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Original Article

Molecular Detection of Hepatitis C virus (HCV) Genotypes and Viral Loads in Chronic HCV Infected Patients in Kirkuk, Iraq

Sarmad Falah Hasan, Zeyad Khalaf Maded, Muhannad Abdulla Alazzawy[1]

Gynecological and Pediatric Hospital, Kirkuk Health Directorate, Iraq, [1]College of Medical Technology, AL-Kitab University, Kirkuk, Iraq

Abstract

Background: Hepatitis C is a disease that has a significant global impact. The World Health Organization estimates that over 150 million people are chronically infected with the hepatitis C virus (HCV). **Objectives:** The purpose of the study was to make a quantitative determination of the hepatitis C viral load and genotype using real-time polymerase chain reaction (RT-PCR) and to determine how these factors relate to the HCV chronicity. **Materials and Methods:** A cross-sectional hospital-based study was carried out in Kirkuk city, Iraq. A total of 50 patients with chronic hepatitis C whose ages ranged from 20 to 75 years were the subjects of the study. The control group consisted of 30 apparently healthy persons admitted to the blood bank for blood donation. Five milliliters of blood were drawn for the molecular detection of HCV load and genotype by RT-PCR, detection of hepatitis C antibodies by enzyme-linked immunosorbent assay (ELISA), and biochemical analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total serum bilirubin (TSB). **Results:** According to the research, 86% of chronic hepatitis C patients with ELISA-detected anti-HCV obtained positive PCR results. Statistically significant ($P = 0.01$). According to the study, more chronic hepatitis C patients (31 out of 43) had genotype 4 HCV than genotype 1a (27.91%). According to the study, genotype 4 chronic hepatitis C patients exhibited significantly higher viral loads than genotype 1a patients (1901.3 vs. 1693.41 IU/mL; $P = 0.01$). About 41.94% of men and 58.06% of females with chronic hepatitis C had genotype 4, whereas 33.3% of males and 66.67% of females had genotype 1a. Chronic hepatitis C patients had higher ALT, AST, ALP, and TSB levels than the control group, and the difference was very significant. ALT values were 64.69, 54.8, 285.5, and 4.33 mg/dL. This study found a connection between chronic hepatitis C viral load and ALT, AST, ALP, and TSB levels. Viral load rose as fibrosis progressed, according to this study. Patients without fibrosis had the lowest mean viral load (1235.5 IU/mL), followed by those with stage 1 fibrosis, whereas cirrhosis (stage 4) had the highest (2088.1 IU/mL). Non-fibrotic patients had the lowest virus burden. **Conclusions:** Genotype 4 of HCV was the most predominant genotype in Kirkuk city and there was a strong positive correlations of ALT and viral load with stages of fibrosis in patients with chronic hepatitis C.

Keywords: HCV, Kirkuk, PCR, viral load

Introduction

A global, epidemic, hepatitis C virus (HCV) affects millions of individuals annually. Over 150 million people have a chronic hepatitis C infection, according the World Health Organization (WHO). Overall, the available data suggest that the prevalence of HCV infection is approximately 2.2%–3.0% worldwide. While individual estimates from different regions or countries have undergone some changes since the first estimates were made by the WHO in 1997, the overall picture is still similar, with the highest prevalence of HCV infection found in the African and Eastern Mediterranean regions.[1] Africa and the Western Pacific have a significantly greater incidence rate than the Americas and Europe.[2] Between 2 and 5 million persons in Europe are thought to have HCV infection. By the way of illustration, those who rely on injectable medications are more vulnerable.

Hepatitis C, the chronic version of the virus, is the most frequent cause of liver disease in both Europe and the

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Address for correspondence: Dr. Muhannad Abdulla Alazzawy, PhD, College of Medical Technology, AL-Kitab University, Kirkuk, Iraq. E-mail: drmuhannadalazzawy@gmail.com

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United States. Hepatitis C virus (HCV) infection is a leading cause of liver transplantation in these areas. It is difficult to estimate the total number of new HCV infections, because most acute infections are not clinically noticeable. Recent data from Europe show that there is still a widespread outbreak of acute HCV, especially among intravenous drug users and men who have sex with men.[3] The possibility of developing a long-term HCV infection is high. 75%–100% of persons remain HCV RNA positive after being treated for acute hepatitis C.[4]

During future monitoring, most of these patients will be found to have persistently elevated liver enzymes. Following the 6 months of viral persistence after an assumed HCV infection, the condition is considered chronic. There is little hope that a persistent infection will resolve on its own once it has taken hold. Most persons with a prolonged infection will experience either no symptoms at all or very mild, non-specific signs if liver cirrhosis is not present. Nucleotide sequence comparisons of HCV in different populations have uncovered at least seven distinct HCV genotypes and an even a larger number of subtypes within each of these.[5] Major HCV genotype 1, 2, 4, and 5 strains have been found in Sub-Saharan Africa, whereas genotypes 3 and 6 are more widely distributed in Southeast Asia. One can conclude that the many different HCV genotypes have their roots in these geographic areas.[6] The purpose of the study was to make a quantitative determination of the hepatitis C viral load and genotype using RT-PCR and to determine how these factors relate to the HCV chronicity.

Materials and Methods

During the period beginning on September 10, 2020 and ending on January 10, 2021, the city of Kirkuk played host to a hospital-based cross-sectional research study. The range of ages of the patients with hepatitis who participated in the study was from 20 to 75 years old, and they all had chronic hepatitis C infection. Patients like this were taken to the hepatology and gastroenterology facilities in Kirkuk. These patients participated in an interview that was conducted using a questionnaire form created by the investigator. The fibrosis stage of patients with hepatitis who were enrolled in the study was defined by referring to the patients' datasheets and also by asking the patients' specialized physicians about the patients' conditions. The control group consisted of 30 people who were admitted to the blood bank for the purpose of donating blood. These individuals appeared to be free of any infections and were matched up with the patients. Each participant in the study had a vein puncture performed using a syringe capable of holding 5 mL so that 5 mL of blood could be extracted. Two tubes, one of which contained the anticoagulant ethylenediaminetetraacetic acid, were used to hold the blood samples that were taken. The second portion of the sample was 3 mL, which were placed in plane tubes, allowed to sit for 30 min at 37°C for clotting, and then centrifuged at 3000 revolutions per minute for 15 min. The obtained plasma from the first tube was aspirated using automatic micropipette and transferred to Eppendorf tubes and stored in deep freeze for molecular test of HCV viral load and genotyping.

Ethical approval

Approval permission was presented to the director of Kirkuk Health Directorate according to the document number 21247 (including the number and the date in 8/9/2020). An interview was conducted with these patients using a questionnaire form created by the investigator, which included demographic information such as age, sex and so on.

Results

In this investigation, 50 patients were diagnosed with chronic HCV infection using an HCV enzyme-linked immunosorbent assay (ELISA). According to the study, 86% (43 of 50) of patients with chronic hepatitis C who had anti-HCV as determined by ELISA had a positive PCR result. The outcome was significant (P = 0.01), as shown in Table 1.

When compared to the patients with genotype 1a, 72.09% (31 of 43) of patients with chronic hepatitis C were infected with HCV genotype 4 (27.91%) [Figure 1].

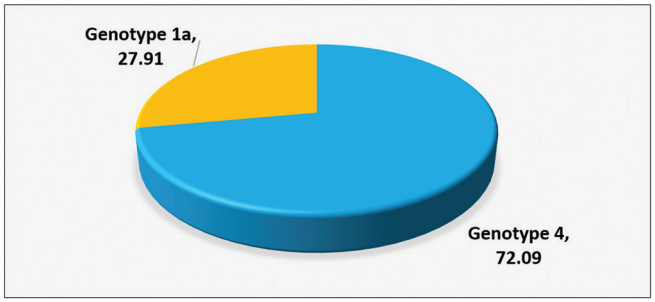

Figure 1: Frequency of patients with chronic hepatitis C concerning genotypes

Table 1: Comparative analysis of ELISA and PCR for evaluating HCV patients

Anti-HCV ELISA positive	Real-time PCR assay				Total samples	
	Positive		Negative			
	No.	%	No.	%	No.	%
Chronic HCV (n= 50)	43	86	7	14	50	100

P < 0.01

The study found a highly significant difference in viral loads between individuals with chronic hepatitis C genotype 4 and those with genotype 1a (1901.3105.6 vs. 1693.4108.4 IU/mL), with a significant difference ($P = 0.01$) [Table 2].

In the current study, 41.94% of males with chronic hepatitis C had genotype 4 and 58.06% of females with chronic hepatitis C had genotype 4; 33.33% of males and 66.67% of females had genotype 1a, according to Table 3.

The current study found that patients with chronic hepatitis C had the highest mean of ALT, AST, ALP, and TSB when compared to the control group, with a significantly significant relationship between the groups [Table 4].

The current investigation found a favorable relationship between ALT level and viral load in patients with chronic hepatitis C ($r = 0.63$, $P = 0.01$) [Figure 2].

The current investigation discovered a favorable relationship between AST level and viral load in patients with chronic hepatitis C ($r = 0.59$, $P < 0.01$) [Figure 3].

The current investigation found a favorable relationship between ALP level and viral load in patients with chronic hepatitis C ($r = 0.58$, $P = 0.05$) [Figure 4].

The current investigation found no link between TSB levels and viral load in chronic hepatitis C patients ($r = 0.22$, $P > 0.05$) [Figure 5].

Table 2: Relation of viral load with genotype in patients with chronic HCV

HCV viral load (IU/mL)	Genotype 4 ($n = 31$)	Genotype 1a ($n = 12$)
Mean ± SD	1901.3 ± 105.6	1693.4 ± 108.4

$P < 0.01$

Table 3: Distribution of HCV genotypes in patients with chronic hepatitis C according to sex

Chronic HCV	HCV genotype			
	Genotype 4		Genotype 1a	
	No.	%	No.	%
Males	13	41.94	4	33.33
Females	18	58.06	8	66.67
Total	31	100	12	100

$P > 0.05$

Table 4: Levels of liver function tests in chronic HCV patients versus the control group

Parameters	Patients ($n = 43$)	Control ($n = 35$)	*P* value
ALT	75.3	9.61	0.004
AST	63.2	10.63	0.005
ALP	266.1	57.6	0.033
TSB	4.28	0.36	0.033

According to the findings of this study, the level of viral load increased as fibrosis progressed. Patients with the first stage of fibrosis had the lowest mean viral load (1235.5 IU/mL), followed by the patients with the second stage, and the patients with cirrhosis (stage 4) had the highest mean viral load (2088.1 IU/mL). Patients without fibrosis had the lowest mean viral load [Table 5].

Discussion

In its guidelines, the European Association for the Study of the Liver recommended that individuals who meet the criteria for HCV testing should first be tested for HCV antibodies, and if the findings are positive, they should then be tested for HCV RNA.[7] Indications for testing with an HCV nucleic acid test include a positive HCV serology (to confirm current active HCV infection), patients who are immunocompromised (including those on chronic hemodialysis), even if an HCV serology test comes back negative, and individuals who have been exposed to HCV within the past 6 months but may not have developed detectable HCV antibodies. Additionally, testing for HCV RNA should be performed on all HCV patients undergoing antiviral therapy, as well as at regular intervals both during and after treatment.[8] Studies indicated an increase in the viral load of patients' sera; nevertheless, it was noticed that only (25%) of patients had a high viral load; this study demonstrated that 71.9% of participants had a high viremia.[9] Regarding the PCR-positive results, Abbas *et al*.[10] discovered that 56.66% of seropositive individuals yielded positive HCV-RNA results. The discrepancy between the antibody positive and HCV-RNA negative cases may be due to the fact that HCV may be present in peripheral blood mononuclear cells in those cases and not in serum or plasma, as has been reported by Caudai *et al*.,[11] who detected HCV-RNA in human peripheral blood mononuclear cells in 10.5% out of 38 plasma viremia negative patients, and also due to the fact that spontaneous viral clearance was occur in 20% of individuals who were exposed to the virus. A sustained virological response rate of roughly 60% has been generated by the combination of an interferon and ribavirin, which is utilized in the conventional treatment of viral infections.[12] According to the results of the research conducted, a greater number of patients with chronic hepatitis C (31 out of 43) were infected by the genotype 4 strain of HCV as compared to the patients with genotype 1a (27.91%). This study concurred with Osoba[13] in the Kingdom of Saudi Arabia (KSA) to determine the prevalence of HCV genotype in a number of different population groups in the KSA. The frequency of the HCV genotype 4 was found to be the highest across all of the different patient groups that were investigated for this study. The frequency of the genotype 4 ranged from 40% to 74% of the population. In example, one can gain a better understanding of the varied patterns of serological

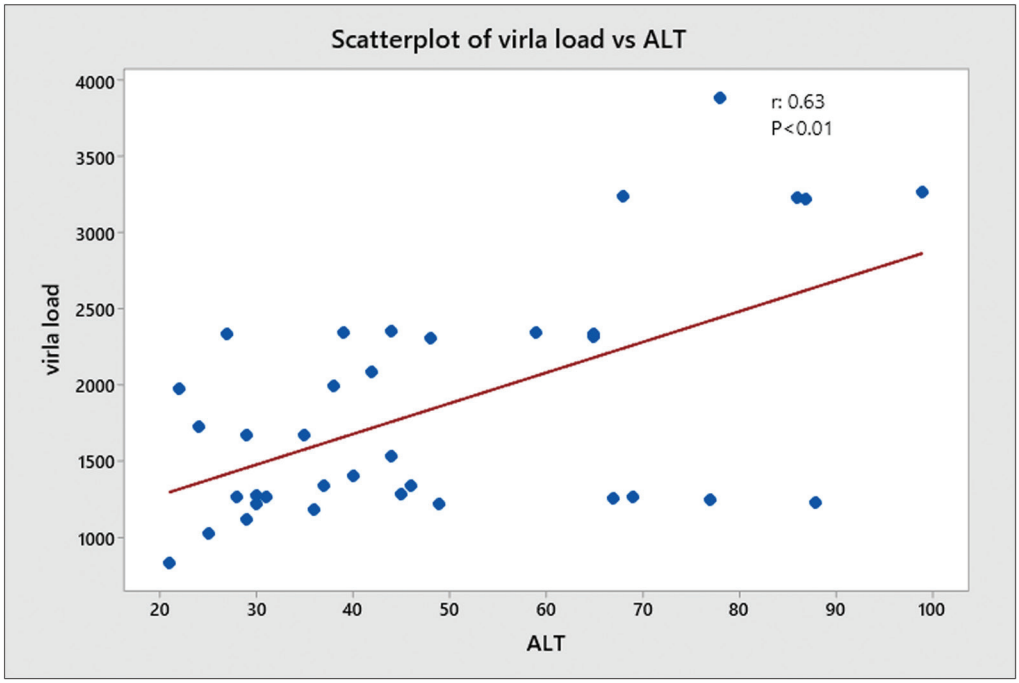

Figure 2: Correlation between viral load and ALT in chronic HCV patients

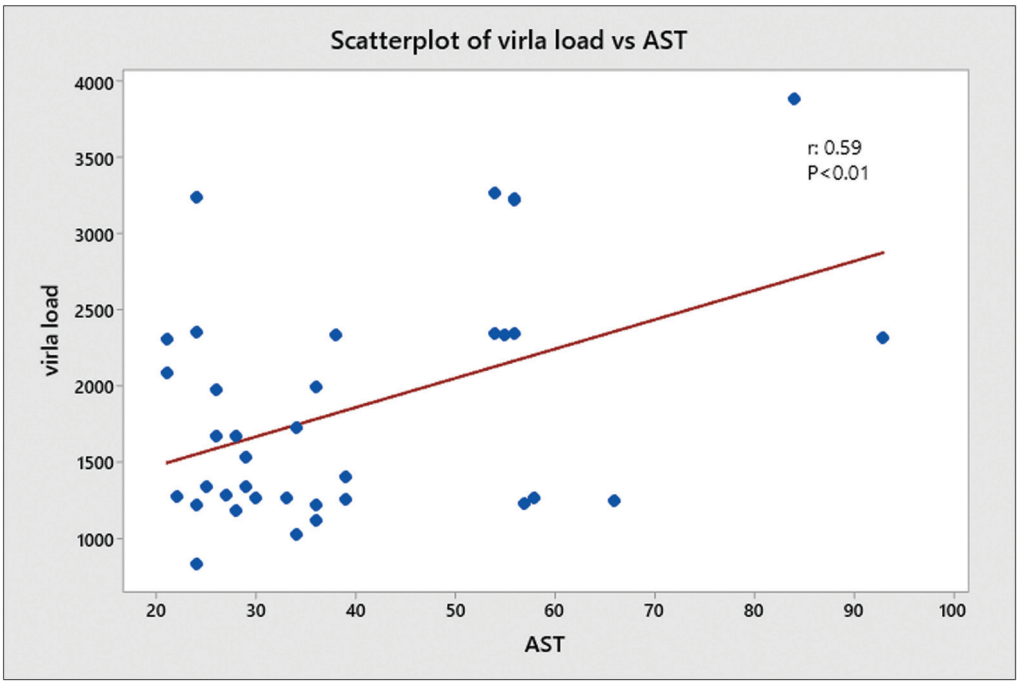

Figure 3: Depicting the correlation between viral load and AST level in chronic HCV patients

reactivity as well as how the body reacts to treatment by looking at the diversity of sequences.[14] There is much evidence to support the theory that HCV is capable of demonstrating a variety of pathogenic potentials. This theory is supported by the fact that different types and subtypes of HCV have been shown to have different responses to the treatment interferon.[15] On the other hand, some sources claim that the genotypes 1a and 1b are more prevalent in Middle Eastern countries that are not Arab.[11] According to the findings of Bozday *et al*.[16] study, the HCV genotypes 1b and 3a that are identified in Turkey are the ones that are most common in the countries that are nearby. A study that was carried out in Lebanon by Abou Rached *et al*.[17] came to the conclusion that the HCV genotypes found in Syrian patients who were suffering from HCV were evenly split between HCV

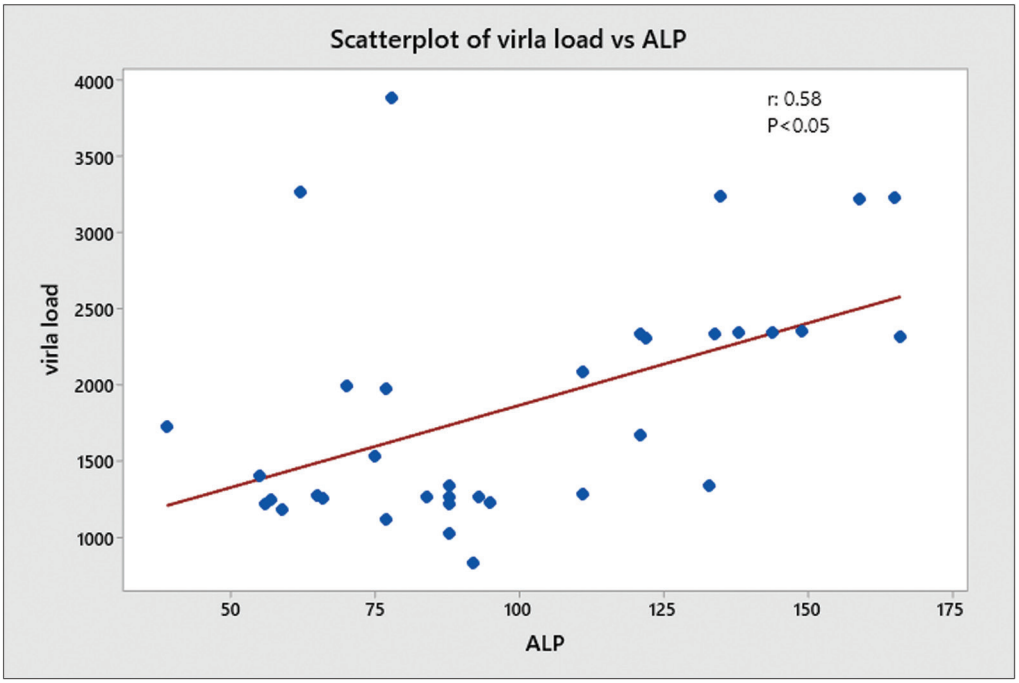

Figure 4: Depicting the correlation between viral load and ALP level in chronic HCV patients

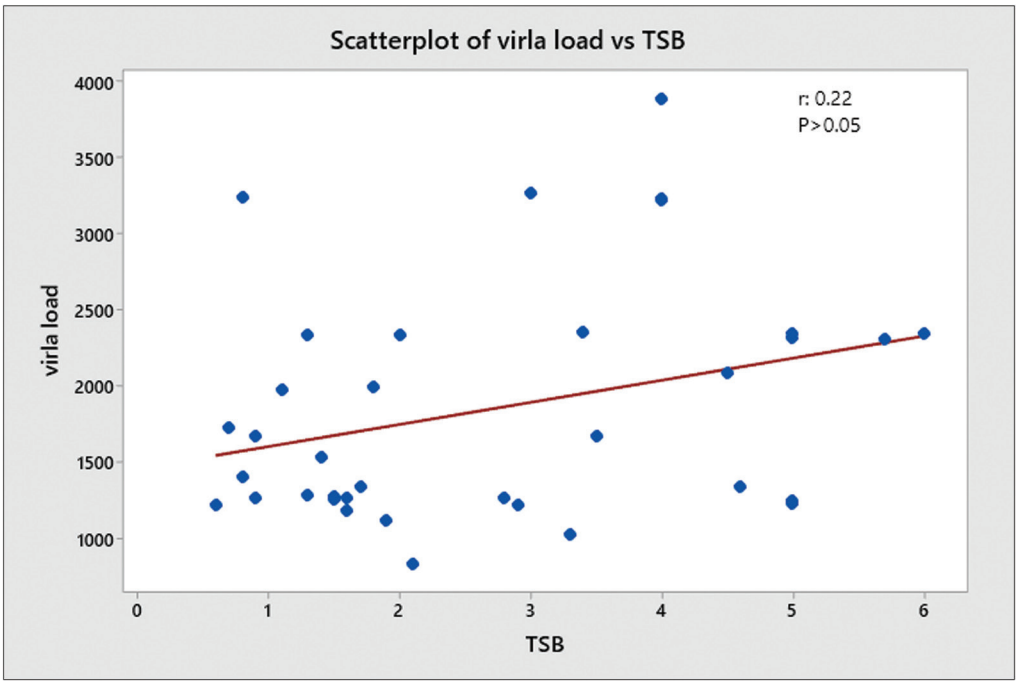

Figure 5: Depicting the correlation between viral load and TSB level in chronic HCV patients

genotype 1 and HCV genotype 4. None of the samples that were examined contained the genotypes 2, 5, or 6. None of the samples contained these genotypes. There is no evidence that can be considered as certain that these genotypes do not exist in Iraq. This is because there is no proof that these genotypes exist in Iraq. According to the findings of Ali *et al.*,[18] serum ALT levels were found to be elevated in individuals with acute hepatitis C at much higher rates than in healthy control patients. Mehta *et al.*[19] found that HCV patients had significantly higher levels of transaminases in their blood. Numerous studies have demonstrated that there is a substantial correlation between high levels of the virus in the blood and cirrhosis of the liver brought on by the hepatitis C virus type. This connection has been shown to be significant in both humans and animals. Because of the link that existed

Table 5: Relationship between viral load and fibrosis phases in chronic HCV patients

Viral load (IU/mL)	Stage of fibrosis*					*P* value
	0	1	2	3	4	
No.	18	8	7	6	4	0.0001
Mean	1235.5	1442.5	1677.8	1871.4	2088.1	
SD	120.9	116.4	206.9	210.9	122.6	

*highly significant

between the two different conditions, we were able to draw these results. Until it reaches its most advanced stage, which is cirrhosis, which may eventually lead to what is known as liver cancer, the degree of cirrhosis will increase in direct proportion to the percentage of the virus that is present in the blood until it reaches its most advanced stage, which is cirrhosis. Cirrhosis may eventually lead to what is known as liver cancer.[20,21]

Conclusions

The highest rate of patients with chronic hepatitis C had HCV Ab as detected by ELISA were positive by RT-PCR. Genotype 4 of HCV was the most predominant genotype in Kirkuk city. Strong positive correlations between viral load and stages of fibrosis in patients with chronic hepatitis C were observed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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