a public health threat by 2030 and has proposed a target of a 90% reduction in incidence and 65% reduction in mortality due to chronic HBV and HCV infections⁷. The higher prevalence of HBV and HCV in developing countries is also attributable to fragile health and hepatology services², in addition to multiple community-based risk factors.

Injectable drug usage (IDU) and associated sharing of contaminated injecting equipment⁸, blood donation⁹, unscreened blood transfusion¹⁰, surgical procedures¹¹, sharp object injuries¹², tattooing¹³ and barbering¹⁴ have all been reported as risk factors for the occurrence and transmission of HBV and HCV infections. The cumulative risk of HCV infection in a population often increases with age as well¹⁵.

Pakistan has the second highest prevalence of HCV (5%) after Egypt and the second highest number of people suffering from HCV after China¹⁶. In 2007–08, a nation-wide survey reported a general population prevalence of 2.5% and 4.9% for HBV and HCV, respectively. At a provincial level, Punjab has the highest burden of hepatitis¹⁷. The current study was conducted to report on the prevalence and risk factors associated with HBV and HCV infections in Punjab. The findings of this study will help in further understanding of the local epidemiology of hepatitis and its prevention and control.

Methods

Ethical approval and consent to participate

Ethical approval was obtained from the institutional review board (IRB) of the Pakistan Kidney and Liver Institute and Research Center (PKLI-RC; approval # PKLI-73). A copy of the consent form is provided as *Extended data*. Written consent was obtained from all the participants prior to interviews for the

use of their epidemiological data for research and publication. Parental consent from minor participants was obtained for participants aged 16 years or below. It was signed by the parent or guardian who accompanied the patient on behalf of the minor. This consent form was provided in both the local language and in English. A copy of the minor consent form duly signed by the principle investigator of study was also provided to the parents of the minor participant. In cases where parents were not willing to participate in the study and did not sign the minor consent form, the patients were not included in the study.

Study design and settings

Punjab is one of the most populated provinces of Pakistan, with a population of 110 million¹⁸, and has a high burden of hepatitis B and C. The PKLI-RC is state-of-the-art tertiary care center in Punjab's provincial capital, Lahore. The hospital is currently providing clinical services for liver and kidney diseases, including kidney transplants. Given the burden of hepatitis B and C in the province, the PKLI-RC has established an outreach program (Hepatitis Prevention and Treatment Program), which has been providing free-of-charge, evidence-based hepatitis B and C preventive, screening, diagnostic, vaccination and treatment services to the population of Punjab since March 2017. The Hepatitis Prevention and Treatment Program's (HPTP) services are being delivered through 24 Hepatitis Prevention and Treatment Clinics (HPTCs), which are based in Lahore and 23 other districts of the Punjab province. In addition to the PKLI-RC's HPTP, two other similar government-funded hepatitis control programs are providing necessary care services to the population of Punjab. This cross-sectional study is based on the data of individuals who visited the HPTCs from March 2017 to August 2018 for screening, diagnosis and treatment of hepatitis.

Study participants

All the visiting individuals were screened for HBV and HCV after their registration in the HPTCs' Electronic Medical Record (EMR). Patients were included in the study after signing the consent form once the study had been explained to them by the investigator¹⁹. Patients who did not sign the consent form to participate in the study were screened and treated but they were not included in the study analysis. Patients were included irrespective of their age, gender, ethnicity and locality within Punjab Province. Patients were excluded from the study if they were unwilling to participate in the study or came from any other province of the country. Face-to-face interviews were conducted after voluntary informed consent was given. A total of 141,705 individuals were registered and screened in at all HPTCs and were then interviewed at the health center, the majority being carried out at the HPTC-Lahore clinic. After signing the consent form, patients were interviewed about their exposures to risk factors by trained interviewers and/or medical officers using a questionnaire²⁰. Each question was made clear to the patient in order to get the true answer according to the patient's knowledge and memory. The criteria for inclusion in further analysis was no previous history of hepatitis. Of the 141,705 patients interviewed, only 12,427 met the criteria and only their data was used in the final analysis.

Data variables

The predictor variables included in the analyses were: age (in years); gender (male/female/transgender); ever having had intravenous (IV) injections (and number of injections in the last five years), ever having had blood transfusions, ever having had dental procedures, being hospitalized within the last five years, ever having had surgery, having taken recreational drugs in the last five years, having had a circumcision performed by a barber (males only), shaving by barbers, visiting a beauty parlor within the last five years, ever having had hijama therapy, having a body piercing in the last five years, having a tattoo in the last five years and ever having performed self-flagellation with sharp objects. The outcome variables were serostatus for hepatitis B (HBsAg) and hepatitis C (anti-HCV).

Data collection and management

The data was extracted from a centralized electronic medical records (EMR) database in Excel format. An epidemiological questionnaire, also part of the EMR system, was used to collect the data on individuals' demography and hepatitis-related risk factors. The questionnaire was piloted on 30 participants to obtain estimates about the expected response rates, data quality, validity and comprehensibility of the questionnaire. These participants were patients who visited these clinics routinely and were coming for a screening or checkup. They were provided with a consent form for their willingness to participate in the study. After signing the consent form, they were interviewed by medical officers using the pilot questionnaire. After reviewing the results of piloted questionnaire, a few variables were excluded from the final questionnaire (are you taking drugs; have you been bitten by any animal in last five years; are you taking cannabis) because these variables were found to be irrelevant and response rate for these variables were very low. The overall response rate was 96.66% (29/30) and the data quality was good in terms of dimensions such as 'consistency', 'accuracy', 'completeness', and 'timeliness'. The internal consistency was measured by Cronbach's alpha, with an alpha value of 0.87. The normality of data was assessed for the pilot survey to evaluate the fitness of data.

Questionnaires were completed by staff during face-to-face interviews, conducted in the local language by a trained interviewer. Interviewers were bachelor nurses or medical officers who had been trained by an epidemiologist to carry out interviews. The data was exported to Epi-Data for revisions such as data cleaning and data validation. Where >10% of all variables in any patient's questionnaire were missing or not provided, the patient was excluded from analysis.

Serum sample collection and diagnosis

A 5ml blood sample was collected from each patient using disposable vacuum syringes by nurses at the health centers. Samples collected at the sentinel sites were transported daily to the central laboratory for laboratory diagnosis. Serum specimens from all samples were separated using standard protocols and stored at 20°C on the same day until tested for HBsAg or Anti-HCV in the central PKLI laboratory in Lahore following WHO standard procedures. HBsAg and anti-HCV analyses were performed, the details of which are given below.

A chemiluminescent micro particle immunoassay (CMIA) anti-HCV assay (ARCHITECT Anti-HCV assay, ABBOTT Diagnostics; Catalog no. 6L47) was conducted for the qualitative detection of antibodies against HCV in the patients' serum and plasma. Recombinant HCV sample antigen-coated paramagnetic micro-particles and anti-human acridinium-labeled conjugates are fixed to create the reaction mixture and then incubated for 5 minutes at 25°C. Ancillary wash buffer was added to the mixture after washing, followed by another wash cycle and incubation for 5 minutes at 25°C. Pre-Trigger (Pre-Trigger Solution containing 1.32% w/v hydrogen peroxide) and Trigger solutions (containing 0.35N sodium hydroxide) are added to the mixture. The resulting chemiluminescent reaction was measured in relative-light-units (RLUs) using a luminometer. The presence of anti-HCV antibodies is determined by comparing the signals shown in the reaction to an active-calibration curve. If the signal in the specimen is greater or equal to the cutoff signal, the sample is then considered anti-HCV positive and vice versa. The ARCHITECT Anti-HCV assay calculates a result based on Sample RLU/Cutoff RLU (S/CO). The cutoff calculation is done such that; $\text{calibrator 1 Mean RLU Value} \times 0.074 = \text{Cutoff RLU}$. Specimens with S/CO values <1.00 are considered nonreactive by the ARCHITECT Anti-HCV assay and need not be tested further. Specimens with S/CO values ≥ 1.00 are considered reactive by the ARCHITECT Anti-HCV assay. A two-step immunoassay for qualitative detection of HBsAg using CMIA technology was used. The ARCHITECT HBsAg assay (Catalog no. 6C36) utilized a four parameter logistic curve-fit (y-weighted) to generate a calibration curve. Specimens with concentration values < 0.05 IU/mL was considered nonreactive by the criteria of ARCHITECT HBsAg. Specimens with concentration values ≥ 0.05 IU/mL were considered reactive by the criteria of ARCHITECT HBsAg.

Statistical analysis

Data was analyzed using SPSS version 22 (IBM, NY, USA). Results are reported as percentages and odds ratios. Descriptive statistics of prevalence are reported in Table 1. To analyze the association of independent variables with the outcome variables, logistic regression was performed²¹. Variables with a significance level of <0.25 were retained in the final logistic model. Regression analysis of the data was conducted using multivariable logistic regression model. Goodness of fit of the model used was assessed using the Hosmer-Lemeshow test and the likelihood-ratio test. QGIS Version 3.2.2²² was used for geographical distribution and heat maps were developed based on coordinates data collected during interviews to present the seroprevalence of HBV and HCV at the district level.

Results

A total of 141,705 individuals were included in the analysis, including 64,622 (45.6%) male, 77,066 (54.3%) female and 17 transgender (0.01%) patients²³. All the patients were screened for both HBV and HCV. The study population was distributed into five age groups, with 31–45 making up around 34% of the total, as highlighted in Table 1. Of these, data from 12,427 participants were included in further analysis²⁴. Overall 11,157 (89.7%) participants self-reported having had IV injections, with around 31% reported having more than 10 IV injections.

Table 1. Hepatitis B virus (HBV), hepatitis C virus (HCV) and co-infected cases in study population, 2017–18 (N=141,705).

Variable	Screened population, n	HBV reactive, n (%)	HCV reactive, n (%)	Co-infection reactive, n (%)
Total patients	141705	11839 (8.4)	60543 (42.7)	2553/60543 (4.2)
Age (years)				
≤15	12394	382 (3.1)	566 (4.6)	22 (3.8)
16–30	34845	3995 (11.5)	9416 (27.0)	480 (5.0)
31–45	48532	4306 (8.9)	24275 (50.0)	1035 (4.2)
46–60	35138	2414 (6.9)	19901 (56.6)	756 (3.7)
≥61	10796	742 (6.9)	6385 (59.1)	260 (4.0)
Gender				
Male	64622	7491 (11.6)	24068 (37.2)	1380 (5.7)
Female	77066	4346 (5.6)	36465 (43.3)	1173 (3.2)
Transgender	17	2 (11.8)	17 (58.8)	0 (0)

HBV, hepatitis B virus; HCV, hepatitis C virus; n, number of participants.

Seroprevalence of HBV, HCV and co-infection

Descriptive statistics were **Figure 1** gives the overall prevalence of HBsAg and anti-HCV in the study population, which was 8.4% and 42.7%, respectively. Of those with HCV infection, seroprevalence of co-infection with HBV was recorded to be 4.2%. The seroprevalence data stratified by various categories is presented in **Table 1**. Briefly, the prevalence of co-infection was higher in males (5.7%) than females (3.2%). The transgender population had a significantly higher seroprevalence of HBsAg (11.8%) and anti-HCV (58.8%) compared to other gender groups. The chi-square test found a directly associated trend of increased HCV and co-infection was found with increasing age. Geographical heat maps for the seroprevalence of HBV and HCV are presented in **Figure 2**. Muzaffargarh district had the highest HBV seroprevalence at 26%, followed by Rajanpur (20.3%), Lodhran (10.6%), and Shujabad (10.3%). HCV seroprevalence was significantly higher (P value <0.05) in Shujabad district (66.4%), followed by Muzaffargarh (65.2%), Nankana Sahab (62.5%), and Lodhran (61.8%).

Risk factors for HBV and HCV

In the multivariable logistic model, age group (16–30 years) was found to be a significant (p-value <0.05) risk factor (OR=4.2) for HBV occurrence, followed by age groups 31–45 years (OR=3.4), and >61 group (OR=3.2). Males had 2.1-fold higher risk of HBV infection than females. The other risk factors found to be significantly associated with the occurrence of HBV included exposure to hijama therapy, circumcision performed by barbers, barber shaving, recreational drug use, tattooing, beauty parlor visits, IV injections and having >10 injections (**Table 2**).

HCV occurrence was significantly associated with an increase in age of the patients. Belonging to the older age group (>61) was found to be a risk factor (OR=56.5; 95% C. I.=40.6-78.6) for HCV infection. Exposure to hijama therapy was also recorded

PKLI-RC= Pakistan Kidney and Liver Institute & Research Center clinics
ELISA= ABBOTT PRISM HBsAg; ABBOTT Anti-HCV 2.0
Coinfected=Positive for both HBV & HCV

Figure 1. Flow diagram of study population and hepatitis status on screening at HPTP clinics, Punjab, Pakistan, 2017–18.

as a risk factor (OR=6.0). Females had a 1.3-fold higher risk of HCV than males. The other significant risk factors for HCV included circumcision by barbers, recreational drug usage, tattooing, piercing, blood transfusion, dental procedures, surgical procedures and prior hospitalization (**Table 3**).

Age showed a significant association with HBV and HCV co-infection, as before. Patients in the >61 years age group had a 29.3-fold (95% C. I.=6.9-124.6) higher risk of co-infection than the younger age groups. The other significant risk factors included barber shaving, self-flagellation, recreational drug use, circumcision by barbers, piercing, beauty parlor visits, history of blood transfusion and having >10 IV injections (**Table 4**).

Figure 2. Left: map of Pakistan showing Punjab province outlined in red. Right: heat maps of HBV (A) and HCV (B) seroprevalence in population visiting HPTP clinics in 2017/18, Punjab Province, Pakistan (maps are based on the data collected in this study during interviews).

Discussion

Viral hepatitis is an important global public health problem. Several community-based studies report a higher prevalence of viral hepatitis in Pakistan both in Sindh and Punjab Province^{13–14,25}. Studies on understanding the epidemiology of viral hepatitis and related risk factors in Pakistan are limited. The present study is an attempt to assess the epidemiology and the potential risk factors in individuals who visited PKLI-RC's clinics during 2017–18.

We have found a very high prevalence in our clinic cohorts compared to the previous study reports in Pakistan^{25,26}. Several social factors are responsible for the higher prevalence of HBV and HCV, including lack of health and safety standards due to unsatisfactory awareness and knowledge of the disease in the general population, as reported by 27. The poor literacy rate of 43% in Pakistan²⁵ is also a reason for a higher prevalence of viral hepatitis. Areas with low literacy rates were found to have a higher prevalence (P value <0.05), as the awareness level of the population in these areas is low and these areas are exposed to the driving risk factors at a higher rate as compared to developed areas^{25,28}. The higher prevalence of HBV and HCV in the transgender population could be due to a lower socio-economic status and behavioral factors including alcoholism, promiscuity and IV drugs usage^{25,28}. These behavioral factors are practiced more in transgender community and among the

groups who are in contact with transgender community at regular basis⁷. The increasing prevalence of HBV and HCV in the transgender community is a concerning situation for an otherwise healthy population. Furthermore, the higher prevalence of hepatitis is also linked to the improper disposal of hospital waste in Pakistan²⁹. This matter is now being aggressively tackled with the approval and implementation of the Hepatitis Act in Punjab.

Epidemiology of hepatitis risk factors

We have found age to be a significant risk factor associated with the occurrence of HBV (Table 2). The younger age group (16–30 years) was at a 4.2-fold higher risk of HBV than the older age groups. The variation in the seroprevalence of HBV in different age groups could be due to the changing immune response of the body to infectious agents at certain ages, as also supported by the findings reported previously (Cheng *et al.*, 2007). We also found males to be at a higher risk and barber shaving was significantly associated with HBV occurrence. Exposure to barber shaving, IV injections, and hijama therapy could be promoting the risk of HBV occurrence in males in our study population. Frequent exposure to barber shaving, surgical and dental procedures, blood donation and a higher number of injections being important risk factors for HCV infection has been reported elsewhere¹⁰. Our results are in line with the findings reported from other regions of the world^{21,25,30}.

Table 2. Multivariable logistic regression of risk factors for hepatitis B in the study population, 2017–18 (N=12,427).

Variables	HBV infection (HBsAg)		
	Reactive/non-reactive	OR	95% CI
Total patients	745/11682		
Age (years)			
≤15	36/1744	Ref	
16–30	228/2641	4.2	2.9–5.9
31–45	274/3866	3.4	2.4–4.8
46–60	160/2728	2.8	1.9–4.1
≥61	47/703	3.2	2.0–5.0
Gender (male)	470/5283	2.1	1.7–2.4
IV injection	672/10485	1.1	0.8–1.3
No. of IV injections			
≤10	462/6877	0.8	0.6–1.1
>10	211/3605	1.0	0.7–1.3
Blood transfusion	71/1220	1.1	0.8–1.4
Dental procedure	294/4561	1.0	0.8–1.1
Hospitalization	229/3774	1.1	0.9–1.2
Surgical procedure	188/3081	1.0	0.8–1.2
Recreational drug use	31/311	1.5	1.1–2.3
Circumcision performed by:			
Barbers	375/4170	2.7	1.3–5.3
Clinicians	9/273	1.8	1.5–2.1
Barber shaving	377/4170	2.5	2.0–3.1
Beauty parlor visit	63/273	1.4	1.1–1.8
Hijama therapy	2/3497	2.9	0.2–35.9
Body piercing	211/1346	1.0	0.9–1.2
Tattooing	24/291	1.3	0.8–1.9
Self-flagellation	14/254	1.1	0.6–1.9

OR, odds ratio; C, 95% confidence interval; HBV, hepatitis B virus; IV, intravenous.

Hepatitis C viral infection has been reported to be an emerging epidemic in Pakistan²⁵. Here, an elevating trend of HCV seroprevalence was recorded with an increase in age (Table 3). The highest seroprevalence of HCV was recorded in age group ≥61 years, followed by age group 46–60. This is likely due to a cumulative effect whereby the exposure to multiple risk factors increases with age. For instance, the number of child births, recreational drug use, exposure to barbers and numbers of injections all increase with age. These results are in line with the findings of 29. Hijama therapy was identified as a potential risk factor for HCV infection. This might be due to unhygienic environments where the procedure is carried out and the use of unsterilized instruments that are not properly autoclaved and are used on multiple individuals²⁷. Circumcision performed by barbers was also recorded as a risk factor in our study.

This could be attributable to the use of contaminated instruments on multiple individuals²⁶. Other exposure variables found in this study have previously been reported, such as history of hospitalization, surgical procedures, recreational drug use, IV injections, tattooing, piercing, barbers shaving, beauty parlor visit, blood transfusion and self-flagellation with sharp objects^{31,32}.

Strengths and limitations of the study

The main strength of this study is that this involved 24 clinics based in various parts of the province and had a large sample size of 141,705 individuals, who were screened for HBV and HCV infections through ELISA. Data was collected through a centralized EMR system assuring the accuracy, completeness, consistency, reliability and repeatability of the data. Of

Table 3. Multivariable logistic regression of risk factors for hepatitis C in the study population, 2017–18 (N=12,427).

Variables	HCV (anti-HCV)		
	Positive/negative	OR	95% CI
Total patients screened	5019/7408		
Age (years)			
≤15	45/1735	Ref	
16–30	761/2108	13.9	10.2–18.9
31–45	2065/2075	38.3	28.3–51.9
46–60	1702/1186	55.3	40.7–75.0
≥61	446/304	56.5	40.6–78.6
Gender (female)	2919/3755	1.3	1.2–1.4
IV injection	4513/6644	0.9	0.8–1.0
No. of IV injections			
≤10	2993/4346	0.9	0.8–1.1
>10	1525/2291	1.1	0.9–1.1
Blood transfusion	587/704	1.2	1.1–1.4
Dental procedure	2050/2805	1.1	1.0–1.2
Hospitalization	1644/2359	1.1	0.9–1.1
Surgical procedure	1375/1894	1.1	1.01–1.1
Recreational drug use	161/181	1.3	1.0–1.6
Circumcision performed by:			
Barbers	1671/2874	1.3	1.2–1.4
Clinicians	15/267	0.1	0.07–0.1
Barber shaving	1775/2099	1.1	0.9–1.2
Beauty parlor visit	591/818	1.1	0.9–1.2
Hijama therapy	33/22	6.0	1.1–30.9
Body piercing	1369/2338	1.2	1.1–1.3
Tattooing	151/164	1.3	1.0–1.7
Self-flagellation	102/166	1.1	0.8–1.4

OR, odds ratio; CI, 95% confidence interval; HCV, hepatitis C virus; IV, intravenous.

the 141,705-study population, we only interviewed 12,427 participants for identification of risk factors associated with viral hepatitis. However, this sizeable number is still large enough to provide us reliable estimates (Table 2–Table 4) on the role of various risk factors in the occurrence and spread of hepatitis in the local population. The limitations of the study are that all 141,705 individuals were not interviewed due to loss to follow up or low response levels in signing the consent form.

Conclusions

There is an urgent need for the implementation of preventive and control strategies to halt the rapid increase in seroprevalence of HBV and HCV in Punjab. A considerable amount of

work is already being done to tackle the issue including, but not limited to, screening of donated blood, proper sterilization of health care instruments, implementation of hospital waste management systems, training of barbers, improving the uptake of birth dose of hepatitis B vaccination, and the implementation of the Punjab Hepatitis Act. These initiatives also need to be supported by improvements in operational research, such as in evaluating and monitoring their impact in decreasing the spread of viral hepatitis. Health professionals must be trained to disseminate accurate information about viral hepatitis. Sustainable and adequate funding of the public-sector hepatitis programs is also an extremely important area that should not be neglected.

Table 4. Multivariable logistic regression of risk factors for co-infection with HBV and HCV in the study population, 2017–18 (N=12,427).

Variables	Co-infection with HBV & HCV		
	Positive/negative	OR	95% CI
Total patients screened	211/4808		
Age (years)			
≤15	2/43	Ref	
16–30	35/726	10.9	2.6–45.7
31–45	81/1984	17.7	4.3–72.2
46–60	69/1633	21.7	5.3–88.8
≥61	24/422	29.3	6.9–124.6
Gender (male)	117/1983	0.6	0.5–0.9
IV injection	191/4322	1.0	0.6–1.7
No. of IV injections			
≤10	130/2863	0.9	0.5–1.5
>10	62/1463	1.1	0.8–1.4
Blood transfusion	25/562	1.1	0.7–1.7
Dental procedure	86/1964	1.0	0.8–1.4
Hospitalization	67/1577	1.0	0.7–1.3
Surgical procedure	56/1319	1.0	0.7–1.3
Recreational drug use	8/153	1.4	0.6–2.8
Circumcision performed by:			
Barbers	92/1579	1.3	0.9–1.7
Clinicians	1/14	0.2	0.0–1.6
Barber shaving	101/1674	1.6	1.0–2.3
Beauty parlor visit	18/573	1.3	0.8–2.2
Hijama therapy	1/32	0.9	0.1–6.7
Body piercing	52/1317	1.3	0.9–1.7
Tattooing	6/145	1.1	0.4–2.5
Self-flagellation	3/99	1.5	0.4–4.8

OR, odds ratio; CI, 95% confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; IV, intravenous.

Data availability

Underlying data

Figshare: Epidemiology of viral hepatitis B and C in Punjab, Pakistan: a multi-center cross-sectional study, 2017–18 (Extended Data File). <https://doi.org/10.6084/m9.figshare.9891260.v1>²³

This project contains the following underlying data:

- Extended Data File F1000.xlsx (raw data for all 141,705 individuals interviewed)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Figshare: Epidemiology of Viral Hepatitis B and C in Punjab, Pakistan: A Multicenter Cross-sectional Study, 2017–18. <https://doi.org/10.6084/m9.figshare.9702845.v1>²⁴

This project contains the following underlying data:

- Data file for F1000.xlsx (raw data for the 12,427 individuals included in final analysis)

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Extended data

Figshare: Questionnaire.docx for Epidemiology of viral hepatitis B and C in Punjab, Pakistan: a multi center cross-sectional study, 2017–18. <https://doi.org/10.6084/m9.figshare.9993044.v1>²⁰

Figshare: Epidemiology of viral hepatitis B and C in Punjab, Pakistan: a multicenter cross-sectional study, 2017–18 (Consent Form). <https://doi.org/10.6084/m9.figshare.9730592.v2>¹⁹

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgements

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Reviewer Report 29 June 2020

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? **Mohammad Farahmand**

Department of Virology, School of Public Health, Tehran University of Medical Science, Tehran, Iran

The manuscript entitled: "Epidemiology of viral hepatitis B and C in Punjab, Pakistan: a multicenter cross-sectional study, 2017-18" was aimed to assess the prevalence of HBV and HCV infections in individuals who visited the Hepatitis Prevention and Treatment Clinics (HPTCs) in Punjab, Pakistan. The authors also performed a multivariate analysis to analyze potential risk factors. The study was conducted on a large sample size of 141,705 individuals. After reviewing the above manuscript in detail, I consider the manuscript well-written, and the arguments are developed in an orderly manner. Still, there are some concerns about this paper to be addressed.

1. In the "Statistical analysis" section, the authors claimed that variables with a significance level of <0.25 were maintained in the final logistic model, but all variables are included in tables 2,3, and 4. Please explain whether all variables in all tables have a significance level of <0.25 .
2. In addition to multivariate analysis, please include the univariate logistic regression model results in tables 2,3, and 4.
3. In the result section, part: "Seroprevalence of HBV, HCV and co-infection" line 1, it seems figure 1 was not appropriately cited in the text.
4. In the discussion section, the authors try to compare their results that using samples from hospitals with hepatitis disorders (current manuscript), with the data from Akhtar *et al.* (ref 25) that estimates the prevalence of HCV and HBV infection in transgender males. Any comparison between these two groups, because of using different sampling population is not valid.
5. In the discussion section, part: "Epidemiology of hepatitis risk factors": for probable reasons for elevating trend of HCV seroprevalence with an increase in age, the authors should consider that about 70% of people infected with HCV will develop chronic HCV

infection. So, they may remain seropositive for several years.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical virology and epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 14 April 2020

<https://doi.org/10.5256/f1000research.22160.r61796>

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Arshad Altaf

Global HIV and Hepatitis Dept, World Health Organisation, Geneva, Switzerland

Abstract

Background

1. Please use HBV and HCV estimates from the WHO fact sheets which are more updated.

Results

1. For clarity it is suggested to expand what the authors mean by "intravenous injections" as many readers might associate it with intravenous drug use. If it is intravenous therapeutic

or medical injections please state that.

Conclusions

1. Please make the conclusion more objective. When authors say "monitoring mechanism" please clarify monitoring of what? Public sector funding for what?

Introductions

1. Please use the HBV and HCV estimates from these WHO factsheets.

HBV

HCV

2. The most common terms nowadays is people who inject drugs (PWIDs) or injection drug user (IDU) however, PWID has replaced IDU.

Data variables

1. Please clarify if the question only asked intravenous injections and information regarding receiving intra muscular injections was not collected? In order to be clear it is suggested to rephrase ever having had intravenous (IV) injections (and number of injections in the last five years) to number of intravenous injections received in the last five years. Rephrase: ever having had blood transfusions to ever had blood transfusion. Applicable to all similar variables.

Data collection and management

1. Generally, so much detail about piloting is not required. In the interest of "economy of words" it is suggested to simply say, "the questionnaire was piloted and participants of the pilot were not included in the actual study."

Serum sample collection and diagnosis

1. I am not qualified to review this section. However, if it can be reduced to fewer lines by the authors it will be beneficial for the reader. But if the authors are convinced that it should stay, I am okay with that.

Results

1. What do the authors mean by descriptive prevalence?
2. The standard way of presenting results is by describing demographic characteristics of the subjects which can also be developed by hepatitis status wise.
3. Tables should provide more information about the participants.
4. Not clear what does a "heat map" mean? It seems like a distribution map of hepatitis status of the patients district wise. The map has been developed based on hepatitis status and district of origin of the patients.
5. How GPS coordinates were collected when all interviews were conducted at the health facility?
6. The flow chart provides the process of study i.e. the number of patients came in the health centres during the study period, number refused, number screened then perhaps the number interviewed. At the moment the flow chart and the tables seem the same. They have to be different.
7. In "seroprevalence of HBV, HCV and co infection" there is incorrect use of Chisquare. Chisquare shows association between two variables not trends.

8. Tables 2 to 4 showing crude OR as adjusted OR? Confidence intervals have 1 in the range which is insignificant but it is shown as significant.

Discussion

1. Discussion should be in this pattern:
 1. First para describing the key findings of the study.
 2. Subsequent paras talk about key findings in light of other peer reviewed studies.
 3. Penultimate para should be the limitation in which also authors have to mention "recall bias".
 4. In conclusion please mention the use of availability of direct acting antivirals (DAAs) in Pakistan particularly for hepatitis C.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Both experimental and non experimental research. However, the statistical section certainly needs to be reviewed by a qualified person.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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