Hepatitis C Virus (HCV) Gentyping in Infected Patients From Albaha Region

Shaia S. R. Almalki, PhD* Hanan E. Alyahyawi, PhD* Raed A. Alharbi, PhD* Waled AM Ahmed, PhD** Mohammad A. Izhari, PhD* Saleha K. Alatawi, PhD*** Faisal Klufah, PhD*

ABSTRACT

Background: Hepatitis C virus (HCV) is a significant public health issue in Saudi Arabia, with an estimated prevalence of 1.4% in the general population. The preponderance and distribution of HCV genotypes varies globally, and in Saudi Arabia. Genotypes 1a, 1b, and 4 are most commonly found. HCV genotype is predictive of the response of HCV-infected patients to antiviral therapy. Thus, the genotype should be determined so that the patient can receive the most appropriate therapy regimen.

Objective: To examine the distribution of HCV genotypes in infected patients from Albaha region.

Setting: Medical Laboratory Department, Central laboratory and blood bank, Albaha.

Design: A retrospective cross-sectional study.

Method: Forty suspected cases of HCV collected from Central laboratory and blood bank at Albaha from January 2020 to April 2020 were included in the study. All samples were analyzed with real-time RT- qPCR for HCV RNA detection as described previously. HCV RNA positive samples were further analyzed. The HCV genotype-specific multiplex PCR was performed using Abbott RealTime HCV Genotype II assay.

Result: Between January 2020 to April 2020, 40 suspected cases of of HCV were analysed; 21(52.5%) were HCV RNA negative and 19 (47.5%) were HCV RNA positive. Genotype 1a was the most prevalent among all the genotypes observed followed by 1b and genotype 2. Genotype 4 and 5 were found in 5% of our samples.

Conclusion: HCV genotype 1a is highly prevalent in Albaha region. These findings will help medical professionals to prescribe more appropriate treatment for the HCV infection, and they will also develop plans for more efficient disease control.

Keywords: Prevalence, Genotype, Hepatitis C Virus

INTRODUCTION

Hepatitis C virus (HCV) infection is a global puplic health issue, causing of chronic liver disease^{1,2}. The World Health Organisation (WHO) estimated that about 3% of the human population have been infected with HCV. With 12 million chronically infected individuals worldwide³. The Eastern Mediterranean Region and the European Region have the highest disease burden. An estimated 10 million people in each region of the Western Pacific and South-East Asia are chronically infected. Five million people in the Americas and nine million in Africa are chronically infected. According to WHO, 350,000 individuals died from HCV mostly from cirrhosis and hepatocellular

*	Department of Laboratory Medicine
	Faculty of Applied Medical Sciences
	Al-Baha University
	Saudi Arabia.
	E-mail: shalmalki@bu.edu.sa
**	Department of Nursing
	Faculty of Applied Medical Sciences
	Al-Baha University
	Saudi Arabia.
***	Department of Optometry
	Faculty of Applied Medical Sciences
	Al-Baha University
	Saudi Arabia.

carcinoma (primary liver cancer)^{3,4}.

HCV is a positive single- stranded RNA virus of flavivirus with a high genetic diversity worldwide⁵. The majority of research have shown that HCV is an endemic medical condition, and several precautions are taken to reduce the danger of this infection. Most frequently, the virus is spread via surgical procedures including organ transplants, blood transfusions, or injections using infected syringes. Despite the availability and effectiveness of HCV therapy, the distributions of viral infection characteristics such as genotype, age, and race are increasing globally.

Seven gentotypes are labeled with numbers $(1-7)^{6,7}$, e.g., genotype 1 to genotype 7 and around 80 subtypes ^{8,9}, with varied geographic prevalence, have been recognised^{10,11}. For example, Genotype 1 is the predominant genotype in North and South America and Europe. Genotype 2 circulates in Europe, North America, and Jaban. Geontype 3 is common in South Asia, Australia and Europe¹². Genotype 4 is prevalent in Egypt, the Middle East, and Africa¹³.

In Saudi Arabia, the prevlent genotype is type 4^{14,15}, but other genotype are also present^{16,17}. Genotype 4 accounts for about 53% of all HCV infections. Genotypes 1a and 1b are also prevalent, occurring in 31% and 15% of individuals, respectively. Other HCV genotypes, such as 2, 3, 5, and 6, are relatively rare in Saudi Arabia^{16,18,19}. The distribution of HCV genotypes in Saudi Arabia varies by region. For example, in the western and southern regions, genotype 4 predominates, while genotypes 1a and 1b are more common in the central and eastern regions^{16,20}. The severity and aggressiveness of the liver infection, as well as the patient's responce to therapy, are all significantly influenced by the patient's HCV genotype²¹. In addition, The genotype of HCV can be used for determing the HCV distribution in society, hence, studying the mode of transmission. The aim of this study is to examine the distribution of HCV genotypes in infected patients from Albaha region.

METHOD

A retrospective cross-sectional study was carried out between January 2020 to April 2020 in Central laboratory and blood bank, Albaha, Saudi Arabia. This study recruited 40 suspected cases of HCV obtained from the diagnostic Medical Laboratory at Central laboratory and blood bank, Albaha, Saudi Arabia, for the HCV RNA detection and genotyping. No patient sample was specifically collected for the purpose of this study. Only HCV samples were obtained and used in the project. No Patient's clinical data were collected at the time of the study. The inclusion criteria was any HCV RNA positive, no exclusion criteria was carried out. HCV RNA were analysed from 40 serum samples.

Since no samples were directly collected from the patients for the purpose of our study and only the recruited HCV samples were used in the study, no consent form was required. The ethical approval for the study was obtained from Deanship of Scientific Research Review Board at Albaha University, Albaha, Saudi Arabia.

RNA was extracted from serum and reverse-transcribed to cDNA and the core region of HCV genome was targeted and amplified, serum HCV RNA levels tested by real-time RT-qPCR, (Bio-Rad CFX96 Dx Real-Time PCR Detection Systems for In Vitro Diagnostics (IVD), The Primerdesign genesig Kit for Hepatitis C Virus (HCV) Bndrgen, Madinah, Saudi Arabia). The prevalence of different genotypes in the subject population was analyzed by type-specific multiplex PCR targeting the core region of HCV genome (Abbott RealTime HCV Genotype II assay, bndrgene, Madinah, Saudi Arabia(.

Statistical analysis was carried out using Microsoft Excel software version 16.71. All variables were calculated as frequency and percentage.

RESULT

All samples were analyzed with RT-PCR for HCV RNA detection. Of the total 40 individuals, 19 were found to be HCV RNA positive while 21 were negative (Figure 1).

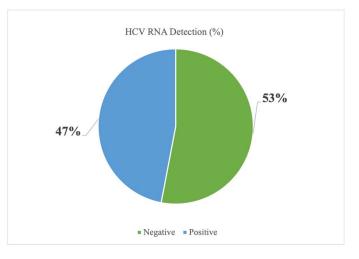


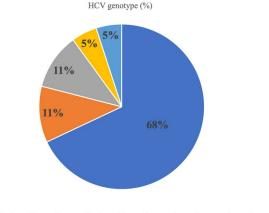
Figure 1: Percentage of HCV RNA detection

The prevalence of different genotypes in the subject population was analyzed by type-specific PCR targeting the core region of HCV genome. HCV genotype 1 was detected in 79% of the HCV RNA positive patients, the most prevalent genotype in the studied area. HCV genotype 2 was observed in 11% and genotype 4 and 5 were 5% (Table 1).

Table 1: The	presented	data	illustrate	the	identified	HCV	genotype	
--------------	-----------	------	------------	-----	------------	-----	----------	--

Identified HCV genotype	Total	Percentage
Genotype 1	15	79%
Genotype 2	2	11%
Genotype 4	1	5%
Genotype 5	1	5%
Total	19	100%

The genotype 1a was found to be the major causes of infections in the study (Figure 2).



Genotype 1/ subtype 1a Genotype 1/ subtype 1b Genotype 2 Genotype 4 Genotype 5a

Figure 2: Genotype 1/ subtype 1a accounts for the majority of identified HCV genotype in this study

DISCUSSION

The current study describes the prevalence of HCV genotypes in infected patients from Albaha region, Saudi Arabia. To our knowledge, there had been no studies of HCV genotypes in Albaha, Saudi Arabia.

The prevalence of HCV genotypes can vary depending on several factors, including geographic location, mode of transmission, and patient population^{22,23}.

Our data showed that genotype 1a (68%) is the most prevalent, followed by genotype 1b and 2 (11%) in Albaha region, Saudi Arabia. In addition, several studies have been conducted to determine the prevalence of HCV genotypes in Saudi Arabia. It has been found that genotype 4 is the most prevalent HCV genotype in Saudi Arabia²³⁻²⁶. However, genotype 4 (5%) was the least common in this study. The high prevalence of HCV genotype 4 in Saudi Arabia may be due to several factors, including the country's location at the crossroads of global trade routes, the high prevalence of blood-borne transmission, and the widespread use of unscreened blood and blood products^{15,24}. Additionally, Saudi Arabia has implemented several measures to control the spread of HCV infection, including the introduction of blood screening programs, the promotion of safe injection practices, and the expansion of access to HCV treatment²⁷⁻³⁰. These efforts have contributed to a decline in the overall prevalence of HCV infection in the country.

Despite these efforts, challenges remain in addressing HCV infection in Saudi Arabia, including the need to improve access to HCV testing and treatment, especially in remote and underserved areas. Furthermore, there is a need to strengthen prevention efforts among high-risk populations, such as people who inject drugs and those with a history of blood transfusion³¹.

The prevalence of HCV genotypes in Saudi Arabia has significant implications for the management and treatment of HCV infection. Different HCV genotypes have different responses to antiviral therapy, and the choice of treatment regimen depends on the genotype of the virus. Therefore, accurate information on the prevalence of HCV genotypes is essential for developing effective prevention and treatment strategies.

Several limitations remained in the current study. First, this is a onelabortory center study that examined only small number of samples with the lack of patient's clinical data. Consequently, the results may not be representative across the area. Although genotype 1a continues to dominate, the distribution and prevalence of HCV genotypes are changing in this region mainly due to population increase.

Additional research is needed to understand the prevleance, risk factors and outcomes for the varition in the HCV genotypes and subtypes. Further data are required for genomic sequences of HCV isolates from Albaha region to assess the clinical significance of HCV genotypes in the area. Furthermore, gathering information on HCV genotypes from a large population can help healthcare professionals and administrators monitor and control HCV infection.

CONCLUSION

Overall, the prevalence of HCV genotypes in Albaha, Saudi Arabia appears to be dominated by genotype 1a. This has important implications for the development of treatment strategies, as different genotypes may require different antiviral regimens. Therefore, accurate and up-to-date information on the prevalence of HCV genotypes is essential for the effective management and treatment of HCV infection in Saudi Arabia.

Author Contribution: All authors share equal effort contribution towards (1) substantial contribution to conception and design,

acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version should be published. Yes.

Financial Support: The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number MOE- BU- 1- 2020.

Ethical Approval: Approved by the Deanship of Scientific Research Review Board at Albaha University, Albaha, Saudi Arabia.

Potential Conflict of Interest: None.

Competing Interest: None.

Acceptance Date: 26 April 2023

REFERENCES

- 1. Ireland G, Mandal S, Hickman M, et al. Mortality rates among individuals diagnosed with hepatitis C virus (HCV); an observational cohort study, England, 2008 to 2016. Eurosurveillance 2019;24(30):1800695.
- Hall EW, Schillie S, Vaughan AS, et al. County-Level Variation in Hepatitis C Virus Mortality and Trends in the United States, 2005-2017. Hepatology 2021;74(2):582-90.
- 3. World Health Organization (WHO). Hepatitis C. 2022.
- 4. Lazarus JV, Roel E, Elsharkawy AM. Hepatitis C Virus Epidemiology and the Impact of Interferon-Free Hepatitis C Virus Therapy. Cold Spring Harb Perspect Med 2020;10(3):a036913.
- Galdino AS, Santos JC, Souza MQ, et al. A Novel Structurally Stable Multiepitope Protein for Detection of HCV. Hepat Res Treat 2016;2016:1-9.
- Simmonds P, Alberti A, Alter HJ, et al. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 1994;19(5):1321-4.
- Murphy DG, Sablon E, Chamberland J, et al. Hepatitis C Virus Genotype 7, a New Genotype Originating from Central Africa. J Clin Microbiol 2015;53(3):967-72.
- 8. Kuiken C, Simmonds P. Nomenclature and Numbering of the Hepatitis C Virus. Methods Mol Biol 2009;510:33-53.
- Smith DB, Bukh J, Kuiken C, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource. Hepatology 2014;59(1):318-27.
- Messina JP, Humphreys I, Flaxman A, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015;61(1):77-87.
- 11. Schnell G, Krishnan P, Tripathi R, et al. Hepatitis C virus genetic diversity by geographic region within genotype 1-6 subtypes among patients treated with glecaprevir and pibrentasvir. PLoS One 2018;13(10):e0205186.
- 12. Sharafi H, Rezaee-Zavareh MS, Miri SM, et al. Global Distribution of Hepatitis D Virus Genotypes: A Systematic Review. Hepat Mon 2020;20(2).
- Ghaderi-Zefrehi H, Gholami-Fesharaki M, Sharafi H, et al. The Distribution of Hepatitis C Virus Genotypes in Middle Eastern Countries: A Systematic Review and Meta-Analysis. Hepat Mon 2016;16(9):e40357.
- Al Ashgar H, Khan M, Al-Ahdal M, et al. Hepatitis C genotype
 4: Genotypic diversity, epidemiological profile, and clinical relevance of subtypes in Saudi Arabia. Saudi J Gastroenterol 2013;19(1):28.

- Abdel-Moneim AS, Bamaga MS, Shehab GMG, et al. HCV Infection among Saudi Population: High Prevalence of Genotype 4 and Increased Viral Clearance Rate. PLoS One 2012;7(1):e29781.
- Anonymous. Distribution of hepatitis C virus (HCV) genotypes in a Saudi Arabian hospital during the 2015-2020 period. Infezioni Medicina 2021;29(3):450-5.
- 17. Memish ZA, Knawy B Al, El-Saed A. Incidence trends of viral hepatitis A, B, and C seropositivity over eight years of surveillance in Saudi Arabia. Int J Inf Dis 2010;14(2):e115-20.
- Abozaid SM, Shoukri M, Al-Qahtani A, et al. Prevailing genotypes of hepatitis C virus in Saudi Arabia: a systematic analysis of evidence. Ann Saudi Med 2013;33(1):1-5.
- Chaabna K, Cheema S, Abraham A, et al. Systematic overview of hepatitis C infection in the Middle East and North Africa. World J Gastroenterol 2018;24(27):3038-54.
- Bawazir A, AlGusheri F, Jradi H, et al. Hepatitis C virus genotypes in Saudi Arabia: a future prediction and laboratory profile. Virol J 2017;14(1):208.
- 21. Zein NN. Clinical Significance of Hepatitis C Virus Genotypes. Clin Microbiol Rev 2000;13(2):223-35.
- 22. Mahmud S, Al-Kanaani Z, Chemaitelly H, et al. Hepatitis C virus genotypes in the Middle East and North Africa: Distribution, diversity, and patterns. J Med Virol 2018;90(1):131-41.
- 23. Ali Hazazi, Mohammed Almubarak, Fawaz Albloui, et al. Hepatitis C Virus Genotyping in Saudi Population. J US China Med Sci 2019;16(3).

- 24. Al-Qahtani AA, Baele G, Khalaf N, et al. The epidemic dynamics of hepatitis C virus subtypes 4a and 4d in Saudi Arabia. Sci Rep 2017;7(1):44947.
- 25. Khan A, Al Balwi M, AlAyyar L, et al. Tracing the epidemic history of hepatitis C virus genotypes in Saudi Arabia. Inf Gene Evol 2017;52:82-8.
- 26. Sievert W, Altraif I, Razavi HA, et al. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver International 2011;31(2):61-80.
- 27. Alghamdi A, Sanai F, Ismail M, et al. SASLT practice guidelines: Management of hepatitis C virus infection. Saudi J Gastroenterol 2012;18(7):1.
- Alzahrani MS, Ayn Aldeen A, Almalki RS, et al. Knowledge of and Testing Rate for Hepatitis C Infection among the General Public of Saudi Arabia: A Cross-Sectional Study. Int J Environ Res Public Health 2023;20(3):2080.
- 29. AlMutairi HH, AlAhmari MM, Al-Zahran BH, et al. Prevalence of serological markers and nucleic acid for blood-borne viral infections in blood donors in Al-Baha, Saudi Arabia. J Inf Dev Countr 2016;10(6):619-25.
- 30. Sallam TalalA, El-Bingawi H, Alzahrani K, et al. Prevalence of hepatitis B and hepatitis C viral infections and impact of control program among blood donors in Al-Baha region, Saudi Arabia. Saudi J Health Sci 2020.
- 31. Alzahrani MS, Ayn Aldeen A, Almalki RS, et al. Knowledge of and Testing Rate for Hepatitis C Infection among the General Public of Saudi Arabia: A Cross-Sectional Study. Int J Environ Res Public Health 2023;20(3):2080.