National Journal of Biological Sciences

Received: 15th February, 2024

Revised: 28th May, 2024

Accepted: 5th June, 2024

Published: 27th June, 2024

DOI: https://doi.org/10.37605/njbs.v5i1/2

RESEARCH PAPER

TITLE

SERO-PREVALENCE OF HCV IN THE POPULATION ATTENDING GOVERNMENT HOSPITALS OF DISTRICT BANNU PAKISTAN

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ABSTRACT

Hepatitis C virus is endemic throughout the world, with approximately 170–200 million unhygienic cases of which Pakistan contributes 17 million cases. HCV is known as the primary origin of liver disorders (Cirrhosis and Hepatocellular Carcinoma). HCV is responsible for causing something like 27% of cirrhosis and 25% of hepatocellular carcinoma cases throughout the world. Every year about 350,000 cases faces to death because of HCV. This revision was performed in common residents of District Bannu, Khyber Pakhtunkhwa Pakistan. A total of 793 patently cases were examined containing 481 males and 312 females under the age of 1–75 years which are classified in to 5 groups. These cases were examined for HCV by ICT (Immuno chromatographic) technique and further conformed was done by PCR technique. Out of total 793 samples, 418 (52.71%) cases are with 245 (50.93%) males and 173 (55.44%) females were PCR positive. The maximum Hepatitis C infection was recognized in age-group of 1-15 and minimum infection was recognized in age-group of 31-45. Second largest HCV infection was demonstrated in 61-75 and 46-60, sensible infection was determined in 16-30 of age-group. Shortage of suitable blood test infrastructure in district Bannu and lack of awareness regarding to the potential routes factors of HCV are giving a grand contract in the direction of broaden of disease between the societies. Appropriate sterilization and test practice should be made to avoid a far superior risk of further Hepatitis C infection in upcoming era.

KEYWORD: HCV (Hepatitis C virus), ICT (Immuno Chromatographic Test), PCR (Polymerase Chain Reaction), Quant.PCR (Quantitative PCR), SPSS (Statistical Software), Bannu.

1. INTRODUCTION

HCV infection poses a significant global health threat, with an estimated 170–200 million cases reported worldwide, including 17 million in Pakistan (Saleha *et al*., 2014). Pakistan ranks 134th out of 174 countries globally in HCV infection rates due to lack of awareness (Azam et al., 2023) (Siddiqi et al., 2002). Hepatitis C is a primary cause of liver disorders globally, including liver cirrhosis and hepatocellular carcinoma, accounting for 27% of cirrhosis cases and 25% of hepatocellular carcinoma cases (McGlynn et al., 2015) (Schütte et al., 2009). Approximately 4 million cases of HCV are reported annually worldwide, resulting in 700,000 deaths per year (Lanini et al., 2016) (Thrift et al., 2017) HCV, with its positive single-stranded RNA genome belonging to the Flaviviridae family, exhibits significant genetic variability, resulting in six genotypic forms (Wu et al., 2015) (Kato, 2001) . HCV genome is about 9.6 kb long, which is programmed for polyprotein of approximately 3010 amino acids and is lined by short untranslated regions (UTRs) at the 5′ and 3′ end (Idrees & Riazuddin, 2008).

HCV, with its positive single-stranded RNA genome belonging to the Flaviviridae family,

exhibits significant genetic variability, resulting in six genotypic forms (Pawlotsky, 2003) (Echeverría et al., 2015). It is a major cause of infectious liver diseases, including hepatocellular carcinoma and cirrhosis (Perz et al., 2006) . HCV is a widespread health problem affecting all demographics, including age, gender, and race, with related cases even reported in chimpanzees (Kawo et al., 2012). There are also some related cases of HCV, reported in chimpanzees having symptoms similar to that of human (Rauf et al., 2013). Notably, 90% of HCV cases arise after blood donation (Chen & Morgan, 2006). Acute HCV infection, lasting up to six months, is often asymptomatic, with only 20% to 30% of patients showing symptoms such as malaise, weakness, anorexia, and jaundice. Chronic HCV infection, lasting more than six months, leads to severe complications, including liver failure, hepatocellular carcinoma, liver cirrhosis, liver fibrosis, and extra-hepatic manifestations. During this case of infection there is 70% to 80% of patient had no symptoms (asymptomatic). Only 20% to 30% patients with acute HCV infection may symptomatic (Gerlach et al., 2003) (Hoofnagle, 1997). The general symptoms may consist of malaise, weakness, anorexia and jaundice. HCV RNA can be detected in the serum within 6 up to 12 days after revelation (Aslam et al., 2019). HCV infection that becomes ancient in the body more than six months transfer in to chronic case. A huge quantity of HCV infected persons, ranging from 75% to 85% expand toward chronic HCV, infection and leads to liver failure, hepatocellular carcinoma (HCC), Liver cirrhosis, liver fibrosis, and HCV related extra-hepatic tricky situation (El-Feky) (Sneller et al., 2012) (Ali et al., 2011).

Particularly in progressive countries like Pakistan, HCV is a highly contagious and significant health concern, leading the prevalence in the Oriental region, including Nepal, India, Myanmar, Afghanistan, and Iran, due to limited healthcare resources and lack of awareness (Bostan & Mahmood, 2010). Pakistan becomes at the top in the prevalence of HCV in the oriental region such as Nepal, India, Myanmar, Afghanistan and Iran (Idrees et al., 2008). The percentage of HCV infection is rising day by day due to underprivileged health care recourses and lake of awareness in society. The frequency of HCV in Pakistan is increasing, reaching 6% due to inadequate healthcare resources and low awareness levels (Ilyas & Ahmad, 2014) (Khan et al., 2008).

2. MATERIALS AND METHODS

- **Study area**

This study was planned in district Bannu of Khyber Pakhtunkhwa province of Pakistan. It was nominated as district during British Raj in 1861. District Bannu is situated between Lakki Marwat to the Southeast, Karak to the Northeast, North Waziristan to the Northwest, and South Waziristan to the Southwest. The total number of residents of district Bannu is 1.168 million. District Bannu is branched into 4 tehsils i.e., Bannu Tehsil, Domel Tehsil, Miryan Tehsil, and Bakakhel Tehsil, and 49 union council.

3. DATA COLLECTION

The data was collected from both genders referred by physicians and medical officers.

All the individuals were aged between 1-67 years. There were 793 samples collected from DHQ Hospital, Women and Children Hospital, and Khalifa Gulnawaz Hospital. All the samples were analyzed through ICT (Immune Chromato Graphic Test) and further verification of viral load and severity of disease was performed by PCR (polymerase chain reaction).

- **ICT Procedure**

On the basis of age, all the participants were categorized into 5 sections. The serum was isolated from each blood sample and was tested for anti-HCV antibodies through an immune chromatographic (ICT) assay (Khan et al., 2011)

- **Molecular diagnosis**

HCV RT Quant DX kit was used to extract the HCV RNA. The serum was extracted from each blood sample and shifted into a serum cup. 300ul lysis buffer was added into 150ul serum-containing serum cup. After that, the samples were incubated for five minutes at 70°c. 30ul ethanol was to each sample. Thus, the samples were transferred to a fresh Eppendorf tube and centrifuged at 8000rpm for sixty seconds and pellets were discharged. After that 300ul of washing buffer 1 (RAW) was added and centrifuged at 8000 rpm for sixty seconds and discharged the pellets. For more purification, the washing buffer 2 (RAW3) was added and centrifuged at 12000 rpm for five minutes and discharged to the pellets. Finally, place the supernatant in a new Eppendorf tube and placed it at 70°c for sixty seconds. The elution buffer 60ul was added into the tube and centrifuged at 12000 rpm for sixty seconds. The extracted purified DNA was shifted to a new PCR tube. And 8ul of the master mix was added to 8ul of DNA sample and run it into the smart cycler (made in the USA) for 90 minutes and the reading were noted as a quantity of HCV anti-bodies (Jan et al., 2020) (Waheed et al., 2009) (Nafees et al., 2009).

4. RESULTS

A total of 793 apparently healthy individual of the age-group 1-75 years were randomly sampled for the study. Out of the total 793 samples examined, 312 were females and 481 from male population. The entire samples were classified into 5 age groups. The serum were segregated from all the blood samples and consequently tested for anti-HCV antibodies by immune chromato graphic (ICT) test, by using ICT strips from Accurate (USA). The results showed that 418 (52.71%) out of 793 cases had anti-HCV antibodies in their blood sera and 375 (47.28%) have no anti-HCV antibodies in their sera. The given results were then confirmed by PCR.

4.1. Sex-wise Prevalence of Hepatitis C Virus in District Bannu

During the present research work, the sex-wise occurrence of Hepatitis C infection was demonstrated. The PCR-positive individuals include 173 (55.44%) females and 245 (50.93%) males. It shows that the prevalence of HCV infection with respect to gender is higher in females than in males (Figure 1).

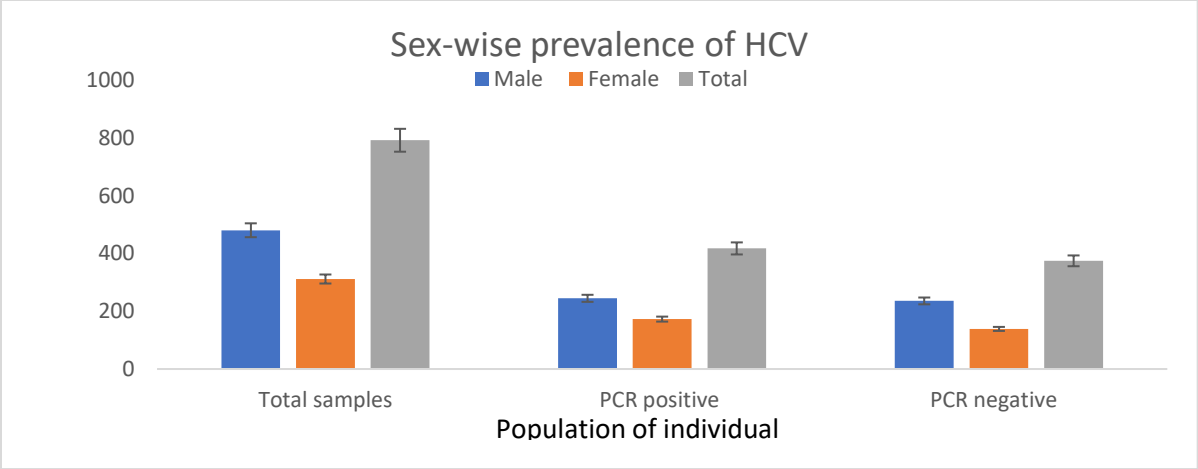

Figure 1: Sex-wise Prevalence of HCV in District Bannu

4.2. Age-wise Prevalence of Hepatitis C in District Bannu

Age-wise ratio of HCV infection was also taken into consideration. The individuals of 1-75 years of age categories were five age groups. After demonstration, it was found that the prevalence rate of HCV was advanced in immature age and moderate in mature age while high rate in old age (Figure 2). The highest HCV infection was recorded in the age group of 01-15 years (58.33%), while the lowest infection was found in 31-45 years (51.42%).

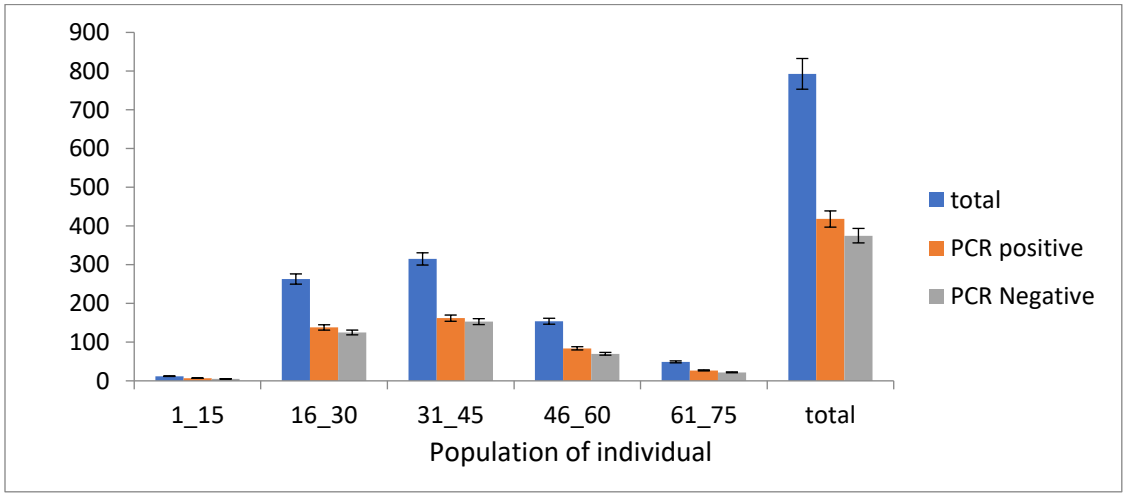

Figure: 2 Age-wise Prevalence of Hepatitis C in District Bannu

4.3. Prevalence of HCV in District Bannu with Reference to Various Risk Factors

The various risk factors linked with the infection of HCV were also taken into consideration in the current study. The maximum quantity of risk factors were shown in patients with history of dental surgery (15.63%) (Figure 3). The PCR positive patient were exposed to body piercing and tattooing (2.90%), use of

contaminated blade repeatedly (11.34%), general surgery (10.71%), reuse of syringes (5.42%), drug addiction (10.46%) and interfamilial prevalence each (1.51%). All the data was analyzed through SPSS Software.

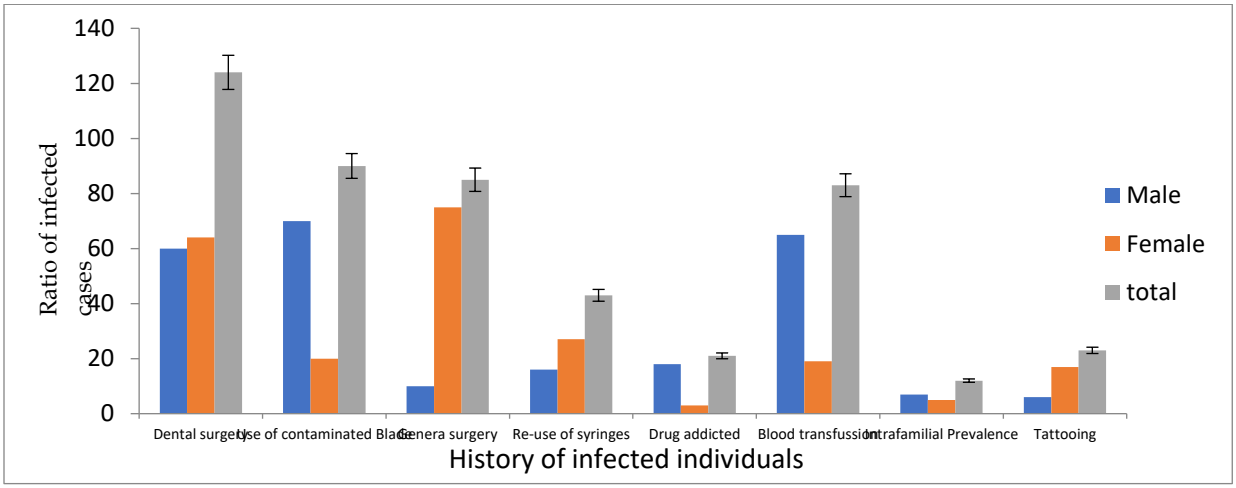

Figure 3: Frequency of risk factors for HCV in PCR-positive individuals of District Bannu

5. DISCUSSION

Hepatitis is one of the most common health issues in the recent era of developing countries such as Pakistan. The current study testifies that the female population of district Bannu is more affected by HCV as is the result of a prevalence is 55.44% in the female population. Conversely, the male population is less affected with 50.93% compared to the female population. The advanced infection ratio of HCV infection in males in comparison to females is due to their different exposure status to different risk factors which acted as a transmission source to Hepatitis C infection. This was discovered from the manner of life and history of the persons tested for the study. Our results are completely different from (Jan et al., 2020) who reported the prevalence of HCV as 66.93% in males and 33.07% in females in 25944 patients admitted to Medical units of District Head Quarter Teaching Hospital (DHQTH). Similarly the prevalence of HCV in 2.8% of male and 1.5% of female in 1016 patients referred by a physician (Jan et al., 2020). Our study is also oppose to (Ali et al., 2010) who led their research on IDPs in the War against Terrorism in South Waziristan Agency KPK and confirmed 26% of active HCV infections in males and 15% in females. The research indicates that HCV is less prevalent (51.42%) among the age group of 31-45 years this is due to good insusceptibility and having consciousness in society. The second most conscious age group is 16-30 years in which the prevalence ratio is about (52.47%). The next age groups are also moderating group such as 46-60 years (54.54%) and 61-75 years (55.10%). The highest risk age group is 1-15 years in which the prevalence ratio is about (58.33%). This high prevalence is due to a single and major reason; that, they have absence of consciousness about the transmission and causative agents of Hepatitis C infection. These results show deviation from the

previous studies such as (Idrees et al., 2008) (Jan et al., 2020). This study also shows a little bit connection to dental surgery as compared to other risk factors. The major risk factors identified in our sampled individuals were dental surgery (15.63%) this one is supported by various studies such as (Jan et al., 2020). Dental surgery is one of main risk factor for HCV in Pakistan. This is because the dental surgery instrument is not properly sterilized in hospitals and clinics (Ali et al., 2010). The other risk factors groups show a great variation with previous studies such as use of contaminated blade (11.34%), general surgery (10.71%), re use of syringes (5.42%), Drug addiction (2.64%), Blood transfusion (10.46%), and interfamilial prevalence (1.51%).

6. CONCLUSION

Hepatitis C Virus increased progressively with the lack of awareness. We can say that awareness against HCV is directly proportional to the spread of HCV in a society. Such as the highest occurrence ratio was documented in the age groups of 1-15 years which is because of the lack of awareness. Similarly, the prevalence in females was higher than in the male population because, in recent medical reports, most females face caesarian (C-section) delivery cases. Which is performed by unqualified staff and unsafe surgical instruments. But the Prevalence of HCV has no relation to seasonal changes. The HCV epidemic in district Bannu continues due to a lack of awareness and lack of education and shortage of medical and pathological staff and lack of health facilities. This growing HCV widespread it is possible to grow extensively increased dose.

Limitation of the study

The study's sample may not be fully representative of the entire population of Bannu district, Khyber Pakhtunkhwa, Pakistan. It might have excluded certain demographic groups or individuals who didn't have access to hospitals or healthcare facilities, leading to a biased estimation of HCV prevalence.The study utilized Immuno Chromato Graphic Test (ICT) and Polymerase Chain Reaction (PCR) techniques for detecting HCV antibodies and viral load, respectively. While these are common methods, they may have limitations such as false positives or false negatives, impacting the accuracy of prevalence estimates. The study provides age and gender breakdowns but lacks detailed demographic information such as socioeconomic status, education level, occupation, or lifestyle factors, which could influence HCV prevalence and transmission patterns. Without this information, it's challenging to understand the full scope of the epidemic and design targeted interventions effectively. The study adopts a cross-sectional design, capturing data at a single point in time. This limits the ability to establish causality or determine temporal relationships between risk factors and HCV infection. Longitudinal studies would provide a more comprehensive understanding of the dynamics of HCV transmission over time

7. Recommendations

Recommendations for future research and interventions based on the findings of the study include:

Conduct longitudinal studies to track the trends of HCV prevalence over time in Bannu district. Longitudinal data will provide

insights into the dynamics of HCV transmission, the effectiveness of interventions, and changes in risk factors over time. Investigate a broader range of risk factors contributing to HCV transmission, including socioeconomic status, educational level, occupation, healthcare access, and behavioral practices such as injection drug use, unsafe medical procedures, and blood transfusions. Understanding these factors will help tailor interventions to address specific vulnerabilities in the population. Foster collaboration between government agencies, non-governmental organizations, healthcare providers, and community stakeholders to implement coordinated and sustainable interventions to address the HCV epidemic in Bannu district. Pooling resources, expertise, and efforts will maximize the impact of interventions and ensure long-term success in combating HCV transmission.

8. Authors' Contribution (ACF):

Muhammad Kamran Khan. Conducted the research, sampling and laboratory activity

Tayyiba Ali: Contributed in conceptualization and validation of the study

A. Abbas, and Sidra Sattar, Shah: Participated in writing, and edited the methodology.

9. Conflict of interest:

All the authors mentioned in the manuscript have no conflict in the research work and compilation.

Abdul Basit critically reviewed along with manuscript writing

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