Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i8.7405

UNVEILING THE HEPATITIS B AND HEPATITIS C LANDSCAPE IN JALALABAD: INSIGHTS FROM NUCLEIC ACID AMPLIFICATION TESTING

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Abstract
The aim of the current study was to determine the epidemiological state of HBV and HCV infection in the Afghanistan, province of Nangarhar. Over a six-month period, approximately 17600 patients were treated for various diagnoses. 538 of the samples tested positive for HBs Ag and HCV Ab; the ICT screening was used to identify the positive samples, and PCR was used to confirm the results. The entire patient population (17600) had a prevalence of both HBV and HCV of (n=538, 3.056%), while each had a prevalence of (n=198, 1.125%) for HCV Ab and (n=340, 1.9318%) for HBs Ag. The nucleic acid-based prevalence for viral hepatitis was lowered to (n=171, 0.9715%) for HCV, (n=53, 0.3011%), and (n=118, 0.670%) for HBV. The research findings indicate that the prevalence of HCV is higher in females than in males. The age range between 31 and 40 has the highest prevalence; based on information provided in the questionnaire, women in this age group have had many deliveries, including multiple blood transfusions. Men were more likely than women to be affected by HBV. The data gathered suggests that dental work up and family interaction with any affected individual may be contributing factors.

Key words: HBV, HCV, Prevalence, PCR, Epidemiology

Introduction
Globally, hepatitis B and C (Hepadnaviruses) viruses are among the most frequent causes of liver disease. More than 300 million people worldwide are infected with the hepatitis B virus (HBV), which is a major contributor to liver disease and liver cancer [1]. Around 2 million deaths are caused by different liver diseases, out of which 1 million are due to viral hepatitis and hepatocellular carcinoma the latter is one of the leading complications of viral hepatitis [2]. HBV is a DNA virus that targets human only and is comparatively contagious to other human viruses as HCV and HIV.[3] Hepatitis B viruses results in chronic hepatic infections being run for months, which mostly result in liver failure, about 25% results in hepatocellular carcinoma (HCC) around 0.5% of the hepatitis B infections result in death.[4]. HCC is one of the leading cancers worldwide Around one third of the world population is infected with hepatitis B infection out of which 400 million are carriers [5]. Being an RNA virus the hepatitis c virus belongs to the family flaviviruses, targeting the hepatocytes and B

lymphocytes, the number of individuals being infected yearly is around 170 million worldwide, the infection with hepatitis C is five times more widespread than infection with Human immunodeficiency virus type 1 (HIV-1). [6]. HCV is considered as one of the leading causes of post transfusion hepatitis the most focused group is thalassemia patients. [7] Human immunodeficiency virus (HIV), and hepatic viruses as Hepatitis C virus (HCV) and Hepatitis B virus (HBV) are major health concerns, as they share same route of infection, HIV-HBV and HIV-HCV coinfection are common[8] According to some studies co infection of HBV and HCV exists, the percentage of which is 10% to 15% of individuals with HBV, the prevalence varies based on different population.[9, 10] coinfection alters the history of viral related liver diseases[9, 11]

MATERIAL AND METHODS

Study Description

The study was done in the city Jalalabad of the province Nangarhar Afghanistan, Between August 2023 and April 2024, over 17600 patients received treatment for a variety of diseases. Multiple clinical and specialized laboratory tests were performed as part of the investigations to rule out viral hepatitis. Of the samples tested, 538 tested positive for hepatitis B and hepatitis C. Among the patients included in this group were those who were from regional hospitals and had undergone PCR testing.

Immunochromatography (ICT)

An immunochromatographic device, whose principle is based on capillary action chromatography, is used to detect the presence of HBs Ag [12]. The device's cassette has a strip that has been precoated with anti-HBs Ab in the test line region. A line on the control area (C) is required in both positive and negative scenarios when the sample flow from the sample window on capillary action and the HBs Ag react with the test line (anti HBS Ab) to produce a colored line in the test region [13, 14]. ICT devices of the company Abbott and ACON (Acon laboratories Inc., San Diego, USA)[13] were used.

Nucleic Acid Extraction (DNA/RNA)

After immunochromatography, the positive samples were prepared for PCR. During this procedure, MN and Qiagen extraction kits were used to extract the nucleic acid from the samples in accordance with standard practices [15].

Real Time PCR

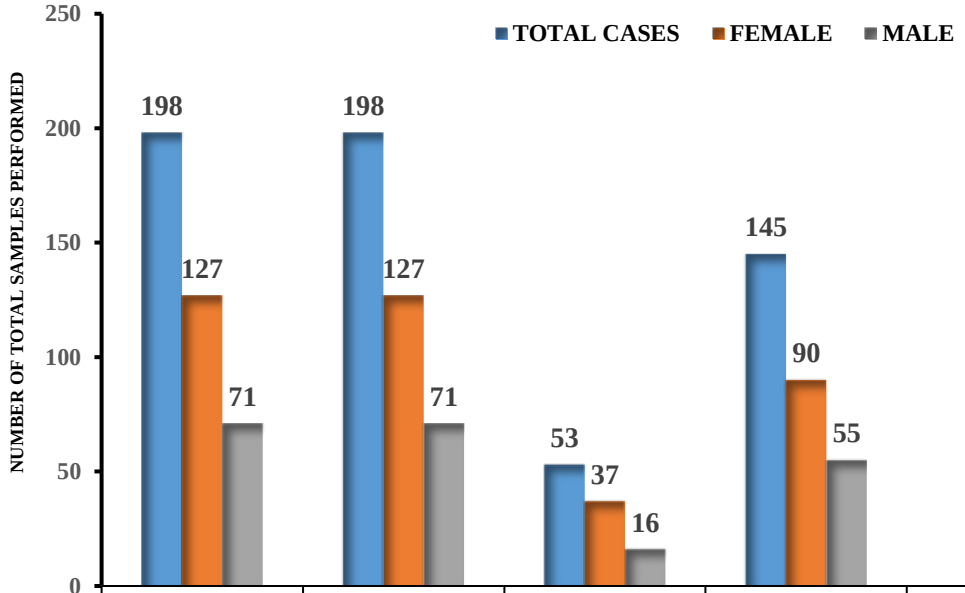
The polymerase chain reaction (PCR) has been as the gold standard for amplifying a number of different templates**[16]** Detection is based on specific and nonspecific fluorescent probes **[17]**. To quickly and easily amplify DNA fragments PCR is one of the methods of choice.**[18]** PCR was performed in a clinical laboratory and all procedures were done using ISO 17822:2020. A total number of 198 samples post confirmed for HCV Ab and 340 for HBs Ag by ICT Rapid Devices were processed for PCR at Kamawi Medical Laboratory (KML), the patients were properly registered with their respective territories, age, gender, symptoms, previous surgeries, tooth extractions and any family member infected previously. The sample was properly taken, the blood was drawn into a yellow top tube to isolate serum, the serum was isolated and about 1 ml of serum was refrigerated at -20 C in an Eppendorf until a weekly batch of HBV and HCV PCR was run, the samples were thawed at the day batch was run they were properly treated with MN and Qiagen Extraction Kits to Extract their genome (DNA and RNA) using SOPs provided by their kit inserts, after their extractions the genome was added in a small 0.2ml of PCR tubes and were placed in an 36 slots rotor of Qiagen-5 plex HRM thermocycler and various cycles and steps were programmed to complete Denaturation, Annealing and Extension of a specific target of genome, Each batch was run with a Positive Control, Negative control and Non Template Control (NTC) to get accurate and reliable reports the data was then compared with the calibrator data to get values in IU/ul which were then converted into U/Ml and Copies/Ml , The results were interpreted using internal control data along with the calibrators data.

Results

The samples were tested by ICT and PRC both for confirmation. In the present study a total of 198 samples for HCV and 340 for HBV PCR were collected in which 53 (26.7%) were positive for HCV RNA PCR and 118 (34.7%) were positive for HBV DNA PCR out of 198 and 340 respectively the total number of samples were all positive by ICT, the patients appeared to get tested for various diseases and turned positive for HBV and HCV. Both methods used for confirmation, for HCV the comparison ratio for Positive cases of ICT on PCR was 26.7% while it was 34.7% for HBV.

HCV RNA by PCR (Data Representation)

The data shows total cases performed by ICT and PCR, total 53 (26.7 % of the total) cases were detected positive with 37 (29.13 %) females and 16 (22.53 %) males in the study. The total 145 negative cases were recorded including 90 and 55 females and males, respectively. The graphical data is presented below;



	PERFORMED BY ICT	PERFORMED BY PCR	POSITIVE BY PCR	Negative by PCR	
TOTAL CASES	198	198	53	145	
FEMALE	127	127	37	90	
MALE	71	71	16	55	

Fig. 1: Figure shows the detection of HCV by ICT and PCR in male and female patients.

Table. 1: Table shows the percentages of PCR in male and female individuals.

Total samples positive by ICT	Positive by PCR (N%)	Negative by PCR (N%)
198	53 (26.7%)	145 (73.2%)
Male 71	16 (22.53%)	55 (77.46%)
Female 127	37 (29.13%)	90 (70.86%)

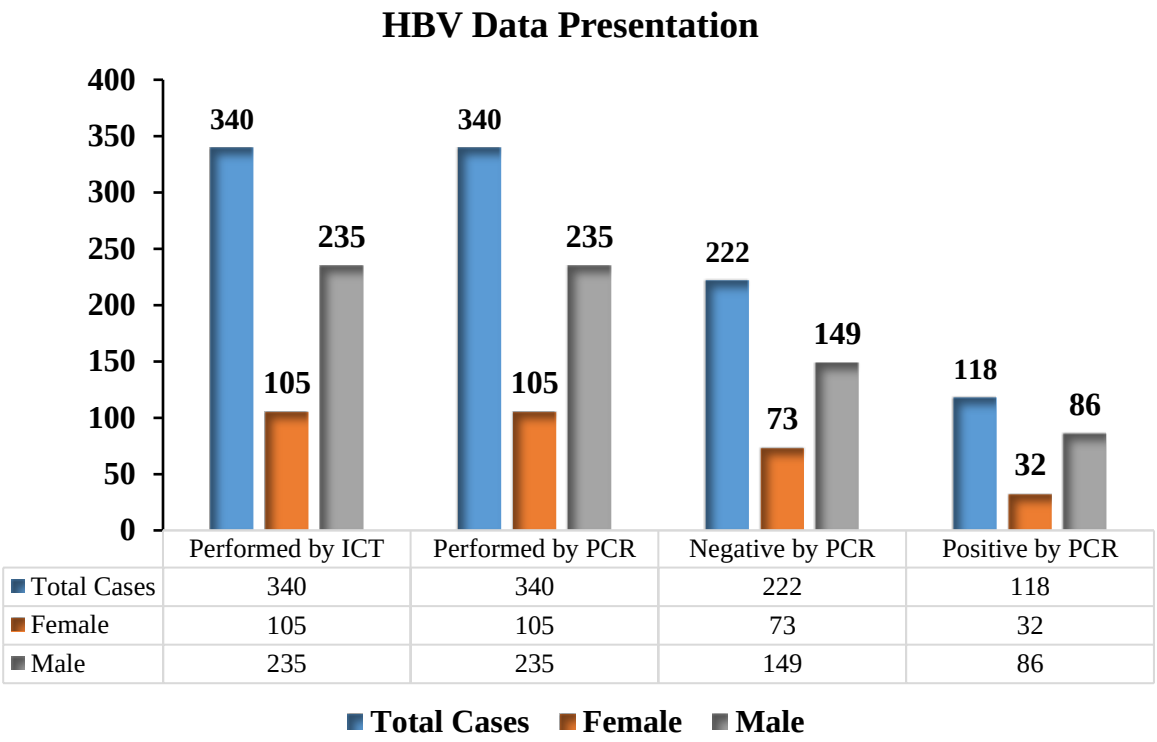


Fig. 2: The representation of HBV data among the patients test with dual methods.

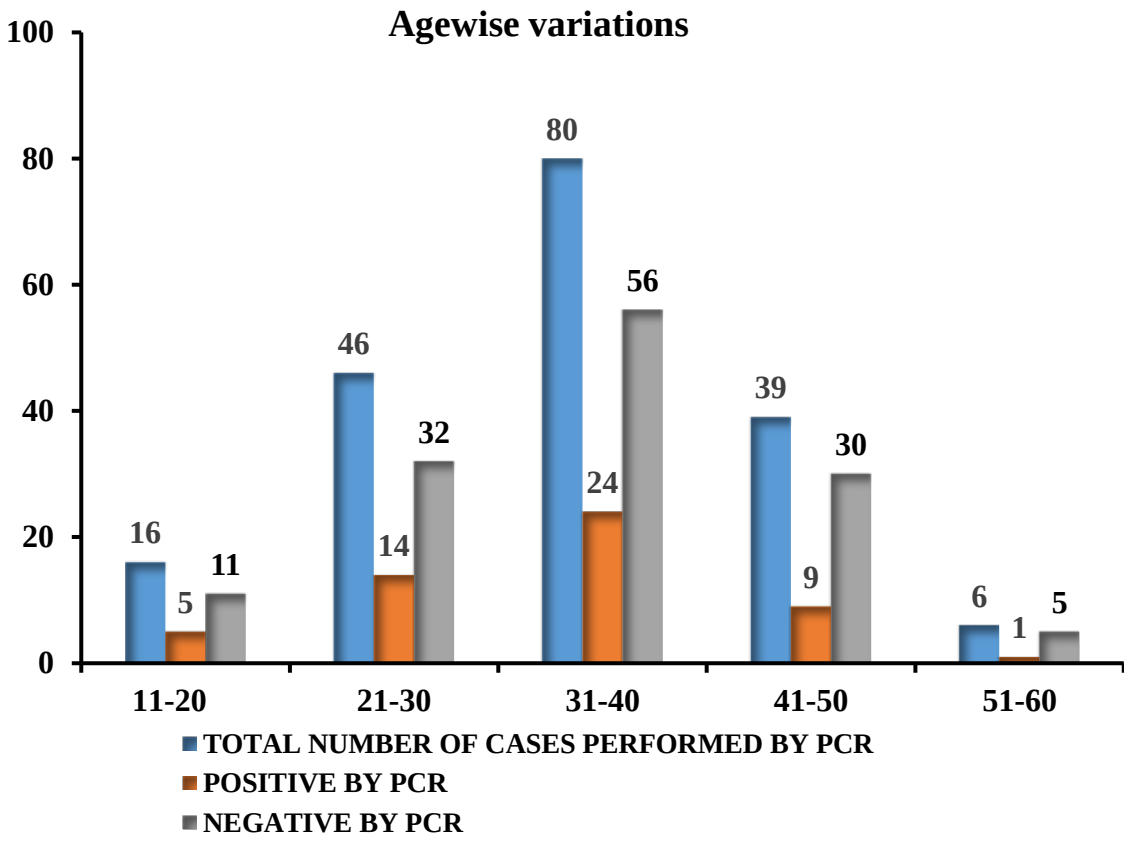


Fig. 3: Shows age wise representation of HCV RNA by PCR

The age wise distribution of Hepatitis C cases in the study provides important insights into different demographic categories. Five of the 16 cases in the age range of 11 to 20 were positive, remaining 11 cases negative. The incidence was greater in the 31–40 age range (24 out of 80 cases tested

positive, and 56 were negative). In the age group 21-30 total of 14 cases were tested positive among 46 samples. The least positive cases were reported in age group ranges from 51-60 with only one case positive among the six tested samples. The moderate number of positive cases were reported in age group ranges from 41-50 with 9 positive cases among the 39 tested samples.

Discussion

In the present study the prevalence of HBV and HCV was determined in the people of Jalalabad City of Nangarhar Afghanistan who visited Kamawi Medical Laboratory to get tested for a number of different investigations and turned positive for HBV and HCV with ICT kits, which were later processed for Quantitative RNA and DNA PCR to get accurate RNA and DNA quantities. Our study found higher HVC positive cases in females.

Overall, there are no differences between men and women according to meta-analyses of HCV prevalence studies. These cross-sectional prevalence studies do, however, have difficulties when it comes to determining the actual HCV burden [19]. Research using prospective cohorts on people who inject drugs (PWID) has yielded mixed results, with some research suggesting a higher incidence in females and others in males [19]. These variations are probably caused by social and behavioral risk factors. Even though female susceptibility and incidence are lower overall, biological variables such as oestrogen receptors and genetic differences may play a role [20]. Moreover, spontaneous HCV clearance is more common in women [19]. Moreover, our study shows increased rates of HBV infection than men which is different than HCV. In the current study the women participated had gone through normal delivery or surgical procedures, where they received blood from different donors. Our study is in line with the different studies done for HBS prevalence. Across a wide range of populations, the existing epidemiological data consistently demonstrates that males are more likely than females to get HBV infection, even though the exact mechanisms are still being revealed. This gender gap is probably influenced by immunological responses as well as sex hormones. An estimated 2.36% of Iranian men and 1.47% of Iranian women were predicted to have HBsAg nationwide, according to a meta-analysis [21]. Additionally, males had a higher prevalence of anti-HBc Ab (15.21%) than females (12.77%), indicating prior exposure. Males had a 0.41% HBsAg prevalence compared to females' 0.28% (OR 1.98, 95% CI 1.2–3.2) in a cohort study involving 31,990 blood donors from Crete. Men had a higher anti-HBc prevalence (9.16%) than women (5.86%), suggesting more exposure [22]. Men who work in certain occupations seem to be more susceptible to contracting the hepatitis B virus (HBV). Numerous studies have revealed that regular contact with blood and needles increases the occupational risk of HBV infection in healthcare workers, particularly physicians [23]. The danger and the amount of blood-needle contact during regular work are highly correlated [24]. 6.8% of 2,910 public safety employees—including police, firemen, sheriffs, and prison guards—who participated in the study stated that they had come into contact with blood or contaminated objects at work at least once in the preceding six months. Depending on their line of work, 7.4% of police officers reported having at least one exposure [23]. The degree of blood-needle contact during every day work was shown to be closely connected with the occupational risk of HBV infection, which was found to be high across several occupational groups in a study conducted on hospital staff [25]. Our study shows higher detection of HBS and HCV in younger and middle-aged individuals. The age range of 21 to 50 years is associated with a higher prevalence of infections caused by the hepatitis B and C viruses for a number of reasons. Different studies HBS is primarily transmitted through contact with infected bodily fluids, such as during unprotected sex, sharing needles or syringes, or from mother to child during childbirth [26, 27]. These risk factors are more common in young and middle-aged adults. Young and middle-aged adults have higher exposure risks due to their higher levels of social interactions, sexual activity, and involvement in high-risk behaviors like injectable drug use compared to children and the elderly [28, 29]. Systematic screening is more frequently performed on younger adults, increasing the age group's detection rates in comparison to those of children and the elderly [29].

Conclusion

All things considered, this investigation on the frequency of HBV and HCV infection in Nangarhar, Afghanistan, revealed the age distribution and the two approaches used to monitor HBV and HCV infection. The findings will be helpful in the prevention and treatment of HBS and HCV infection for epidemiologists and national health workers. In order to provide further insight on the epidemiology of viral diagnoses throughout the nation, we also advise the systematic screening of HBS and HCV in the remaining regions of Afghanistan.

Conflict of Interest

The authors declare no conflicts of interest.

Authors Contribution:

Mr. Kamavi has collected all the data and analyzed the who samples. He also participated in writing the original draft. Dr. Ilyas analyzed the samples and contributed to the writing of the original draft. Dr. Habiba arranged the whole data and modified it in presentable form. Dr. Niaz participated as correspondence author, he mainly guided the entire study and modified the original manuscript. Dr. Ali contributed as correspondence author and mainly contributed to writing the original manuscript and finalizing the final version.

Ethical Responsibility

This is original study and has not been submitted to any other journal.

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