Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 1, 249-254

Determination of Viral load of Hepatitis C Virus and Evaluation of Some Liver Function Markers and Interlukin-27 in Hepatitis C Patients

[1]Ramy Z. Nasser, [1]Saife D. Al-Ahmer

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

Received: September 11, 2023 / Accepted: October 22, 2023/ Published: September 23, 2024

Abstract: The Hepatitis C virus (HCV) is the main contributor to chronic liver diseases and affects over 71 million people worldwide that primarily leads to significant morbidity and mortality through its predisposition to liver fibrosis, cirrhosis, and liver cancer. The present study aim to early detection of HCV and determine the viral load that can be contributed in the viral hepatitis infection, as well as estimation of some liver function markers include TSB, ALT, AST and ALP, and evaluation of IL-27 role in immune response against the hepatitis C virus. The present study was include 50 patients infected with the HCV and 50 apparently healthy individuals. The RNA was extracted from serum samples of the HCV patients and the viral load of HCV were determined using one-step qRT-PCR. The biochemical tests include TSB, ALT, AST and ALP, were done for patients and control groups. In addition, the IL-27 levels were estimated using ELISA. The detection of HCV viral load showed that 31(62%) of HCV patients were negative, while 19(38%) out of 50(100%) of HCV patients were positive for the assay. Also, the results of TSB, ALT, AST and ALP tests of showed significant differences between the patients and control groups. In addition, the results of interlukine-27 revealed that there were significant differences between the patients and control groups. The HCV viral load in acute HCV males patients were range from (126-2310000 IU/ml) while the viral load in acute HCV females patients were range from (137-2180000 IU/ml). It was concluded that the results of liver function tests showed that there was a non-significant correlation between these results and viral hepatitis infections. In addition, the estimation of IL-27 levels showed there was significant correlation between these results and viral hepatitis infections.

Keywords: Viral hepatitis, Liver function test , Interleukine-27.

Corresponding author: (Email: ramynasser1981@gmail.com).

Introduction

Hepatitis is the emergence of an inflammatory condition in the liver tissue as a result of viral infection. There are several different types of viral hepatitis as a result of the virus that causes the disease. In most cases, the source of the disease is the result of infection of three basic viruses' implication: the hepatitis A, B, or C virus, and with less frequent infections caused by Hepatitis D and E viruses (1,2). The hepatitis C virus (HCV) has a significant global impact, with 58 million people chronically infected and about 1.5 million new infections occurring per year (3). Hepatitis C virus (HCV) is transmitted by blood or body fluids. The most common modes of transmission are through sharing needles among drug users and any blood transfusion before 1992, the year that standardized testing was implemented on blood products (4,5). Chronic

infections with HCV could result in chronic inflammation of the liver tissue. This liver injury ranges from minimal necro-inflammation to cirrhosis, as well as late complications of cirrhosis such as hepatocellular carcinoma (HCC). Treatment of HCV reduces long-term complications, cirrhosis, HCC, and all-cause mortality. Most patients with acute HCV infection will develop chronic infection (about 85%), whereas the virus will be cleared spontaneously in about 15% of this population (6,7). Patients infected with HCV will have positive HCV antibodies but only the positive viral load (PCR) confirms active infection. Since these antibodies persist, patients can test positive for cases of past infection (spontaneous clearances), previously treated infection, or false-positive results. Therefore, the presence of active HCV infection, or relapse of underlying infection, can only be confirmed by positive RNA PCR. All patients suspected of HCV infection should be tested for antibodies and if the antibodies are positive, further testing for HCV RNA is warranted (8,9 and 10). The Interleukins are a type of cytokine first thought to be expressed by leukocytes alone but have later been found to be produced by many other body cells. They play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. They also have pro-inflammatory and anti-inflammatory properties. The primary function of interleukins is, therefore, to modulate growth, differentiation, and activation during inflammatory and immune responses. Interleukins consist of a large group of proteins that can elicit many reactions in cells and tissues by binding to high-affinity receptors in cell surfaces. They have both paracrine and autocrine functions. Interleukins are also used in animal studies to investigate aspects related to clinical medicine (11, 12 ,13).

The emergence of new, pandemic-level viral threats has brought to the forefront the importance of viral immunology and the continued improvement of antiviral therapies. Interleukin-27 is a pleiotropic cytokine that regulates both innate and adaptive immune responses. Accumulating evidence has revealed potent antiviral activities of IL-27 against numerous viruses, including HIV, influenza, HBV, and more. IL-27 contributes to the immune response against viruses indirectly by increasing the production of interferons (IFNs) which have various antiviral effects. Additionally, IL-27 can directly interfere with viral infection both by acting similarly to an IFN itself and by modulating the differentiation and function of various immune cells. This review discusses the IFN-dependent and IFN-independent antiviral mechanisms of IL-27 and highlights the potential of IL-27 as a therapeutic cytokine for viral infection (14, 15 and 16).

Materials and methods

Samples collection

The present study was conducted from the beginning of December 2021 to the end of February 2022 on 50 patients infected with the Hepatitis C virus (Patient group) and 50 apparently healthy individuals (Control group), the blood samples were collected from the patient's group who were attended to the Gastroenterology and Liver Hospital in the Medical City according to ethical considerations and the hospital approval.

Serological method

All the samples of hepatitis C virus patients (Patients group) and apparently healthy individuals (Control

group) were subject to the HCV rapid test for diagnosis of HCV infection (17).

Biochemical methods

The biochemical tests that include total serum bilirubin (TSB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were done for the hepatitis C virus patients and apparently healthy individuals.

Molecular methods

Detection of hepatitis C virus by one-step qRT-PCR The RNA was extracted from serum samples of the hepatitis C virus patients using QIAamp viral RNA mini kit (Qiagen/ Germany), and then the extracted RNA was subjected to detect the hepatitis C virus and determine its viral load in the hepatitis C virus patients by using one-step qRT-PCR kit (Coyote, China).

Extraction of RNA

The RNA was extracted from serum samples of the hepatitis C virus patients using the QIAamp viral RNA mini kit (Qiagen/ Germany) according to the Procedure of kit

Determination of viral load of hepatitis C virus by one-step qRT-PCR

The one-step qRT-PCR kit adopts the PCR method combined with fluorescence probe in vitro amplification technology. In this method, the HCV probe contains a fluorescent reporter dye FAM at the 5' end of the probe and a quencher dye BHQ at the 3' end of the probe. The internal reference probe contains a fluorescent reporter dye ROX. When the probe is intact, the proximity of the reporter dye to the quencher dye suppresses the reporter fluorescence. Probe cleavage during the PCR reaction spatially separates the reporter dye from the quencher dye, thereby allowing the detection of the reporter dye fluorescence. The fragments of reporter dye are displaced from the target, resulting in an increase in fluorescence. This step, enables the fluorescence signal accumulation and PCR products to form synchronously, thus achieving qualification detection of the HCV in the infective patients' serum samples, which provides auxiliary means for the HCV in the treatment of the patient. Moreover , the use of standards provided in the kit, combine with the software quantification function to provide quantitative results for samples according to the Procedure one-step qRT-PCR kit Coyote /China.

Immunological method

Estimation of interleukin-27

All the hepatitis C virus patients (Patients group) and apparently healthy individuals (Control group) of the current study were subjected to detect the interleukine-27 level by the enzyme-linked immune sorbent assay (ELISA) method using Human Interleukin-27 Elisa kit (BTLAB/ China). All the reagents, standard solutions, and samples were prepared and brought to room temperature as instructed before use. The assay was performed at room temperature.The number of strips required for the assay was determined and the strips were inserted into the frames for use.

Results and discussion

All 100 subjects (Patients and control groups) were at age ranged from ≤ 30, 31-40, 41-50 and ≥ 60 the results exhibited that more than 50% of patients are present the ≤ 30 and 31-40 age groups. The results of A total of 50 hepatitis C virus patients (Patients group) that were include 29(58%) male and 21(42%) female in addition 50 apparently healthy individuales (Control group) that were include 30(60%) male and 20(40%) female. The results of HCV detection showed that 31(62%) out

of 50(100%) of HCV patients were negative for the one-step qRT-PCR who include 18(58.06%) males and 13(41.93%) females out of 31(100%) of HCV patients (Chronic patients). Whearse 19(38%) out of 50(100%) of HCV patients were positive for the one-step qRT-PCR who include 11(57.89%) males and 8(42.10%) females out of 19(100%) of HCV patients (Acute patients) (Table 1). The biochemical tests that include total serum bilirubin (TSB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were current study showed significant differences between the patients and control groups. But were no significant between the HCV patients (Table 2). The results of interleukin-27estimation showed that the concentrations of interleukin-27 in hepatitis C virus patients that range (0.405-1.848 ng/L) were lower than the interleukin-27 concentrations in apparently healthy individuals that range (0.419-1.864 ng/L). Also, the results showed that the concentrations of interleukin-27 in acute hepatitis C virus patients that range (0.442-1.848 ng/L) were higher than the interleukin-27 concentrations in chronic hepatitis C virus patients that range (0.419-1.799 ng/L). In addition, the results of interleukin-27 that estimation for the patients and control groups revealed that there were significant differences between the Chronic hepatitis C virus patients and apparently healthy individuals, While there were no significant differences between healthy peoples and acute hepatitis C virus patients. Also the results of Pearson correlation were no significant according to viral load of HCV, IL-27 concentrations of acute and chronic infected patients (Tables 3, 4).

Table (1): The results of statiscal analysis of hepatitis C virus detection and viral load determination in the acute hepatitis C virus patients.

Variables		No.	Percentage	P value
Gender	Male	11	57.9%	0.491 NS
	Female	8	42.1%	
Age groups	≤ 30	2	10.5%	0.188 NS
	31-40	8	42.1%	
	41-50	3	15.8%	
	≥ 50	6	31.6%	
Total		19	100%	

Table (2): The statistical analysis of Liver function tests concentration of hepatitis C virus patients and healthy individuals.

Variables		GOT	GPT	TSB	ALK
		mean ± SD	mean ± SD	mean ± SD	mean ± SD
Status	Patient	35.16±16.87	36.51±23.41	0.99±0.75	124.37±53.54
	Control	25.19±5.5	28.56±7.3	0.64±0.26	76.58±16.13
	Sig	0.000**	0.024**	0.002**	0.000**
Gender	Male	29.39±13.34	32.64±18.37	0.84±0.57	99.84±45.67
	Female	31.3±13.7	32.39±16.95	0.77±0.6	101.4±47.23
	Sig	0.489 NS	0.945 NS	0.568 NS	0.868 NS
Type of infection	Acute	38.95±13.61	36.42±18.63	1.15±0.8	145.31±45.93
	Chronic	32.83±18.41	36.58±26.21	0.89±0.71	111.54±54.48
	Sig	0.217 NS	0.982 NS	0.244 NS	0.023 *

Table (3): The results of statiscal analysis of interlukine-27 estimation for the patients and control groups.

No.	Status	No.	Mean ± SD	P value
1	**Patient (A+C)**	50	0.777±0.312	0.007**
	Control	50	0.974±0.394	
2	**Patient (C)**	31	0.724±0.295	0.003 **
	Control	50	0.974±0.394	
3	**Patient (A)**	19	0.861 ± 0.338	0.245 NS
	Control	50	0.974±0.394	

Table (4): The results of Pearson correlation according to viral load of HCV, IL-27 concentrations of acute and chronic infected patients.

Variables		PCR HCV	IL-27
PCR HCV	Pearson Correlation		-0.077
	Sig. (2-tailed)		0.593 **Non Siginificant**

Conclusion

It was concluded that the results of liver function tests showed that there was a non-significant correlation between these results and viral hepatitis infections. In addition, the estimation of IL-27 levels showed there was significant correlation between these results and viral hepatitis infections

References

1. Pambuk, C.; Muhammad, F. and Ali, M. (2019). Viral hepatitis: Implication of viral types from A to E. American Journal of Biomedical Science and Research, 4(6): 442-444.
2. Heba F.; Ahmed A.; Abbas M. and Sabeeh A. (2016). Detection of hepatitis C virus in iraqi patients with oral lichen planus. Iraqi JMS. 14(4): 312-319.
3. World Health Organization (WHO), (2022). Global health sector strategy on viral hepatitis 2016-2021. Towards Ending Viral Hepatitis.
4. Guidelines for the screening (GS), (2021). Care, and treatment of persons with hepatitis C infection. Geneva, Switzerland: World Health Organization. Available from: https://apps.who.int/
5. Mohammed, D. (2014). Comparative study between serological and molecular diagnosis test for HBV and HCV in chronic renal failure patients on hemodialysis in Nineveh Government/Iraq. Iraqi Journal of Biotechnology, 13(2): 186-192.
6. Carrat, F.; Fontaine, H.; Dorival, C.; Simony, M.; Diallo, A.; Hezode, C., *et al*., (2019). Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet, 393(10179): 1453-1464.
7. Kanwal, F.; Kramer, J. R.; Asch, S. M.; Cao, Y.; Li, L. and El-Serag, H. B. (2020). Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. Hepatology. 71(1): 44-55.
8. Ghany, M.G.; Morgan, T.R. and AASLD-IDSA Hepatitis C Guidance Panel (2020). Hepatitis C Guidance Panel. hepatitis C guidance update: American association for the Study of liver diseases–infectious diseases society of America recommendations for testing, managing, and treating hepatitis C virus infection. Hepatology. 71(2): 686-721.
9. European Association for the Study of the Liver (EASL), (2020). Recommendations on treatment of hepatitis C final update of the series. Journal of Hepatology, 73(5): 1170-1218.
10. Lissoni, P.; Messina, G.; Pelizzoni, F.; Rovelli, F.; Brivio, F.; Monzon, A., *et al*. (2020). The fascination of cytokine immunological science. Journal of Infectiology and Epidemiology, 3(1): 18-28.
11. Raghad, A.; Ghanima M. and Nawal A. (2009). The Necroinflammatory activity of HCV in liver biopsies of Iraqi patients that detected by In Situ hybridization technique. Um-Salama Science Journal, 6(1): 106-113.
12. Mohammad, D. and Bassima, A. (2012). Hepatitis C virus genotypes in Iraq. Iraqi Journal of Biotechnology, 11 (2): 475-480.
13. Povroznik, J. and Robinson, C. (2020). IL-27 regulation of innate immunity and control of microbial growth. Future Sci., 6(7): 588.

14. Jessica, M. and Cory, M. (2020). IL-27 regulation of innate immunity and control of microbial growth. Future Science, 6(7): 588.
15. Zeuzem, S. (2008). Interferon-based therapy for chronic hepatitis C: current and future perspectives. Nature Clinical Practice Gastroenterology and Hepatology, 5(11): 610-622.
16. Bataller, R. and Brenner, D. A. (2005). Liver fibrosis. The Journal of clinical investigation, 115(2): 209–218.
17. Vetter, B. N.; Ongarello, S.; Tyshkovskiy, A.; Alkhazashvili, M.; Chitadze, N.; Choun, K., *et al*. (2020). Sensitivity and specificity of rapid hepatitis C antibody assays in freshly collected whole blood, plasma and serum samples: A multicentre prospective study. PloS one, 15(12): e0243040.