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THE PREVALENCE OF HEPATITIS B AND C VIRUS CONFIRMATION BY USING PCR-BASED MOLECULAR DIAGNOSIS IN DISTRICT SWAT KHYBER PAKHTUNKHWA PAKISTAN

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ABSTRACT

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major contributors to chronic liver diseases, cirrhosis, and hepatocellular carcinoma and therefore pose crucial health challenges in the world. The purpose of this retrospective study was to elucidate the prevalence of HBV and HCV-infected cases indistrict Swat, 2nd populated district of Khyber Pakhtunkhwa (KPK), Pakistan. This study was planned and conducted at Swat Institute of Nuclear Medicine, Oncology & Radiotherapy (SINOR) Saidu Sharif, Swat, KPK, Pakistan and Amreek Clinical Laboratory (ACL), Saidu Sharif Swat, Pakistan respectively. A total of 10488 samples with immunochromatographic (ICT) confirmed HBV and HCV infections from other labs, with male to female ratio of 1.33:1 were selected at Amreek Clinical Laboratory (ACL), Swat KPK Pakistan from July2019 to December2022. The selected samples for HBV and HCV were tested by real-time quantitative polymerase chain reaction (qPCR). The prevalence of HBV and HCV was analyzed concerning gender and time.

The ICT-based confirmed HBV and HCV cases ratios among all cases were 26.78% (males: 16.75%, females: 10.30%) and 73.22% (males: 40.33%, females: 32.89%) respectively in district Swat, KPK Pakistan. 7.31% of samples were positive for HBV and 21.89% for HCV with an overall 29.20% positive samples, after analyzing all samples with qPCR. The HBV and HCV patients were observed with increased temporal trends in whole duration except a decreased trend in 2020 due to the COVID-19 pandemic. Most HBV and HCV patients were male may be due to comparative greater freedom and social mobility.

The load of HBV & HCV in the district is alarming; therefore, such study will play an important role in infection prevention and future planning to control such viruses in district Swat, KPK Pakistan.

Key-wrds: Hepatitis virus, HBV, HCV, Swat, KPK, Pakistan

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) represent significant global health challenges, as they are major contributors to chronic liver diseases, cirrhosis, and hepatocellular carcinoma (Gunson *et al*., 2003). The World Health Organization (WHO) approximates that nearly 296 million people worldwide are impacted by chronic HBV infection (Organization, 2024), with about 58 million individuals suffering from chronic HCV infection (Perz *et.*, 2006). In Pakistan, these infections are particularly prevalent, especially in rural areas like Swat in KPK, where insufficient healthcare facilities and unsafe medical practices significantly heighten the risk of viral transmission (Qureshi *et al.*, 2010). The significant incidence of HBV and HCV in Pakistan can be linked to various factors (Samo *et al.*, 2021), such as the reuse of unsterilized medical instruments, insufficient blood screening practices, the absence of HBV vaccination programs, and a general lack of public knowledge regarding transmission pathways (Maina and Bii, 2020). Research data suggests that around 2.5–5% of the population in Pakistan is affected by HCV, with 2–4% suffering from chronic HBV infections (Zakki *et al*., 2022). In areas like Swat, these statistics are further intensified by socio-economic difficulties and inadequate healthcare resources (S. Ullah & Naz, Year??). The ICT is a diagnostic test tool for HCV and HBV infections (Almezgagi *et al*., 2020). However in clinical practice normally, if a case is positive on this method, clinicians verify it on enzyme-linked immunosorbent assay (ELISA) technique (Khan *et al*., 2010), and if ELISA is found reactive, then a final and authentic diagnosis is made by molecular diagnostic technique polymerase chain reaction (PCR) due to further confirmation for treatment of the infections. PCR has proven to be the gold standard for diagnosing HBV and HCV infections due to their high sensitivity and

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specificity. PCR is capable of identifying viral DNA (HBV) and RNA (HCV) at minimal concentrations, facilitating early and precise diagnoses that are essential for prompt therapeutic intervention. (Datta *et al.*, 2014). According to WHO estimation about 2.5% (177.5 million individuals) of the world population are infected with HCV (Bernstein *et al*., 2016). Three to four million people in the world newly report cases of HCV each year, 50 -70% of them go into liver complications and cause liver cancer (Basit *et al*., 2014). Similarly, HBV infection is a serious global health problem (Ashfaq and Idrees, 2014). It affects approximately 5% of the world's population (296 million people) including 6 million children under the age of 5 years. Around 820,000 deaths every year were estimated and 25% of chronic HBV infections progress into liver cancer (data and statistic for control and 2022). In Pakistan, the prevalence of HCV ranges from 1.2 to 15.9% (Yendewa *et al*., 2023) and an estimated prevalence of HCV infection in Pakistan is 11.5 % in the adult population (NHRC, 2023). According Pakistan Medical Research Council (PMRC) prevalence of HBV is 2.5% for the general population (Asghar *et al*., 2021). Another study reveals that HBV an estimated HBV prevalence in Pakistan is 7-9 million (3-5%) (Mahmood, Anwar, Khanum, Zaman, & Raza, 2016). According to census-2017 district Swat is the second largest district of KPK, Pakistan, comprise 8,608,378 inhabitants. The primary aim of this study is to reveal the latest prevalence of HCV and HBV concerning gender and time by PCR-based molecular diagnosis (as more authentic method), literally confirmed by ICT method in the district Swat, KPK, Pakistan.

MATERIALS AND METHODS

A total of 10,488 ICT-based confirmed samples [HBV: 2808 & HCV: 7680] were collected from district Swat, KPK, Pakistan from July 2019 to December 2022. All the patients were referred from outdoors by the gastroenterologist, and general physicians. This retrospective study was conducted in Amreek Clinical Laboratory (ACL), Saidu Sharif Swat from July2019 to December2022 and planned at SINOR, Swat, KPK, Pakistan.

For all samples, 2ml whole blood wascollected in an ethylenediaminetetra-acetic (EDTA) tube and then centrifuged at 4000 rpm and supernatant (plasma) was isolated in a clean dry and sterile Eppendorftube and stored at -20 ^{0}C for qPCR analysis. All the samples were first brought to room temperature, thawed and vortexed to homogenizedbefore testing. The samples were tested for the presence of HBV and HCV by real-time qPCR system.Viral nucleic acid extraction wasperformed by auto extraction reagent kit (TAN Bead Taiwan advanced Nanotech Inc.), with smart labassist (SLA-32) using a magnetic bead-based nucleic acid DNA/RNA automated system. 140ul of the sample was added to the respective well, along with 10ul carrier RNA to enhance viral isolation capability.An internal control of HCV and HBV (provided in the reagent kit) was added to each well tube for quality control purposes. After completion, the nucleic acid was collected and brought to the amplification room for viral detection and quantification. For amplification detection and quantification of HBV & HCV reagent kits (SACACE Biotechnologies Inc.) were used. Master mix reactions were prepared following the manufacturer'sprotocol and instructions. The master mix reaction is the mixture of different reagent packages which includes enzyme polymerase, reverse transcriptase, DNTPs, primers and cofactors etc. Separatemaster reactionsets were used for HCV and HBV.

For viral DNA/RNA detection, 12.5ul of extracted nucleic acid was 1stadded to all PCR reaction tubes with the help of a micropipette, next followed by the addition of 12.5ul of master mix reaction. For viral DNA/RNA of HBV& HCV, qPCR was carried out by Cepheid Smart Cycler USA and Ab Quant Gene by SYSTAAQ Diagnostic Product USA. Real-time PCR was monitored at each cycle and the graphs were examined and evaluated according to the manufacturer's instructions.The whole data was arranged systemically and statistically analyzed.

RESULTS AND DISCUSSION

A total of 10488 confirmed infected samples with ICT with male to female ratio of 1757:1051 for HBV and 4230:3450 for HCV before a visit to ACL, Saidu Sharif Swat and infected samples confirmed at ACL SaiduSharif Swat, KPK Pakistan with real-timeqPCR from July2019 to December2022, are shown in Table 1.

Table 1. All HBV and HCV-infected samples with ICT at other labs and qPCR after confirmation at ACL.

Patients Positive with ICT (n = 10488)		**Patients Positive with qPCR (n = 3063) After ICT (n = 10488)**	
HBV	2808	**HBV**	767
HCV	7680	**HCV**	2296
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The cumulative prevalence of HBV infection in district Swat, KPK, Pakistan in suspected patients was recorded at 26.78 %, which is approximately three times lower than HCV infection of 73.22% according to ICT data. As per recorded data analysis, total HCV male patients were higher at 4230 (40.33 %) than female 3450 (32.89 %), also total HBV male patients were higher at 1757 (16.75 %) than female 1051 (10.03 %) according to ICT-based data. However, these were 7.31% for HBV and 21.89% for HCV respectively after qPCR confirmatio n district Swat, KPK Pakistan based on a single data. More HBV and HCV were reported in males due to relatively higher freedom and social mobility, as a part of local culture in the district.

A total samples of 1023, 1838, 3730, 3897 for all patients along with 236, 530, 1037, and 1005 for confirmed ICT-based HBV and 787, 1308, 2693, and 2892 for confirmed ICT-based HCV were tested by qPCR in the years 2019 (half-year), 2020, 2021 and 2022, respectively at ACL Saidu Sharif Swat, KPK Pakistan as shown in Fig.1.

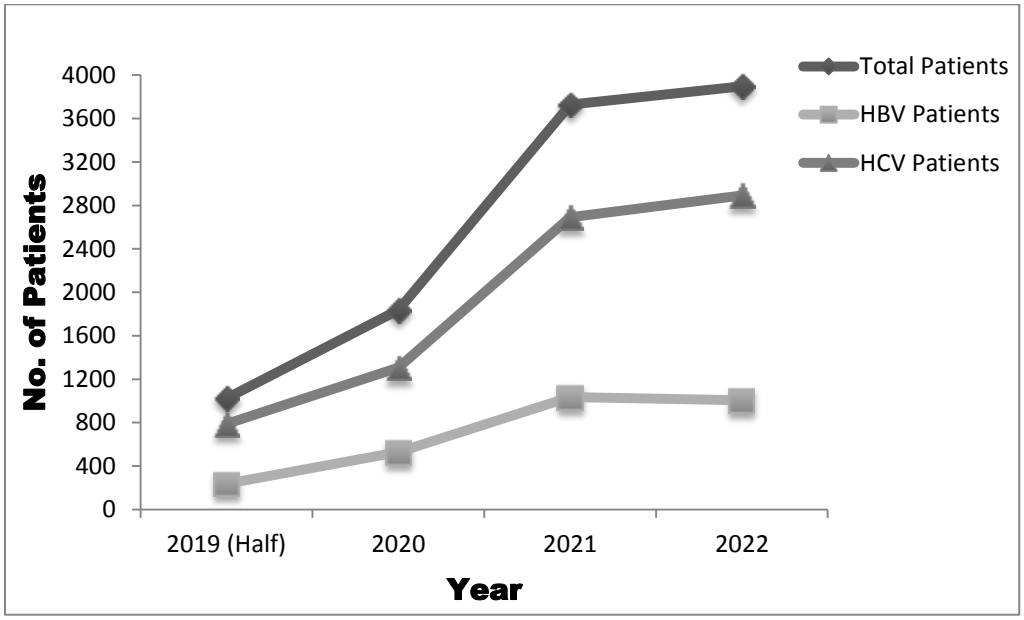

Fig. 1. Total patients along with HBV and HCV tested at ACL in different years.

It was also observed that 27.31% and 29.90% samples were found confirmed positive for HBV and HCV after verification by qPCR at ACL. However, the overall positive samples with real-time qPCR were 29.20% as shown in Table 1. HCV is three times more prevalent than HBV infection. Among these, the active viraemic patients whose qPCR for HCV RNA Positive were 2296/10488 (21.89%) and 5384/10488 (51.33%) patients were found negative for HCV RNA by qPCR. Similarly, 767/10488 (7.31%) whose HBV DNA by qPCR was found positive and 2041/10488 (19.47%) was found negative by qPCR. An increase in HCV and HBV patients was noted during this study which gives a serious indication for the disease burden in the society.

Many studies on the prevalence of hepatitis have been conducted in different regions of Pakistan. However, there are limited studies available on prevalence of hepatitis based on molecular level using qPCR due to cost and other resources in developing countries (Ahmed *et al.*, 2015), like Pakistan. The current study the previous studies conducted on the general prevalence of HBV and HCV infection. The overall prevalence of HCV in the whole of Pakistan is 8.64%. In Punjab, Pakistan it is 5.4%, Sindh 2.55%, KPK 6.07% and Baluchistan 25.77% according to the national hepatitis registry in Pakistan-2023 (Arshad and Ashfaq, 2017). The overall prevalence of HBV in the general population of Pakistan is 4.33%, Punjab 8.0%, 4.8%, Karachi 4.5% (Ali *et al*., 2011). Another study suggests the prevalence of HBV in different regions of the country like in Karachi only at 4.7%, overall Sindh at 6.2% (Mujeeb and Mehmood, 1996), Lahore at 1.52%, Rawalpindi at 2.31%, Peshawar at 4.5%, Mardan 20.79% (N. Ullah *et al*., 2021). According to a research study conducted in swat KPK, 390 adult patients in which 200 were male and 190 werefemale out of which 35.90 % (140) were found positive for HCV RNA by PCR. In Swat, KPK, Pakistan the positive percentage was 67/185 (36.22%), Dir 25/65 (38.46%), Malakand 36/100 (36%) and Mardan 12/40 (30%) (Waqar *et al*., 2014). The rate of HCV infection was also determined in both sexes male and female. The prevalence in males was 81 (40.5%) and in females, 59 (31.05%) was found positive for HCV RNA (Mansha *et al*., 2017). The percentage prevalence HCV and HBV in district Swat in our study is higher according to qPCR based diagnosis as orher reasearches might used other techniques of diagnosis. Also this may be due the small

sample size and single center experience, which is limitation of the study. A high accuracy can be obtained by a large volume of sample size and multicenters data for this type of prevalance.

Another study stated sex-wise distribution in a general population of district Bannu according to which the HBV prevalence in male 75/300 (25%) and in female it is 47/300 (23.5%) samples being tested by PCR (Tawab and Khalil, 2021). A recent studyfindings are HBV was 50.83% HCV was 35.63%. Again the rate of HBV infection based on PCR in district Bannu is higher than in this study which might be due to the low sample size or different region type (Muhammad *et al*., 2022).

Viral infections remain a serious health problem globally. In Pakistan, the viral burden is increasing day to day especially in males. This may be due to a lack of health education, awareness about the viral infection, use of contaminated needles, uneducated and untrained barbers, poor water hygiene sanitation system, imbalanced culture and direct disposal of contaminated waste from private & government hospitals play a crucial role in the spread of disease.

Conclusion

The burden of HBV and HCV is alarming, therefore, this study performs an important role in infection prevention and future planning strategies to control early diagnosis of the infection in the district. By elucidating the molecular epidemiology of these viral infections, the study seeks to enhance public health initiatives and optimize disease management strategies in the area.

REFERENCES

Ahmed, S. S., E. Alp, A. Ulu-Kilic and M. Doganay (2015). Establishing molecular microbiology facilities in developing countries. *Journal of infection and public health,* 8(6): 513-525.

Ali, M., M. Idrees, L. Ali, A. Hussain, I. Ur Rehman, S. Saleem, … . . . S. Butt (2011). Hepatitis B virus in Pakistan: a systematic review of prevalence, risk factors, awareness status and genotypes. *Virology journal,* 8: 1-9.

Almezgagi, M. M., W. H. Edrees, W. A. Al-Shehari, K. Al-Moyed, R. S. Al-Khwlany and A. B. Abbas (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.

Arshad, A. and U. A. Ashfaq (2017). Epidemiology of hepatitis C infection in Pakistan: current estimate and major risk factors. *Critical Reviews™ in Eukaryotic Gene Expression,* 27(1): 63-77.

Asghar, M. S., U. Rasheed, M. Hassan, M. Akram, R. Yaseen and B. Fayaz (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(02): 150-156.

Ashfaq, U. A. and S. Idrees (2014). Medicinal plants against hepatitis C virus. *World Journal of Gastroenterology:* 20(11): 2941.

Basit, A., K. Rahim, I. Ahmad, M. Shafiq, S. Mushtaq, H. Shaheen and I. Khan (2014). Prevalence of hepatitis B and C infection in Pakistan. *J Inf Mol Biol,* 2(3): 35-38.

Bernstein, C. N., A. Eliakim, S. Fedail, M. Fried, R. Gearry, K.-L. Goh, , . . . and S. C. Ng (2016). World gastroenterology organisation global guidelines inflammatory bowel disease: update August 2015. *Journal of clinical gastroenterology,* 50(10): 803-818.

Datta, S., S. Chatterjee and V. Veer (2014). Recent advances in molecular diagnostics of hepatitis B virus. *World Journal of Gastroenterology,* 20(40): 14615.

Gunson, R., D. Shouval, M. Roggendorf, H. Zaaijer, H. Nicholas, H. Holzmann, . . . and W. Gerlich (2003). Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in health care workers (HCWs): guidelines for prevention of transmission of HBV and HCV from HCW to patients. *Journal of Clinical Virology,* 27(3): 213-230.

Khan, J. K., D. S. Lone, A. Hameed, R. Munim, M. Bhatti, A. A. Khattak . . . and M. Munir (2010). Evaluation of the performance of two rapid immunochromatographic tests for detection of hepatitis B surface antigen and anti HCV antibodies using ELISA tested samples. *Annals of King Edward Medical University,* 16(1 SI): 85-87.

Mahmood, M., M. A. Anwar, A. Khanum, N. Zaman and A. Raza (2016). Distribution and clinical significance of hepatitis B virus genotypes in Pakistan. *BMC gastroenterology,* 16: 1-9.

Maina, A. N. and L. C. Bii (2020). Factors affecting HBV vaccination in a Medical training College in Kenya: A mixed methods Study. *BMC Public Health,* 20: 1-12.

Mansha, S., M. Imran, A. M. U. H. Shah, M. Jamal, F. Ahmed, M. Atif, M., . . . and A. Bilal Waqar (2017). Hepatitis B and C virus infections among human immunodeficiency virus-infected people who inject drugs in Lahore, Pakistan. *Viral immunology,* 30(5): 366-370.

Muhammad, W., F. Ali, I. Ullah, U. Nasir, A. U. Rahman and S. Sardar (2022). Prevalence of hepatitis B and C in Malakand division, Khyber-Pakhtunkhwa, Province of Pakistan. *Prevalence*, 29(01): 6827-6834.

Mujeeb, S. A. and K. Mehmood (1996). Prevalence of HBV, HCV and HIV infections among family blood donors. *Annals of Saudi medicine*, 16(6): 702-703.

Organization, W. H. (2024). *Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection*: World Health Organization.

Perz, J. F., G. L. Armstrong, L. A. Farrington, Y. J. Hutin and B. P. Bell (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of hepatology*, 45(4): 529-538.

Qureshi, H., K. M. Bile, R. Jooma, S. E. Alam and H. R. Afridi (2010). Prevalence of hepatitis B and C viral infections in Pakistan: findings of a national survey appealing for effective prevention and control measures. East Mediterr Health J., 16 Suppl:S15-23.

Samo, A. A., Z. A. Laghari, N. M. Baig and G. M. Khoso (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.

Tawab, H. and T. Khalil (2021). Molecular Prevalence and Causes of Hepatitis B Virus Infection in District Bannu Khyber Pakhtunkhwa, Pakistan. *Glob J Clin Virol*, 6(1): 001-006.

Ullah, N., I. Khan, M. Kakakhel, L. Xi, Y. Bai, B. Kalra, . . . and C. Zhang (2021). Serological prevalence of hepatitis B virus (HBV) in Mardan district, Khyber Pakhtunkhwa, Pakistan. *Brazilian Journal of Biology*, 82: e245813.

Ullah, S. and A. Naz (0000). Diseases Related Stigmatization and Discrimination: Investıgating the Perspectives and Experıences of HIV/AIDS Patients in Khyber Pakhtunkhwa, Pakistan. *Multidisciplinary Approaches to Research: Volume* 2: 27.

Waqar, M., A. U. Khan, A. Ali, M. Wasim, M. Idrees, Z. Ismail, . . . and R. U Khan (2014). Prevalence and molecular determination of Hepatitis C infection in Khyber Pakhtunkhwa, Pakistan. *Archives of Clinical Infectious Diseases*, 9(3): e17275.

Yendewa, G. A., G.-M. Wang, P. B. James, S. P. Massaquoi, S. A. Yendewa, M. Ghazawi, . . . and G. F.Deen (2023). Prevalence of chronic hepatitis B virus infection in Sierra Leone, 1997–2022: a systematic review and meta-analysis. *The American journal of tropical medicine and hygiene*, 109(1): 105.

Zakki, S. A., A. Ahmad, M. S. Nazar, J. Muhammad and K. Ullah (2022). Epidemiology of Hepatitis C Virus in district Swat, Khyber Pakhtunkhwa Pakistan. *International Journal of Natural Medicine and Health Sciences*, 1(4): https://doi.org/10.52461/ijnms.v1i4.1030

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