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Research Article

Isolation, identification, and molecular characterisation of Hepatitis B and C viruses in patients from Bahawalpur, Pakistan

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Abstract:

Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are globally linked to chronic liver diseases and spread mainly through parenteral, vertical, and sexual routes. The epidemiology of HBV and HCV infections in Bahawalpur, Pakistan are poorly understood. This study aimed to evaluate epidemiological dynamics and risk factors in Bahawalpur, Pakistan. A total of 263 samples were detected with HCV and HBV among various age groups between 21-80 years via molecular techniques (ELISA and PCR). Results for disease prevalence showed a higher rate for HCV compared to HBV. Further, the gender-wise prevalence of HBV and HCV was also assessed. PCR revealed a 50.1% prevalence rate in males compared to 46% in females, whereas ELISA measured 34.5% and 17.4%. Moreover, results also confirmed the higher infection rate of Hepatitis in males with moderate age compared to young and older age. This study concluded a higher prevalence rate of hepatitis C in the population of Bahawalpur, which has moderate age and causing a considerable loss of economy to the community as well as Pakistan. Our findings improve understanding of the prevalence of HBV and HCV infection in Bahawalpur City, aiding government efforts to minimise associated losses.

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1. Introduction

Hepatitis is one of the most hazardous aspects of the global healthcare system, with an estimated 2.3 billion infections of various hepatitis types. Hepatitis is a viral infection that causes the deaths of about 1.4 million people each year. As per a study directed by the Pakistan Medical Research Council, about 13 million residents of Pakistan were experiencing Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) (Khalid *et al.*, 2021), which incorporates 10 million populations because of hepatitis C infection contamination alone (Asghar *et al.*, 2021). There are five different hepatitis types depending on their causing agents such as A, B, C, D, and E. However, HBV and HCV are the most dangerous of infections that cause around 90% of overall deaths around the world. Despite its deep roots in public health issues, hepatitis was not given much importance. However, now the WHO has taken some serious concerns about its prevention and has therefore declared the "2030 Agenda for Sustainable Development Goals" (Hellard *et al.*, 2017).

Hepatitis usually causes inflammation of the liver that sometimes leads to chronic and even death. HBV is a DNA virus with a diameter of 42-47 nm that commonly enters the liver through blood circulation. HBV is a vaccine-preventable liver infection (Aggeletopoulou *et al.*, 2017; Farooq *et al.*, 2021). However, HCV is an enveloped RNA virus, much smaller in size. HCV has no vaccine yet for treatment, so it is known to be more fatal than HBV (Hayes *et al.*, 2022; Saleem *et al.*, 2022). Various transmission modes for different hepatitis types are reported by different studies. Less common types of hepatitis A and E are generally transmitted through contaminated food and water. Highly fatal types of hepatitis, such as B and C, are transmitted through parental contact with infected body fluids and direct transmission from infected mothers to their babies. However, some modes are common for all types of hepatitis such as blood fusion, use of contaminated equipment for medical procedures, one family member to another, and sexual contact (Kazmi *et al.*, 2022; Ejaz *et al.*, 2023). The developed countries have a declining rate of hepatitis infections (Almezgagi *et al.*, 2020). However, it has been seen that those developing nations, where the public healthcare system is not so efficient, have a much higher prevalence rate of the hepatitis, especially HBV and HCV, which is further increasing daily.

Pakistan is also facing the issue of hepatitis, which was reported with a higher infection rate in many previous studies (Ali *et al.*, 2010; Mehmood *et al.*, 2020). The increase in hepatitis infection is especially reported to increase greatly in the last two decades. The local government of Pakistan has also started a national hepatitis sentry site observation system to assess hepatitis prevalence nationwide (Mehmood *et al.*, 2020). However, this system could not facilitate much due to limited infrastructure and facilities and became limited to regional capitals and Islamabad. Moreover, another limitation of this system was its restriction to screening seropositivity but excluding the high-risk groups.

This comprehensive study aims to molecularly characterise Hepatitis B and C viruses in patients from Bahawalpur, Pakistan. This includes identifying the genotypes and subtypes of both viruses prevalent among the patients, analysing any mutations in the viral genomes that may be associated with drug resistance or disease progression, and gaining insights into the epidemiological patterns of Hepatitis B and C virus infections in Bahawalpur. Additionally, the study aims to correlate viral genotypes and viral load with clinical outcomes to understand the implications for patient management. Ultimately, the study seeks to provide valuable data

that can inform public health strategies and improve the diagnosis, treatment, and prevention of Hepatitis B and C infections in this specific population.

2. Materials and methods

This study aimed to assess the prevalence rate and major risk factors of HBV and HCV in the general population of Bahawalpur City using modern assessment methods such as ALISA and PCR.

2.1. Study area and design

The study was conducted at Pathology Laboratory in Quaid-e-Azam Medical College Bahawalpur from September 2021 to January 2022. All the hepatitis B and C samples were collected from the population of Bahawalpur City along with the information of patients coming from surrounding areas such as Bahawalnagar, Khair Pur, Tamai Wali, Ahmad Pur, Uch Sharif, Yazman, and Lodhran. There was no limitation in sample selection based on gender, location etc. The targeted samples of each person were selected on a random basis. However, only samples screened and detected for HBV and HCV positive from September 2021 to January 2022 were eligible for the study. It was cross-sectional laboratory-based research, where all serum samples were tested for HBsAg and HCV at the laboratory. All samples that tested positive for infections by ELISA were additionally tested by Polymerase Chain Reaction (PCR) to verify anti-HCV and HBsAg positivity. Subject demographics and the risk factors such as age, gender, family history, home hygienic condition, and financial status for all samples were reviewed by examining pre-designed questionnaires. Written consent was also taken from each respondent before including them in this research.

2.2. Sample collection

All the blood tests were gathered from the pathology lab of Bahawal Victoria Hospital situated in Bahawalpur City, Punjab, Pakistan. Each patient's blood sample was taken from a hand vein and transferred into the sterilised vial. Then, the collected samples were correctly labelled. Samples were arranged and centrifuged at 3000 rpm for 2 minutes. The serum is separated and transferred to a plastic vial labelled according to the laboratory number. The entire labelled vials were arranged in racks and then in an ELISA automated machine. During the Sample collection segment, information about patients, for example, calling, age, and orientation, was recorded. The prevalence of hepatitis in various risk factors such as age, sex, and socio-economic conditions was also calculated using the below formula.

$$Prevalence\ (\text{as}\ \%) = 100 \times \frac{Number\ of\ Positive\ Samples}{Number\ of\ Total\ Samples\ Measured}$$

2.3. Laboratory work

All the collected samples were tested for HBV and HCV markers using the HBsAg Sandwich ELISA kits (Antec, UK). The protocol used for all this process was the same as given by the manufacturers using AxSym-Analyzer for hepatitis B surface antigen (HBsAg), total antibodies to hepatitis B core antigen (HBcAb), antibodies to hepatitis B surface antigen (AntiHBs), and for antibodies to Hepatitis C Virus (anti-HCV). To examine HBV positive sera,

an ELISA test was conducted to examine potential antibodies reciprocal to its antigen utilising financially accessible units following the production's convention (BIO KIT, Barcelona-Spain). The HBV-positive samples from ELISA were in this way handled for DNA detachment. The hepanostika HBsAg Uni-FormII ELISA was used to promote HBs Ag subtypes in human serum or plasma (Voller *et al*., 1978). The Anti-HBs CLIA kit (used in this research) works on the principle of solid-phase sandwich enzyme-linked immunosorbent assay.

Multiple epitopes of HCV proteins are bound to the microtiter plate. The HCV antibodies react with recombinant proteins and attach to the solid phase and non-active antibodies are removed from the reagent with the help of buffer. In the end, the subsequent reaction of chromogenic substrate visualises when human IgGs along with antigen react with mouse anti-human IgG peroxidase conjugate. The positive samples visualise dark blue colour to medium whereas no or very pale blue colour indicates a negative reaction. Further, the patients who were HBV positive were also confirmed by using Real-time PCR genotype identification. DNA was first extracted from each sample using (Grimberg *et al*., 1989) protocol and then quantified on gel electrophoresis. Only the samples with good quality and quantity DNA were included in the study. PCR strategy for the assessment of diseases using the technique for DNA enhancement portrayed (Saiki *et al*., 1985). In PCR, a targeted piece of DNA is characterised using a thermostable DNA polymerase. The AB analytical kit was used for PCR detection of hepatitis in the current study.

2.4. Statistical analysis

All the collected data was arranged in Microsoft Excel 365, and then statistical analysis was performed in STATIX software (version 8.1). Descriptive statistics were used in the current study to analyse the data collected here.

3. Results

3.1. Gender-Wise Prevalence of HBV and HCV by ELISA

The prevalence of HBV and HCV detected using the ELISA technique among various genders is listed in Table-1. A total of 15 female and 45 male samples were included in this study and only 1 (7%) female and 9 (20%) males tested positive for HBV. Similarly, 4 (27%) females and 22 (49%) males were recorded to be positive for HCV. The overall results showed 17% and 43% of HBV and HCV positivity prevalence rates, respectively. A graphical representation of the results is also given in Table-1.

Table-1: Gender-wise prevalence of HBV and HCV by ELISA

Gender	No of Samples	HBV Positive	Prevalence (%)	HCV Positive	Prevalence (%)
F	15	1	7%	4	27%
M	45	9	20%	22	49%
Grand Total	60	10	17%	26	43%

3.2. Gender-wise prevalence of HBV by PCR

The results observed from ELISA were further confirmed by PCR genotyping test. A total of

17 female and 60 male samples were included in the PCR test, out of which 8 (47%) and 32 (47%) females were observed to be positive for HBV and HCV, respectively. Similarly, 37 (62%) and 22 (38%) males had HBV and HCV, respectively. Overall results showed 45 (58%) and 54 (43%) positive rates for HBV and HCV by PCR test as listed in Table 2. A graphical representation of the results is also given in Table-2.

Table-2: Prevalence of active HBV and HCV among various genders by PCR test

Sex	HBV Positive	Prevalence (%)	HCV Positive	Prevalence (%)
F	8	47%	32	47%
M	37	62%	22	38%
Grand Total	45	58%	54	43%

3.3. Age-Wise Prevalence of HBV and HCV by ELISA and PCR technique

To calculate the age-wise HBV and HCV prevalence in persons of different age groups were created. The results of the present study showed that total age groups were affected while the prevalence percentage differed in different age groups. The following results were obtained using the ELISA technique. All the screened samples were classified into 6 age groups that drop into different categories: 21 to 80 years or above. The age-wise male and female groups were analysed separately. A graphical representation of the results is also given in Figure 1 and Figure 2.

Figure 1: Prevalence of active HBV and HCV amongst the different age groups by ELISA

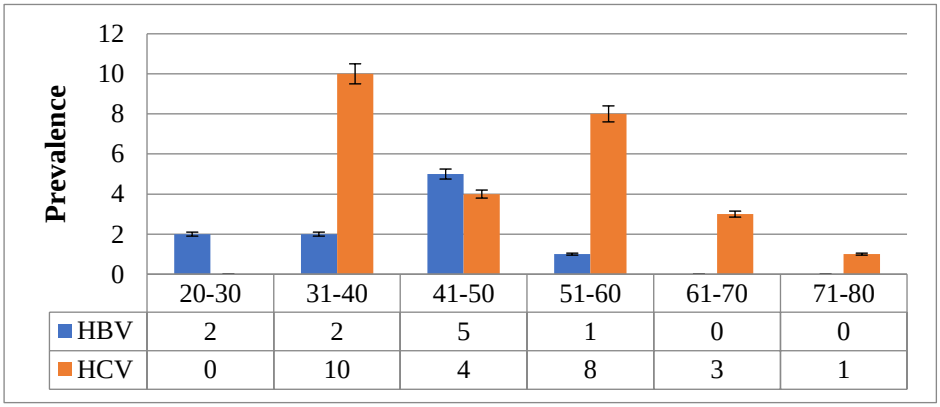
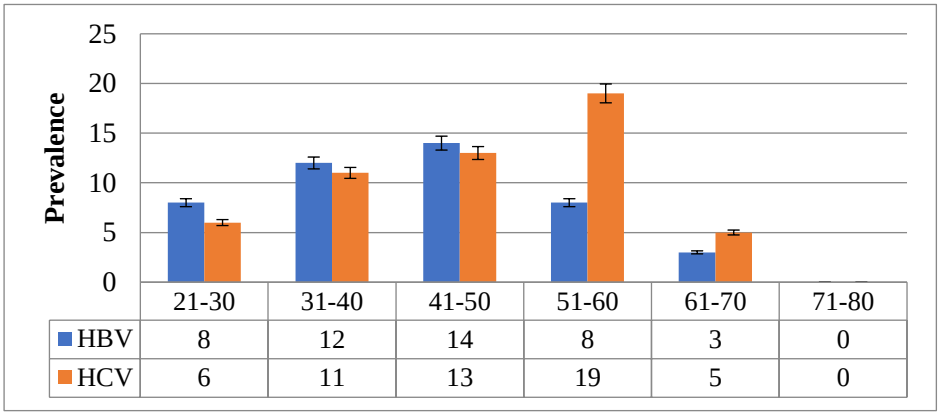


Figure 2: Active HBV and HCV among various age groups of males and females by PCR (N=79)

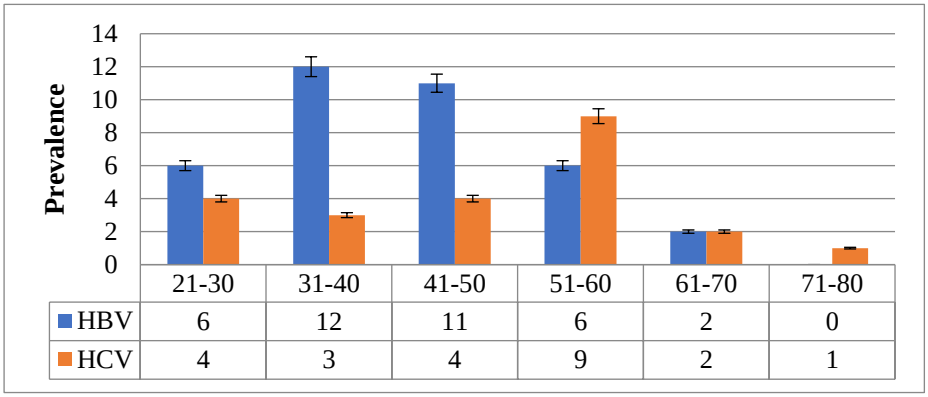


3.4. HBV prevalence in various genders among various age groups by PCR technique

All the collected samples were divided into 6 age groups. Results suggested that among males, most of the positive samples, 12 (63%) were found to be aged 31-40, followed by the 41-50 age group 11(61%) and the rest of the age groups (21-30, 51-60, 61-70 and 71-80) have a much smaller number of overall collected samples for HBV. Similarly, for the HCV, the highest number of positive samples 9 (60%) have been reported in the age group 51-60, followed by 4(40%) among the age group 21-30, 4 (31%) among the 41-50 age group. However, the rest of the age groups have a much smaller number of the overall collected samples or the positive samples to report.

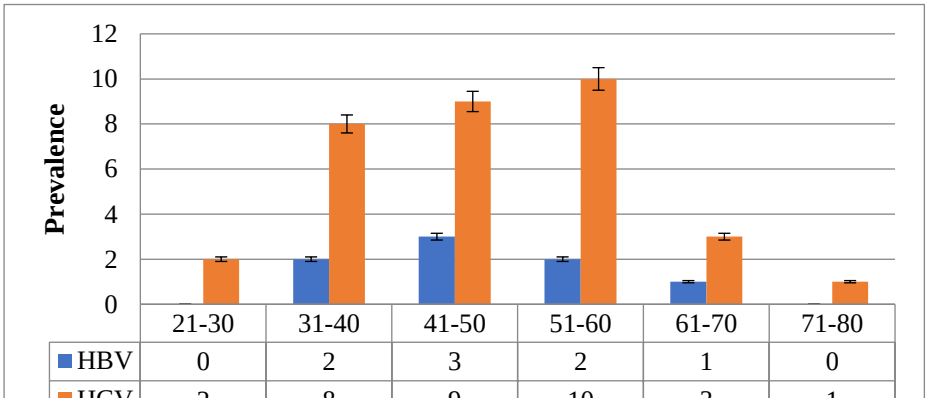
Among females, only 7 samples had positive results for HBV, while a relatively huge number of positive samples for HCV. The highest number of positive samples (10) were detected for the age group 51-60, followed by 41-50 (9) and 31-40 (8), and the rest have only limited numbers. Overall results for male and female positive samples for HBV and HCV among various age groups are listed in the table below. Results are also graphically represented in Figure 3 and Figure 4.

Figure 3: Prevalence of active HBV and HCV among males by PCR



	21-30	31-40	41-50	51-60	61-70	71-80
HBV	6	12	11	6	2	0
HCV	4	3	4	9	2	1

Figure 4: Prevalence of active HBV and HCV among females by PCR



	21-30	31-40	41-50	51-60	61-70	71-80
HBV	0	2	3	2	1	0
HCV	2	8	9	10	3	1

3.5. Risk factors for HBV and HCV infection: positive and negative

There are some risk factors for the HBV and the HCV infections that are revealed by ELISA and PCR as positive or negative as follows:

3.5.1. Smoking

Research findings have unveiled that smoking does not emerge as a significant factor influencing the heightened prevalence of HBV and HCV. The study indicated that merely 06 individuals among the HBV patients and 14 among the HCV patients were categorised as smokers (Figure 5). Under PCR analysis, results provide evidence that smoking has a more substantial contribution to the prevalence of HBV, as observed in 12 HBV-positive samples affected by smoking compared to only seven samples on which smoking did not affect. This suggests that smoking plays a significant role in the prevalence of HBV. Similarly, the study also shows that 12 HCV-positive samples were affected by smoking, while 19 HCV samples did not exhibit any effect of smoking. This indicates that smoking has a relatively more minor contribution to the prevalence of HCV when compared to its impact on HBV (Figure 6).

Figure 5: Risk factors associated with the prevalence of HBV and HCV by ELISA

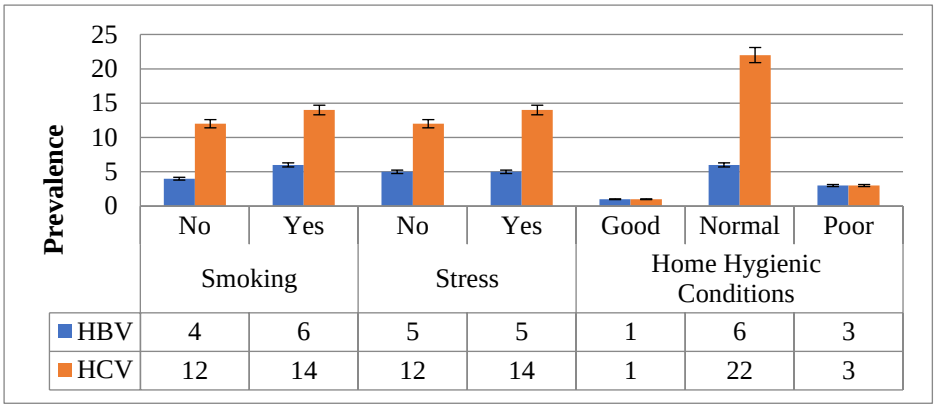
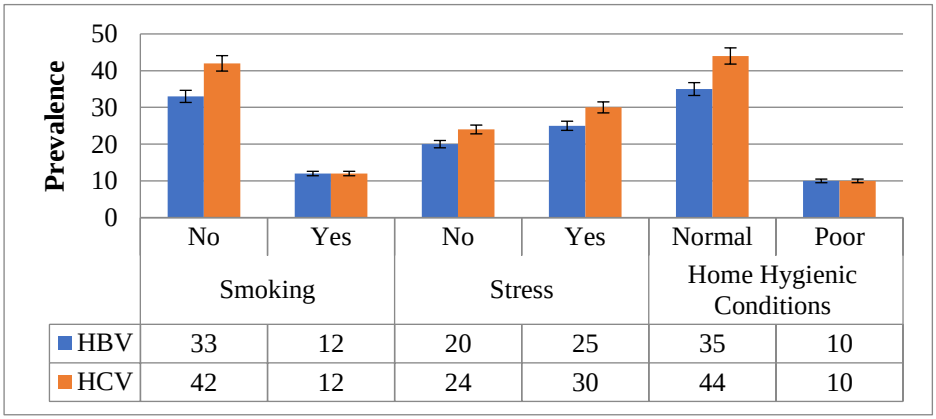


Figure 6: Risk factors associated with the prevalence of HBV and HCV by PCR



3.5.2. Stress

The current study's outcomes have demonstrated that stress conditions exert minimal influence on the prevalence of HBV and HCV. Among the participants, only 05 individuals with HBV and 12 individuals with HCV tested positive for stress (Figure 5). Under PCR analysis, stress conditions have a significant association with the prevalence of both HBV and HCV. Among the participants, 25 HBV-positive samples were affected by stress, while only 17 samples showed no effect of stress, suggesting that stress plays a major role in the prevalence of HBV. Regarding HCV, the study revealed that 30 HCV-positive samples were affected by smoking,

while 32 HCV samples did not exhibit any effect of smoking. This implies that smoking has a relatively more minor contribution to the prevalence of HCV compared to its impact on HBV (Figure 6).

3.5.3. Home hygienic condition

The current investigation has disclosed that the state of household hygiene does not play a significant role in the elevated prevalence of HBV and HCV. The results indicate that among the samples, there were 03 positive cases of HBV and 14 positive cases of HCV that exhibited a similar pattern with smoking. In contrast, 27 HBV samples and 19 HCV samples showed no correlation with smoking. Results are graphically represented in Figure 5. The results from the present study provide valuable insights into the impact of home hygienic conditions on the prevalence of HBV and HCV. Specifically, the study reveals that poor home hygienic conditions contribute significantly, accounting for 50%, to the prevalence of HBV in such conditions. Among the participants, 10 HBV-positive samples were affected by poor home hygienic conditions. In comparison, 10 samples showed no effect, highlighting the major role of poor home hygienic conditions in the prevalence of HBV. Regarding HCV, the study indicates that 10 HCV-positive samples were affected by poor home hygienic conditions, whereas 21 HCV samples did not exhibit any effect, indicating a lesser contribution of poor home hygienic conditions to the prevalence of HCV in Figure 6.

4. Discussion

Several studies have been conducted in past decades in various localities of Pakistan to assess the prevalence rate of HBV and HCV. Various techniques were used to observe their objectives including variation in timeframe, adopted methodologies, and high-risk population groups. Most of these studies have demonstrated a high prevalence rate of hepatitis in various geographical locals of Pakistan. However, this research only focused on Bahawalpur City to assess the epidemiology of Hepatitis B and Hepatitis C using two different molecular techniques including ELISA and PCR. Results from Table-1 showed 17% and 43% of the overall prevalence rate of HBV and HCV in both genders. A previous study conducted by Samo *et al.* (2021) reported a much higher prevalence of HCV in Nawabshah, Sindh, with 14.3% and 6.7% prevalence of HCV and HBV, respectively. However, our results in terms of HBV in women were different from many previous studies where they have found higher prevalence as compared to HCV (Dean, 2010; Kumari *et al.*, 2015). In our study, the prevalence rate of HBV was much lower in females as compared to HCV. This difference might be due to a lack of high-risk categories among females.

In the current study, only a single female out of 15 was observed positive for HBV, while 9 out of 45 males had HBV. Similarly, 27% and 49% of females and males were observed to be affected with HCV, respectively. Such a significant difference among genders in HBV was also observed by Samo *et al.* (2021), but they did not find any difference in the affection rate in various genders. This study also analysed the comparative results of two different molecular techniques, such as PCR and ELISA, to assess HBV and HCV prevalence. Gender-based results for HBV and HCV detected by PCR showed 62% and 47% male and female HBV prevalence, respectively. Similarly, 38% and 47% of males and females were found to be affected by HCV, respectively. Such difference in the prevalence rate of HBV and HCV among genders might be due to more visits of affected males to hospital as compared to females.

Further, high social mobility among males might also be another reason for the higher prevalence in males. These results were similar to many previous studies (Saleem *et al.*, 2020; Desikan & Khan, 2017) and contrasted to (Samo *et al.*, 2021; Chaudhary *et al.*, 2007).

Another critical parameter included in the current study was the prevalence of HBV and HCV among patients of various age groups. Age was classified into 6 groups, starting from 21 to 80 years or above. Results obtained from ELISA observed the highest prevalence of HCB among 31-40 (10) and 51-60 (8) while HBV was observed highest among the 41-50 (5) years age group. Simply, the middle age group has the highest prevalence of hepatitis than older and younger. When these samples were observed with the PCR technique, an almost similar ratio of hepatitis prevalence rate was observed. When HBV samples were observed among males and females based on various age groups, the highest prevalence in males was observed in the age groups 31-40 and 41-50, while in females, the age group 51-60 was observed with the highest hepatitis prevalence. Ahmad *et al.* (2006) also observed almost similar results, where they found the highest prevalence rate among people aged 55-65 while the lowest among people aged 25-35. Our results were also following the results of Samo *et al.* (2021), who observed a higher rate of HCV and HBV in patients aged greater than 30. However, a further increase in age was observed with a decreasing order of disease prevalence, which was in contrast to our results. Such an increase in hepatitis prevalence among older age might be due to greater exposure to hazardous areas by such age groups.

We also cover three major risk factors that have a close relation with hepatitis prevalence, including smoking, stress, and home hygienic conditions. Results showed that all of these risk factors have a relationship with hepatitis infection. However, smoking and home hygiene conditions have a non-significant association, but stress has a close significant relation with hepatitis infection, as listed in the table. Similarly, results were observed by Girgis *et al.* (2012), who stated that these, along with many other risk factors, have a positive relationship with viral infections, including hepatitis. Moreover, Liu *et al.* (2018) and Zaher (2004) also reported an association between the smoking of cigarettes or hookah "Goza" and HCV infection. A recent study on Hepatitis B and C was conducted by Saleem *et al.* (2020) in Punjab, Pakistan where they found non-significant results for the association of smoking with hepatitis. Samo *et al.* (2021) highlighted that the world's regions with poor health and hygiene conditions enlarge viral infections, especially hepatitis. These areas also have high mental stress due to low income and health conditions.

This study concluded that the Bahawalpur population is continuously exposed to HBV and HCV, causing high infection prevalence. Such a high prevalence rate of HBV and HCV is causing a considerable loss of economy to local communities, especially the poor ones. Therefore, there is a dire need to educate the people about the infection's transmission procedure and improve this community's life standard. Moreover, if awareness about hepatitis infection is given through curriculum, this will have much better results than any other type of awareness. It is recommended that an effective team should be sent to various public and private schools to educate people about the routes of transmission of hepatitis.

5. Conclusion

The study aimed to evaluate the epidemiological dynamics and risk factors of HBV and HCV infections in Bahawalpur, Pakistan. The results showed a higher HCV prevalence rate than

HBV, with PCR revealing a 50.1% prevalence rate in males and 46% in females, while ELISA measured 34.5% and 17.4%, respectively. Additionally, the study found a higher infection rate of hepatitis in males with moderate age compared to younger and older age groups. These findings highlight the significant burden of hepatitis C in Bahawalpur, particularly among the male population with moderate age, leading to substantial economic losses for the community and Pakistan as a whole. The study contributes to a better understanding of HBV and HCV infection prevalence in Bahawalpur City, which can aid government efforts to minimise associated losses.

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Author contribution

KAS planned and supervised the research; AD conducted the research work; AS wrote the manuscript; ZA did the static analysis and graphical representation.

Data availability

The raw data are available and will be provided on demand.

Declaration of conflict of interest

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References

Aggeletopoulou, I., Davoulou, P., Konstantakis, C., Thomopoulos, K., & Triantos, C. (2017). Response to hepatitis B vaccination in patients with liver cirrhosis. *Reviews in Medical Virology*, *27*(6), e1942. https://doi.org/10.1002/rmv.1942

Ahmad, I., Khan, S. B., Rehman, H. U., Khan, M. H., & Anwar, S. (2006). Frequency of Hepatitis B and Hepatitis C among cataract patients. *Gomal Journal of Medical Sciences*, *4*(2), 61–64. https://www.gjms.com.pk/index.php/journal/article/view/90

Ali, A., Ahmed, H., & Idrees, M. (2010). Molecular epidemiology of Hepatitis C virus genotypes in Khyber Pakhtunkhwa of Pakistan. *Virology Journal*, *7*(1), 1-7. https://virologyj.biomedcentral.com/articles/10.1186/1743-422X-7-203

Almezgagi, M. M., Edrees, W. H., Al-Shehari, W. A., Al-Moyed, K., Al-Khwlany, R. S., & Abbas, A. B. (2020). Prevalence of hepatitis B virus and hepatitis C virus and associated risk factors among hemodialysis patients in Ibb city-Yemen. *PSM Microbiology*, *5*(2), 32–40. https://doi.org/10.1155/2021/1555775

Asghar, M. S., Rasheed, U., Hassan, M., Akram, M., Yaseen, R., & Fayaz, B. (2021). A cross-sectional screening survey on the seroprevalence of hepatitis B and hepatitis C amongst the general population of rural districts of Sindh, Pakistan. *Arquivos De* Gastroenterologia, *58*, 150–156. https://doi.org/10.1590/S0004-2803.202100000-26

Chaudhary, I. A., Khan, S. S., Majrooh, M. A., & Alvi, A. A. (2007). Seroprevalence of hepatitis-B and C among the patients reporting in surgical OPD at Fauji Foundation Hospital, Rawalpindi: Review of 5-year literature. *Pakistan Journal of Medical Sciences*, *23*(4), 514. https://pjms.com.pk/issues/julsep07/article/article6.html

Dean, A. G. (2010). *Open Epi: open source epidemiologic statistics for public health (version 2.3.1)*. Openness. http://www.openness.com

Desikan, P., & Khan, Z. (2017). Prevalence of hepatitis B and hepatitis C virus co-infection in India: A systematic review and meta-analysis. *Indian Journal of Medical Microbiology*, *35*(3), 332–339. https://doi.org/10.4103/ijmm.IJMM_17_257

Ejaz, S., Abdullah, I., Malik, W. N., Anjum, S., Ashraf, M., Akhtar, N., ... & Sidiq, S. (2023). Screening of hepatitis B and C viral infection, recognition of risk factors, and immunization of patients against hepatitis B virus: a module developed for effective hepatitis control. *Frontiers in Public Health*, *11*. https://doi.org/10.3389/fpubh.2023.1269209

Farooq, A., Waheed, U., Saba, N., Majeed, N., Wazeer, A., Cheema, N. A., ... & Arshad, M. (2021). Molecular and genetic characterization of hepatitis B virus among multi transfused thalassaemia patients in Islamabad, Pakistan. *Journal of Family Medicine and Primary Care*, *10*(2), 998–1002. https://doi.org/10.4103/jfmpc.jfmpc_1880_20

Girgis, N., Farahat, N., & Ahmed, H. (2012). Nursing Intervention to Promote Self Care Management Practices for Clients with Hepatitis "C" Virus. *Journal of American Science, 8*(7), 581–591.

Grimberg, J., Nawoschik, S., Belluscio, L., McKee, R., Turck, A., & Eisenberg, A. (1989). A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Research, 17*(20), 8390. https://doi.org/10.1093/nar/17.20.8390

Hayes, C. N., Imamura, M., Tanaka, J., & Chayama, K. (2022). Road to the elimination of HCV: clinical challenges in HCV management. *Liver International*, *42*(9), 1935–1944. https://doi.org/10.1111/liv.15150

Hellard, M. E., Chou, R., & Easterbrook, P. (2017). WHO guidelines on testing for hepatitis B and C–meeting targets for testing. *BMC Infectious Diseases*, *17*, 1–7. https://doi.org/10.1186/s12879-017-2765-2

Kazmi, S. A., Rauf, A., Alshahrani, M. M., Awadh, A. A. A., Iqbal, Z., Soltane, R., ... & Ansari, Z. (2022). Hepatitis B among university population: Prevalence, associated risk factors, knowledge assessment, and treatment management. *Viruses, 14*(9), 1936. https://doi.org/10.3390/v14091936

Khalid, M., Rehman, S. U., Ali, A., Azam, S. M., Yaqoob, R., Faizan, M., ... & Tahir, M. M. (2021). Prevalence of Hepatitis B & C in Bahawalpur Region, Punjab, Pakistan. *Frontiers in Chemical Sciences, 2*(2), 175–182. https://doi.org/10.52700/fcs.v2i2.23

Kumari, K., Seetlani, N. K., & Akhter, R. (2015). The emergent concern of seropositive status of hepatitis-B virus and hepatitis-C virus in pregnant females attending a tertiary care hospital. *Journal of Ayub Medical College Abbottabad, 27*(1), 155–57. https://pubmed.ncbi.nlm.nih.gov/26182764/

Liu, X., Baecker, A., Wu, M., Zhou, J. Y., Yang, J., Han, R. Q., ... & Zhang, Z. F. (2018). Interaction between tobacco smoking and hepatitis B virus infection on the risk of liver cancer in a Chinese population. *International Journal of Cancer, 142*(8), 1560–1567. https://doi.org/10.1002/ijc.31181

Mehmood, S., Raza, H., Abid, F., Saeed, N., Rehman, H. M., Javed, S., & Khan, M. S. (2020). The national prevalence rate of hepatitis B and C in Pakistan and its risk factors. *Journal of Public Health*, *28*, 751–764. https://doi.org/10.1007/s10389-019-01081-5

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., & Arnheim, N. (1985). Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, *230*(4732), 1350–1354. https://doi.org/10.1126/science.2999980

Saleem, H. A., Roman, U., & Mughal, H. H. (2020). Prevalence of Hepatitis B and C and associated risk factors in rural areas of Punjab. *PJMHS, 14*(4), 2062–2063 https://pjmhsonline.com/2020/oct_dec/2062.pdf

Saleem, U., Aslam, N., Siddique, R., Iqbal, S., & Manan, M. (2022). Hepatitis C virus: Its prevalence, risk factors and genotype distribution in Pakistan. *European Journal of Inflammation, 20*, 1721727X221144391. https://doi.org/10.1177/1721727X221144391

Samo, A. A., Laghari, Z. A., Baig, N. M., & Khoso, G. M. (2021). Prevalence and risk factors associated with hepatitis B and C in Nawabshah, Sindh, Pakistan. *The American Journal of Tropical Medicine and Hygiene*, *104*(3), 1101. https://doi.org/10.4269/ajtmh.20-1228

Voller, A., Bartlett, A., & Bidwell, D. E. (1978). Enzyme immunoassays with special reference to ELISA techniques. *Journal of clinical pathology*, *31*(6), 507-520. https://doi.org/10.1136/jcp.31.6.507

Zaher, R. (2004). Frequency of anti–HCV in the general population and selected groups in Egypt. Paper presented at the *Afro–Arab Conference on Liver and Biliary Disease, Cairo, Egypt* (pp. 6–13). https://doi.org/10.1111/liv.13186