Epidemiological Screening And Genotyping Analysis Of Hepatitis C Virus In Jazan Region, Saudi Arabia

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Abstract

Globally, HCV infection is a significant public health concern leading to chronic liver disease (cirrhosis, hepatocellular carcinoma) resulting in substantial morbidity and mortality. HCV genotyping is the primary tool for clinical evaluation, disease pathogenesis, treatment, and follow-up of infected cases. The variations in HCV genotype prevalence present challenges to developing vaccines and therapeutics. To identify the distribution of HCV genotypes and subtypes in the Jazan region, a hospital-based prospective study was conducted. A total of 52 HCV clinically infected patients that had previously been positively tested by ELISA were randomly selected and were subjected to genotyping analysis and liver biochemical marker function test. GT-4 and GT-1 were predominated in Jazan region with the highest infection rates observed in adults between 26-65 years old who are married and of Saudi nationality. Relatively, no significant difference between other demographic factors tested. The biochemical markers tested suggest liver dysfunction with elevated albumin, bilirubin, ALP, and ALT levels. Therefore, our results may require further investigation to determine the underlying cause of the liver abnormalities.

Key words: Hepatitis C Virus, epidemiological screening, genotyping, Jazan region, Saudi Arabia.

INTRODUCTION

Hepatitis C Virus (HCV) is a small, enveloped single stranded positive sense-RNA virus belongs to the Flaviviridae family and has a high mutation rate (Ganta et al., 2019). It displays substantial genetic variabilities, contributing to its ability to invade the host immune responses resulting in chronic infection (Ma et al., 2006). Once the HCV infect the human, it replicates in the cytoplasm of hepatocytes, involves the synthesis of a complementary negative sense RNA strand for the production of new positive sense RNA genomes (Quinkert et al., 2005). Later, the HCV proteins interact with host cell lipids to form viral particles which are assembled in association with host cell membranes. Through various routes included blood donation, sharing of contaminated needles and syringes, sexual and congenital the HCV has been transmitted (Alter, 2011). Globally, HCV infection is a significant public health concern leading to chronic liver disease (cirrhosis, hepatocellular carcinoma) resulting in substantial morbidity and mortality (Papatheodoridi and Papatheodoridis, 2023). Based on sequence differences, the HCV genome is classified into eleven genotypes (GT-1 to GT-11) and at least 70 subtypes (Landt et al., 2002). The epidemiologic study postulated that, the different HCV genotypes (GT) have distinct geographic distributions, genotypes 1-5 are the most common and types 6-11 are very rare (Waheed et al., 2009, Borgia et al., 2018). The molecular distribution of these genotypes and subtypes depending on the geographic origin and transmission risk factors (Prasad et al., 2023) and has an impact of both current and past migratory patterns (Grossini et al., 2023). Recently, new insights into the distribution of HCV genotypes have been reported in West Europe, Russia, and Israel regions (Wiessing et al., 2023). Across different regions and countries, the prevalence of HCV infections were varied, highest burden observed in developing countries, particularly in Africa, Middle East, and Asia (Tang et al., 2023). Epidemiological screening and genotyping analysis of HCV are crucial aspects in understanding the prevalence, transmission dynamics, and disease outcomes associated with HCV infection in a specific population (Rivera Saldana et al., 2023, Factor et al., 2023). There is a lack of data on the epidemiology and genotypes of HCV in the Jazan region. Therefore, the current epidemiological status, the biochemical marker functions test of HCV infected cases and the distribution of HCV genotypes in the Jazan region were highly needed. Such investigations are crucial and highly necessary to inform the development of effective prevention and control

strategies, ultimately reducing the burden of HCV infection in the region.

Objectives

To examine the prevalence of HCV genotypes in clinically infected patients admitted to the Jazan General Hospital.

To predict the liver involvement among HCV infected patients resident in Jazan region.

MATERIALS AND METHOD

Study Design

To achieve the research objectives: An epidemiological screening and genotyping analysis of the hepatitis HCV in Jazan area, KSA, a hospital-based prospective study was conducted.

Study area and population

Jazan region has various (urban, suburban, and rural) with highest population density providing a diverse population to carry out the current study. The study population included individuals residing in the Jazan region, seeking medical care and admitted to both the inpatient and outpatient hospitals departments, all nationalities, both males and females, adult (<18 years old). Participants were recruited through a random sampling technique, ensuring representation from urban, suburban, and rural areas. Informed consent was obtained from all participants before their inclusion in the study. Before recruitment in the study, the patient, parent, or guardian provided a written informed consent.

Sample

A total of 52 HCV clinically infected patients - that had previously been positively tested by HCV ELISA - who were admitted to Jazan General Hospital were randomly selected. Approximately 5 ml of blood was collected in tubes containing Ethylenediaminetetraacetic acid (EDTA) and the plasma was stored at -70° C until use. All the HCV seropositive selected samples were further subjected to molecular RNA testing, genotyping and liver function tests. PCR confirmed positive and negative samples from Jazan General Hospital were respectively used as control negative and positive for molecular laboratories diagnostic tests.

RNA extraction

The total HCV RNA was extracted from all the plasma selected samples using the Favorgen commercial RNA extraction kit, following the instructions provided by the manufacturer (Catalog number R3-P603-23/9EU, DNA Technology Research and Production, Moscow Region, Russia). Briefly, 100 µL of plasma samples were added to 400 µL of lysis buffer, mixed and were incubated for 12 minutes. Ethanol was added to the mixture, vortexed and was centrifuged. Then, the supernatant solution was transferred into a spin column and was centrifuged at 8000 rpm for 1 minute. Using RNase-free water, RNA was eluted and was stored at -80°C until further analysis.

Reverse transcription

Using a reverse transcription reaction, the extracted RNA was subjected to reverse transcribed proses into complementary DNA (cDNA). Briefly, the extracted RNA, specific primers targeting the HCV genome, dNTPs (deoxynucleotide triphosphates), and reverse transcriptase enzyme were mixed and were incubated at 42-50°C for 30-60 minutes. The resulting cDNA served as the template for quantitative PCR (qPCR) amplification.

Real-time PCR

To amplify and quantify the HCV RNA in the cDNA samples, qPCR also known as real-time PCR was performed. Primers specific to the HCV genome, fluorophore-labeled probes specific to the amplified region, DNA polymerase enzyme, dNTPs, and reaction buffer in a master mix were set up for qPCR reaction. Then cDNA template was added to the reaction mix in a real-time PCR instrument capable of monitoring fluorescence signals during amplification. The thermal cycling (initial denaturation, denaturation, annealing, and extension steps) were set up. The fluorescence signals emitted by the probes were measured at each amplification cycle, allowing real-time monitoring of the PCR amplification. The results of the reverse hybridization assay are analyzed and interpreted to determine the genotype and subtype of the HCV virus present in the sample.

Biochemical markers

All the HCV positive samples were subjected to liver, renal and tissue biochemical marker function test including total protein, albumin, bilirubin, alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate Aminotransferase (AST), blood urea

nitrogen, Creatinine, Creatinine Phosphokinase (CPK) and Lactate Dehydrogenase (LDH).

Ethical approval and informed consent

The study protocol was reviewed and approved by Research Ethic Committee, Jazan Region Ministry of Health, Saudi Arabia. The research was conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki. Informed consent was obtained from all participants before their inclusion in the study, ensuring their voluntary participation and confidentiality of their information.

Results and discussion:

HCV genotypes

Three distinct HCV genotypes and 2 sub genotypes were detected (Table 1). GT-4 exhibited the highest prevalence, (31/59.6%) followed by GT-1a, (12/23.1%). GT-3 was found in 5 cases (9.6%) of the total patients and GT-1b was detected in 4 cases (7.7%). No cases of mixed genotypes were identified. The results of all HCV genotypes and subtypes across various demographic factors were s demonstrated (Table 2).

Biochemical markers

The mean serum level of total protein, albumin, bilirubin, alkaline phosphatase (ALP) and Alanine transaminase (ALT) were reported (Table 3). These liver biochemical markers showed significant elevated levels of albumin, bilirubin, alkaline phosphatase and ALT, on the other hand, the total proteins and AST were fluctuated within the normal levels. The renal biochemical function test showed significant increase (125.737 ± 182.0771) in creatinine and normal level of blood urea nitrogen. Aspartate Aminotransferase (AST), Creatinine Phosphokinase (CPK), Lactate Dehydrogenase (LDH). Tissue biochemical indicators CPK and LDH were fell within the normal range.

Table 1. Frequency of different HCV genotypes detected in clinically infected patients.

Genotype	Frequency	Percent (%)
GT-1a	12	23.1
GT-1b	4	7.7
GT-3	5	9.6
GT-4	31	59.6
Total	52	100.0

Table 2. The socio-demographic data in different HCV genotypes of infected clinically patients.

Demographic data		Genotype (GT)				Total
		GT-1a	GT-1b	GT-3	GT-4	
Age	18-25	1	0	0	2	3
	26-65	8	4	3	24	39
	> 65	3	0	2	5	10
Gender	Male	6	2	3	17	28
	Female	6	2	2	14	24
Nationality	Saudi	10	4	5	27	46
	Non-Saudi	2	0	0	4	6
Marital status	Single	5	0	0	7	12
	Married	7	4	4	21	36
	Widow	0	0	1	2	3
	Divorced	0	0	0	1	1
Province	Central	4	0	1	12	17
	Northern	2	0	0	1	3
	Southern	2	0	2	5	9
	Eastern	1	4	2	5	12
	Western	3	0	0	8	11
Residency	Mountain	0	2	1	2	5
	Rural	7	1	1	13	22
	Urban	5	1	3	16	25

Table 3. Biochemical markers detected in HCV infected blood samples.

Organ	Biochemical markers	Mean	± SD	Normal level	Clinical status

Liver	Total protein	63.985	21.0644	60 to 83 g/L	Normal
	Albumin	38.276	19.4158	3.5-5.5 g/dL	High
	Bilirubin	11.310	6.4330	3.4-20.5 (µmol/L).	High
	ALP	93.121	30.0986	20-40 IU/L	High
	ALT	30.325	15.9760	7-55 IU/L	High
	AST	29.763	17.8124	8-48 IU/L	Normal
Kidney	Blood Urea Nitrogen	4.908	1.2157	2.9-7.1 mmol/L	Normal
	Creatinine	125.737	182.0771	53-97.2 µmol/L	High
Tissue	CPK	101.15	65.012	30-200 IU/L	Normal
	LDH	171.46	32.56	140-280 IU/L	Normal

SD= Standard Deviation.

DISCUSSION

HCV genotyping is the primary tool for the clinical evaluation of the clinical status, course of infection and treatment follow up of diseased cases and future vaccine development. Approximately, 150 million people are HCV infected and more than 300000 individuals are die each year duo to chronic liver involvement worldwide (Nickbakhsh et al., 2023). The variations in HCV genotypes prevalence present challenges to the development of vaccines and therapeutics (Bernal and Soti, 2023). In this study we demonstrated the different and the distinct HCV genotypes among adult (<18 years old), all nationalities and both gender. Of those HCV infected patients, 28 were males, 24 females, 39 (75%) were age (26-65) years old, 46 (88.5%) were Saudi and 36 (69.2%) were married. The current study indicated that GT-4 exhibited the highest prevalence, (31/59.6%) followed by GT-1a, (12/23.1%). Our finding was previously confirmed in Saudi Arabia by many researchers (Boriskin et al., 1999, Abdel-Moneim et al., 2012, Bawazir et al., 2017), furthermore Gt-4 was worldwide distributed (Gower et al., 2014, Messina et al., 2014, Sadeghi et al., 2016). However, Gt-4 is a dominant HCV genotype in Saudi Arabia. GT-3 was found in 5 cases (9.6%) of the total patients and GT-1b was detected in 4 cases (7.7%). No cases of mixed genotypes were identified. The detection of these HCV genotypes were similarly distributed compared with that previously reported in Saudi Arabia over the past decades (Bawazir et al., 2017**,** Al Traif et al., 2013). The results of all HCV genotypes and subtypes across various demographic factors were summarized. The highest numbers of HCV infected individuals were detected in adults (26-65 years old), married and were Saudi nationality. In addition, the data shows a

relatively no significant difference and balanced distribution between gender, province, residency, demographic factors. Our finding indicating that fewer individuals are being exposed to infection and were consistent with other reports from the country (Al Traif et al., 2013). The liver biochemical markers showed significant elevated levels of albumin, bilirubin, alkaline phosphatase and ALT. On the other hand, the total proteins and AST were fluctuated within the normal levels. Higher bilirubin levels indicate impaired metabolism or excretion of bilirubin, which may indicate liver dysfunction. Additionally, elevated levels of ALP and ALT indicate liver damage because these enzymes are normally found in liver cells and can enter the blood stream when liver cells are injured or inflamed (Batool et al., 2023). Blood urea nitrogen levels are within the normal range, indicating that the kidneys are functioning properly in eliminating nitrogen waste products. However, elevated creatinine levels indicate impaired kidney function. Creatinine is a waste product filtered by the kidneys, and elevated levels may indicate reduced filtration and impaired kidney function (Sise et al., 2022).The CPK and LDH value is within the normal range, indicating that there is no obvious tissue damage.

CONCLUSIONS

The most prevalent HCV genotypes in the Jazan region are GT-4 and GT-1, with the highest infection rates observed in adults between 26-65 years old who are married and of Saudi nationality. Overall, the biochemical markers tested suggests liver dysfunction with elevated albumin, bilirubin, ALP and ALT levels.

RECOMMENDATION

The biochemical markers results may require further investigation to determine the underlying cause of the liver and kidney abnormalities.

REFERENCE

I. Ganta, N.M., Gedda, G., Rathnakar, B., Satyanarayana, M., Yamajala, B., Ahsan, M.J., Jadav, S.S. and Balaraju, T., 2019. A review on HCV inhibitors: Significance of non-structural polyproteins. European journal of medicinal chemistry, 164, pp.576-601.

II. Ma, S., Boerner, J.E., TiongYip, C., Weidmann, B., Ryder, N.S., Cooreman, M.P. and Lin, K., 2006. NIM811, a cyclophilin

inhibitor, exhibits potent in vitro activity against hepatitis C virus alone or in combination with alpha interferon. Antimicrobial agents and chemotherapy, 50(9), pp.2976-2982.

III. Quinkert, D., Bartenschlager, R. and Lohmann, V., 2005. Quantitative analysis of the hepatitis C virus replication complex. Journal of virology, 79(21), pp.13594-13605.

IV. Alter, M.J., 2011, November. HCV routes of transmission: what goes around comes around. In Seminars in liver disease (Vol. 31, No. 04, pp. 340-346). © Thieme Medical Publishers.

V. Factor, S.H., Desai, V., Crane, M.A., Dieterich, D.T. and Boffetta, P., 2023. Prevalence of and Risk Factors for Hepatitis C Virus Infection in World Trade Center Responders. La Medicina del Lavoro, 114(2).

VI. Papatheodoridi, A. and Papatheodoridis, G., 2023. Hepatocellular carcinoma: The virus or the liver?. Liver International, 43, pp.22-30.

VII. Landt, O., Lass, U. and Feucht, H.H., 2002. Simultaneous Identification of Five HCV Genotypes in a Single Reaction. In Rapid Cycle Real-Time PCR—Methods and Applications: Microbiology and Food Analysis (pp. 217-225). Berlin, Heidelberg: Springer Berlin Heidelberg.

VIII. Waheed, Y., Shafi, T., Safi, S.Z. and Qadri, I., 2009. Hepatitis C virus in Pakistan: a systematic review of prevalence, genotypes and risk factors. World journal of gastroenterology: WJG, 15(45), p.5647.

IX. Borgia, S.M., Hedskog, C., Parhy, B., Hyland, R.H., Stamm, L.M., Brainard, D.M., Subramanian, M.G., McHutchison, J.G., Mo, H., Svarovskaia, E. and Shafran, S.D., 2018. Identification of a novel hepatitis C virus genotype from Punjab, India: expanding classification of hepatitis C virus into 8 genotypes. The Journal of infectious diseases, 218(11), pp.1722-1729.

X. Prasad, M., Saade, G.R., Clifton, R.G., Sandoval, G.J., Hughes, B.L., Reddy, U.M., Bartholomew, A., Salazar, A., Chien, E.K., Tita, A.T. and Thorp Jr, J.M., 2023. Risk factors for perinatal transmission of hepatitis C virus. Obstetrics & Gynecology, 142(3), pp.449-456.

XI. Grossini, E., Smirne, C., Venkatesan, S., Tonello, S., D'Onghia, D., Minisini, R., Cantaluppi, V., Sainaghi, P.P., Comi, C., Tanzi, A. and Bussolati, B., 2023. Plasma Pattern of Extracellular Vesicles Isolated from Hepatitis C Virus Patients and Their Effects on Human Vascular Endothelial Cells. International Journal of Molecular Sciences, 24(12), p.10197.

XII. Wiessing, L., Sypsa, V., Abagiu, A.O., Arble, A., Berndt, N., Bosch, A., Buskin, S., Chemtob, D., Combs, B., Conyngham, C. and Feelemyer, J., 2023. Impact of COVID-19 & response measures on HIV-HCV prevention services and social determinants in

people who inject drugs in 13 sites with recent HIV outbreaks in Europe, North America and Israel. AIDS and Behavior, 27(4), pp.1140-1153.

XIII. Tang, Q., Chen, Z., Li, H., Zhang, L., Peng, M., Zeng, Y., Liu, X., Liu, Z. and Hu, P., 2023. Molecular epidemiology of hepatitis C virus genotypes in different geographical regions of Chinese mainland and a phylogenetic analysis. Infectious Diseases of Poverty, 12(1), p.66.

XIV. Rivera Saldana, C.D., Abramovitz, D., Beletsky, L., Borquez, A., Kiene, S., Marquez, L.K., Patton, T., Strathdee, S., Zúñiga, M.L., Martin, N.K. and Cepeda, J., 2023. Estimating the impact of a police education program on hepatitis C virus (HCV) transmission and disease burden among PWID in Tijuana, Mexico: A dynamic modeling analysis. Addiction.

XV. Nickbakhsh, S., Leitch, E.C.M., Smith, S., Davis, C., Hutchinson, S., Irving, W.L., McLauchlan, J. and Thomson, E.C., 2023. Geographical variation in hepatitis C-related severe liver disease and patient risk factors: a multicentre cross-sectional study. Epidemiology & Infection, 151, p.e59.

XVI. Bernal, L.A. and Soti, V., 2023. Hepatitis C Virus: Insights Into Its History, Treatment, Challenges, and Future Directions. Cureus, 15(8).

XVII. Boriskin, Y.S., Bakir, T.M., Al-Aska, A.I. and Booth, J.C., 1999. Is hepatitis C virus genotype 4 predominant in Saudi Arabia?. The New microbiologica, 22(3), pp.173-180.

XVIII. Abdel-Moneim, A.S., Bamaga, M.S., Shehab, G.M., Abu-Elsaad, A.A.S. and Farahat, F.M., 2012. HCV infection among Saudi population: high prevalence of genotype 4 and increased viral clearance rate. PLoS One, 7(1), p.e29781.

XIX. Bawazir, A., AlGusheri, F., Jradi, H., AlBalwi, M. and Abdel-Gader, A.G., 2017. Hepatitis C virus genotypes in Saudi Arabia: a future prediction and laboratory profile. Virology journal, 14, pp.1-8.

XX. Gower, E., Estes, C., Blach, S., Razavi-Shearer, K. and Razavi, H., 2014. Global epidemiology and genotype distribution of the hepatitis C virus infection. Journal of hepatology, 61(1), pp.S45-S57.

XXI. Messina, J.P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G.S., Pybus, O.G. and Barnes, E., 2014. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology, 61(1), pp.77-87.

XXII. Sadeghi, F., Salehi-Vaziri, M., Almasi-Hashiani, A., Gholami-Fesharaki, M., Pakzad, R. and Alavian, S.M., 2016. Prevalence of hepatitis C virus genotypes among patients in countries of the eastern Mediterranean regional office of WHO (EMRO): a systematic review and meta-analysis. Hepatitis monthly, 16(4).

XXIII. Al Traif I, Al Balwi MA, Abdulkarim I, Handoo FA, Alqhamdi HS, Alotaibi M, Aljumah A, Al Ashqar HI, Bzeizi K, Al Quaiz M, Alalwan A. HCV genotypes among 1013 Saudi nationals: a multicenter study. Annals of Saudi Medicine. 2013 Jan;33(1):10-2.

XXIV. Batool, A.I., Noreen, A., Naveed, N.H., Inayat, I., Rehman, M.F.U., Idrees, F., Kanwal, M.A., Jabeen, S.H. and Bibi, H., 2023. Biochemical Markers of Liver Toxicity among Coal Mine Workers of Punjab, Pakistan Suffering from HCV. Journal of Bioresource Management, 10(2), p.2.

XXV. Sise, M.E., Goldberg, D.S., Schaubel, D.E., Fontana, R.J., Kort, J.J., Alloway, R.R., Durand, C.M., Blumberg, E.A., Woodle, E.S., Sherman, K.E. and Brown Jr, R.S., 2022. One-year outcomes of the multi-center study to transplant hepatitis C-infected kidneys (MYTHIC) trial. Kidney international reports, 7(2), pp.241-250.