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Hepatitis C Virus RNA and Genotype in Semen of Primitively Diagnosed Patients Infected with HCV-RNA Infection Amongst the Community of Lahore Cantt

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ABSTRACT

Objective: To determine whether Hepatitis C virus is present in the seminal fluid of primitively diagnosed patients and subsequent genotyping of semen-positive samples.

Study Design: Cross-sectional study.

Place and Duration of Study: Darul Shifa Clinic and the PCR Lab, Lahore Pakistan, from Apr 2021 to May 2022.

Methodology: We studied 100 patients with diagnosed Hepatitis C infection via Polymerase Chain Reaction. The reverse transcriptase Polymerase Chain Reaction was used to detect the presence of the Hepatitis C virus in the semen of these sample patients.

Results: Mean age of enrolled 100 male patients was 33.86±7.9 years. Only 4% of semen samples tested HCV RNA positive, while 96% were negative. The results of multiplex Polymerase Chain Reaction revealed that 50% of cases showed genotype 2a and 50% cases showed genotype 3a out of 4 positive cases of HCV RNA.

Conclusion: The observed viral prevalence was relatively low; nevertheless, the potential for pathogen dissemination remains significant. These findings contribute to our understanding of disease transmission dynamics and may have implications for epidemiological modeling and public health interventions.

Keywords: Genotype, Hepatitis C Virus, Ribonucleic Acid, Seminal Fluid.

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INTRODUCTION

Liver disease caused by the Hepatitis C virus occurs as cirrhosis, leading to irreparable liver damage if left untreated as it damages the liver's architecture and may result in liver cancer, due to which approximately 4 million people die yearly[1] with Pakistan having one of the highest rates of Hepatitis C infection in the world, with a prevalence of approximately 8.4% nationwide, and 5.46 % in its most densely populated province, Punjab.[2] An analysis infected people from 117 countries, showed that genotype-1 infected 46% of the population with genotype-3 infecting 30% while combined infectivity of genotypes 2, 4, and 6 was 23%. One third of the people infected globally with genotype-1 were East Asians[3] while genotype-3a is most prevalent in Pakistan.[4] Studies suggest the presence of HCV-RNA in human tears, cerebrospinal fluid, saliva and semen.[5] Liver cirrhosis and scarring can initially be asymptomatic but late stages of liver cirrhosis result in organ failure and cancer.[6] Acute infections in young people typically do not transmit to adults and resolve on their own in 10% to 50% of cases but some patients (80%) can have a persistent viral infection.[7] Cirrhosis is more prevalent in patients who abuse alcohol and in male patients if the patient has HIV or Hepatitis B, along with the risk of developing hepatocellular carcinoma. Early detection and prompt treatment of HCV infection can help to reduce symptoms and stop the disease from spreading to other people as the best course of treatment for patients can be decided using accurate screening techniques.[8,9] The aim of this investigation is to undertake molecular genotyping of HCV in patient seminal secretions by multiplex PCR after detecting HCV RNA in infected patients' semen by RT-PCR confirmatory test.

METHODOLOGY

The study was conducted from April 2021 to May 2022. Ethical approval for conducting the study was granted through letter (IRB-19/022). Sample size was calculated using WHO sample size calculator taking reported prevalence of Hepatitis C virus 53.2%10 after which the sample size came out to be 100. Non-

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probability convenient sampling was used to enroll male patients who had already been diagnosed with Hepatitis C on PCR result.

Inclusion Criteria: Male participants, aged between 18 to 60 years, tested HCV-RNA positive without receiving any antiviral medication were included.

Exclusion Criteria: Male participants with illnesses like Sjogren's syndrome or those who refused the semen analysis were excluded.

Patients' pharmacological and clinical histories were gathered utilizing questionnaires after taking written, informed consent. Selecting individuals with RT PCR-confirmed Hepatitis C virus RNA was challenging, particularly for those who had never received antiviral medication. Free screening camps were set up to collect the required sample size. All patients who tested positive during the screening was asked to do RT PCR Qualitative test where 2-3 samples were taken weekly on two specified days and then sent to the lab for RNA isolation, RT-PCR optimization, and appropriate genotyping.[10] Patients were also requested to provide their semen samples in a sterile container. Samples were sent in an icebox to the laboratory within two hours of ejaculation of which 1 ml of the sample was centrifuged, and supernatant was separated and analyzed for HCV RNA. The genotype was also noted, along with prior reports that were already known, such as ultrasound images of the abdomen. For RNA isolation, one ml of sample (semen) was combined with an equal volume of Trizol reagent and placed in a freezer at -20 °C. RNA extraction was carried out using a commercial viral isolation kit. Reverse transcription was used to create single stranded cDNA for each sample from the isolated RNA sample. The validation of HCV presence or absence in semen was carried out using a nested RT-PCR. Only semen samples that were positively tested for HCV using RT-PCR were genotyped for the virus and sensitive multiplex PCR was used to perform the genotyping. [11] Frequency (n) and percentages (%) were calculated for categorical data by using a Statistical Package for the Social Sciences (SPSS version 22).

RESULTS

There were 100 male patients with a mean age of 33.86±7.96 years. Table-I shows frequency distribution of enrolled patients HCV RNA status, where the number of semen samples which had this virus was only 4(4 %) whereas 96 Semen samples have no HCV RNA.

Table-I: Patient Semen HCV RNA Status Frequency Distribution Based on Laboratory Test Results, (n=100)

HCV RNA Lab Status	Percentage	
	Positive n(%)	Negative n(%)
HCV RNA in Semen	4(4)	96(96)

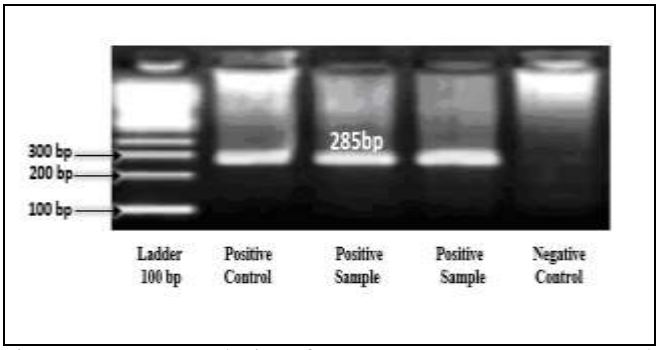

Figure-1: PCR Analysis of HCV-RNA on Agarose Gel Electrophoresis

As shown in Table-I, 4 patients were HCV RNA positive in semen samples. Out of which 2 patients were 2a and 2 patients were 3a positive as shown in Table-II. Figure-1 shows the PCR analysis on Agarose gel. Multiplex PCR was used to genotype the positive samples, as shown in Figure-2. Out of the four positive cases, the results revealed that 2 cases (50%), belonged to Genotype 2a, the genotype with the highest frequency of all those found. Next is genotype 3a, which only accounts for 2 individuals (50%) of the population

Table-II: Frequency Distribution of HCV-RNA Positive Sample Genotypes (n=100)

Genotype	n(%)
2a	2(50)
3a	2(50)
Total	4(100)

The genotyping key for HCV is shown in Figure-2.

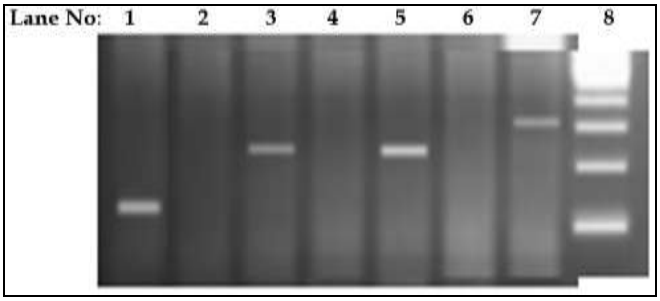

Figure-2: HCV-RNA Genotype via Multiplex PCR, (n=100)

Key of HCV Genotyping

Lane 1: *139 bp (2a)*
Lane 2, 4, 6: *Negative*
Lane 3: *232 bp (3a)*
Lane 5: *232 bp (3a)*
Lane 8: *100 bp Ladder Marker*

DISCUSSION

Hepatitis C virus infections have increased in Pakistan over the past ten years despite recent advancements in the therapeutic field[11] and continues to be a serious health concern in underdeveloped countries even while its prevalence is reducing in industrialized nations.[12] HCV is typically detectable in body fluids of those who have the infection. In this study, HCV RNA was examined in the semen of 100 untreated individuals as the presence of HCV-RNA in semen is confirmed by a small number of worldwide research, but information on HCV-RNA detection with subsequent genotyping is hard to obtain.[13] According to the results of a molecular characterization study, genotype 3(73.9%) was the most common type found in Pakistan, followed by genotype 1(9.7%), and genotype 4(0.3%), which was isolated for the first time in Pakistan[14] with genotype 3a, which made up 39.4% of the population, most frequently found in the population of Lahore, while the most prevalent HCV genotype in Khyber Pakhtunkhwa is 2 and genotype-3 is more prevalent in the provinces of Punjab and Sindh, taking into account our nation's rising genotype-2a prevalence and falling genotype-3a trend.[15] As one of the human viruses with the largest genetic diversity is HCV,[16] genotype-3 is second only to genotype 1, despite being the genotype that is most frequently seen in Pakistan. Another study looked into the possibility of HCV transmission by semen as only 4 of the 32 males who had a chronic HCV infection had HCV RNA present and although there is no concrete proof that the semen samples contributed to infectivity, they do demonstrate that HCV RNA is discharged in the seminal fluid.[17] The epidemiology of Hepatitis C in Pakistan demonstrates a higher prevalence of genotype-3a. Our study found that genotype-2a, in addition to genotype-3a, provides an identical probability of being expelled in secretions like semen.[18] Therefore, healthcare professionals should place a stronger emphasis on advising indivi-duals with genotype-2a with greater caution regarding changes to their behavioral activities, particulalry safe sex practices.

LIMITATION OF STUDY

The major limitations of the study include the sample size along with lack of self-reported HCV infection among family members without laboratory confirmation after enrolment, and the paucity of data about the overall number of family members and the percentage of HCV-infected relatives.

CONCLUSION

Two genotypes in total, 2a, and 3a, were identified in the investigation, with each genotype accounting for half of the patients.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

THK & MUS: Data acquisition, data analysis, critical review, approval of the final version to be published.

AS & SA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MAU: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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