EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

G

ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 15 No. 2 (2023)

Citation: *Egypt. Acad. J. Biolog. Sci. (G.Microbiolog) Vol.15 (2) pp.179- 196 (2023)*
DOI: 10.21608/EAJBSG.2023.393408

Egypt. Acad. J. Biolog. Sci., 15(2):179-196 (2023)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Serological Evidence and Clinical Outcome of Type 1 Herpes Simplex Virus (HSV1) Infection in Chronic Hepatitis C Patients

Ahmed Khedr[1*] and Mohamed Mokhles[2]

[1]Department of Microbial Biotechnology, Biotechnology Research Institute, National Research Centre, Cairo, Egypt.

[2]Department of Internal Medicine, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt.

***E.Mail:** ak.khedr@nrc.sci.eg: dr.a_khedr@yahoo.com

ARTICLE INFO

Article History
Received: 20/10/2023
Accepted:22/12//2023
Available:26/12/2023

***Keywords*:**
Hepatitis C virus;
Type 1 herpes simplex virus;
Co-infection;
APRI biomarker;
Liver fibrosis;
Cirrhosis.

ABSTRACT

BACKGROUNG: Type 1 herpes simplex virus (HSV1) is a ubiquitous pathogen and is known to induce liver dysfunction. Seroprevalence and clinical implications of HSV-1 among chronic hepatitis C virus (HCV)-infected patients are poorly defined **OBJECTIVE:** The study goal was to evaluate HSV1 seroprevalence in chronic HCV patients and healthy controls as well as to demonstrate the clinical outcomes of HCV/HSV-1 co-infection, focusing on the relationship between HSV1 antibodies and liver fibrosis and cirrhosis progression. **METHODS:** The current study involved 120 participants (80 chronic HCV patients and 40 controls). All participants underwent the measurements of HCV RNA and baseline clinical parameters. Serum samples were investigated for HSV1 IgG and HSV1 IgM antibodies by ELISA**.** The APRI score was calculated to evaluate liver fibrosis and cirrhosis. **RESULTS:** A significant increase in the incidence of seropositivity of HSV1 IgG antibodies (*P* 0.004) was detected in HCV patients (80/80,100%), compared to controls (36/40, 90%), whereas the tendency of the increase in seropositivity of HSV1 IgM antibodies was detected in HCV patients (HCV/HSV1 co-infection, 20/80, 25%), compared to controls (4/40,10%). In HCV/HSV1 co-infection, a significant increase in GGT and ALP as well as a tendency of increase in ALT was reported beside a significant decrease in PLC compared to HCV mono-infected patients. At APRI score cutoff ≥ 1.5, HCV patients were identified with late fibrosis (≥ F2, *n* = 40). However, at the APRI score cutoff > 2, HCV patients were identified with cirrhosis (F4, *n* = 24). At high APRI score cutoff values, a non-significant change in seropositivity of HSV-1 IgM antibodies between HCV patients with early fibrosis and those with late fibrosis was observed (*P* > 0.05). At high and low APRI score cutoff values, non-significant changes in seropositivity of HSV1 IgM antibodies among the groups of HCV patients in relation to liver fibrosis and cirrhosis were observed (*P* > 0.05). However, at a low APRI score cutoff value of 1, a significant increased incidence of higher HSV1 IgG antibody titre (> mean value) was observed to be associated with the lower probability of ruling out cirrhosis among chronic HCV patients (Odd's ratio 0.20, 95% C.I. 0.0606 to 0.6604, and *P* 0.008). **CONCLUSION:** Our results shed light on the establishment of HSV-1 reactivation among chronic HCV patients. Increased incidence of HSV-1 IgG antibodies is observed in chronic HCV infection. In the studied cohort of HCV patients, the seroprevalence of HSV1 IgM antibodies is not associated with liver fibrosis and cirrhosis progression. However, the increased incidence of higher HSV1 IgG titre is associated with a lower probability of ruling out cirrhosis.

INTRODUCTION

Type 1 herpes simplex virus (HSV1) exhibits a high worldwide prevalence of about 67%. It is suggested that around 3.7 billion people were seropositive for HSV1 globally (James *et al*., 2020; Zhu and Viejo-Borbolla, 2021). The virus contains a large, linear double-stranded genomic DNA that is protected by an icosahedral capsid, which in turn is surrounded by a tegument as a proteinaceous layer and covered with an envelope that contains the viral glycoproteins (Zhu and Viejo-Borbolla, 2021; Heldwein *et al*., 2008). HSV1 is a highly infectious neurotropic virus (Xu *et al*., 2007; Taha *et al*., 2023), which is traditionally considered a vesicular lesion pathogen (Shen *et al*., 2015; El-Ansary, 2022). HSV1 is transmitted by intimate contact and can cause lifelong infection. The virus is often acquired early in childhood via the orolabial mucosa. Transmission of HSV1 infection via the skin or even mucosa causes inflammation and tissue destruction, resulting in the distinctive herpes blisters (Zhu and Viejo-Borbolla, 2021). Geographical and socioeconomic differences can influence the prevalence of HSV1 in various areas and among various populations (Nasrallah *et al*., 2018; Al-Shuwaikh *et al*., 2019; Taha *et al*., 2023).

During primary infection, HSV1 infects and attacks epithelial cells in the skin or mucosa, then reaches the nerve endings of peripheral neurons, reaching the neuronal cell body. The infection by HSV1 can result in lytic or latent viral replication. During lytic replication or viral reactivation, viral genes are expressed in an organized manner, resulting in the production of infectious viral particles, whereas during latency there is a restriction on viral gene expression without virus particle formation. However, the genome of the virus is capable of reactivation when an appropriate stimulus is present, resulting in the formation of infectious viral particles (Zhu and Viejo-Borbolla, 2021). When HSV1 infects susceptible non-neuronal cells, lytic replication is activated; however, a recent investigation revealed that latency exists in a fraction of non-neuronal cells in vitro. (Cohen *et al*., 2020; Zhu and Viejo-Borbolla, 2021). Infection with HSV1 normally induces immunity to prevent re-infections with the same serotype (Zhu and Viejo-Borbolla, 2021). Following HSV1 infection, immunoglobulin G (IgG) antibodies develop over a varied period of time (14 to 90 days) following the beginning of symptoms, and then HSV1 IgG antibodies remain forever (LeGoff *et al*., 2014; Taha *et al*., 2023). Furthermore, following viral exposure, most people can have detectable levels of HSV1 IgM antibodies within 9 to 10 days. Despite HSV1 IgM antibodies being normally visible for 7 to 14 days, a few cases might last up to 6 weeks (Ashley *et al*., 2002; Taha *et al*., 2023).

Indeed, HSV1 infection can be mild, self-resolving in most immunocompetent individuals, but can also lead to high morbidity and mortality in some cases (Zhang, 2020; Zhu and Viejo-Borbolla, 2021). Symptomatic infection is usually described by oral, ocular, or cutaneous lesions at viral entry site (Taha *et al*., 2023). Moreover, this viral infection can cause severe diseases, including encephalitis and meningitis (Fatahzadeh and Schwartz, 2007; El-Ansary, 2022). The interaction between HSV1 and the host, in particular with the host immune system functions, can determine the outcome of this viral infection (Zhu and Viejo-Borbolla, 2021). Both the innate and adaptive immune responses are fundamental to controlling HSV1 infection and reducing pathogenesis. On the other hand, HSV1 is very well equipped with virulence factors that modulate and evade the immune response (Schulz and Mossman, 2016; Christensen and Paludan, 2017; Zhu and Viejo-Borbolla, 2021). Individuals with deficient T cell immunity are more prone to recurrent meningitis, pneumonitis, and hepatitis (Hull *et al*., 1984; Zhu and Viejo-Borbolla, 2021). HSV1 infection can disseminate to various organs (Glas *et al.*, 2012), including the liver. Even those patients with normal immune function can get HSV1 hepatitis (Wind *et al*., 2012;

Shionoya *et al*, 2023). It is generally accepted that HSV1 infections induce liver dysfunction (Shionoya *et al*, 2023). Previous reports revealed that hepatitis is a rare complication in HSV1 infection and often leads to acute liver failure, liver transplantation, and death (Norvell *et al*., 2007). The risk of HSV-1 hepatitis is heightened by pregnancy, steroid use, renal failure, and co-infection with viruses like human immunodeficiency virus (HIV) (Kusne *et al*., 1991; Kaufman *et al.*, 1997; Azak *et al.*, 2011; Shionoya *et al*, 2023). If recognized early, HSV1 hepatitis is a potentially treatable disorder. Treatment with parenteral acyclovir may reverse the disease process if instituted early (Velasco *et al.*, 1999; Wind *et al*., 2012). According to World Health Organization (WHO) data, 71 million individuals were considered as carriers for HCV chronic infection worldwide (WHO, 2017). Despite the emergence of DAA into practical healthcare in 2011, which contributes to treating more than 90% of HCV patients, the yearly death rate from viral hepatitis is still high (Cooke *et al*., 2019; Yurlov *et al*., 2021). Several HCV patients remain susceptible to the emergence of liver disease progression to cirrhosis and hepatocellular carcinoma (HCC) at different rates (Ravi *et al*., 2017; Roche *et al*., 2018; El Kassas *et al*., 2018; Salum *et al*., 2021). Aspartate aminotransferase to platelet ratio index (APRI) is considered a serum biomarker that is used to diagnose liver fibrosis and cirrhosis (Eslam *et al*., 2016; Catanzaro *et al*., 2021; Dawood *et al*., 2021). According to the METAVIR scoring system, liver fibrosis included four successive stages; the first refers to portal fibrosis without septa or mild fibrosis (F1), the second refers to portal fibrosis with rare septa or moderate fibrosis (F2), the third refers to the presence of numerous septa without cirrhosis or severe fibrosis (F3), and the fourth refers to cirrhosis (F4) (Eslam *et al*., 2016; Dawood *et al*., 2021). It is confirmed that the APRI score is able to predict and diagnose severe fibrosis as well as cirrhosis in chronic HCV infection (Catanzaro *et al*., 2021).

Indeed, few reports have revealed the relationship between the infection of HCV and herpes viruses. Immune changes that follow clearance of HCV have been found to be responsible for the reactivation of herpesviruses (Perelló *et al*., 2016), especially herpes zoster reactivation, which has been observed in patients with HCV infection treated with direct-acting antiviral (DAA) agents (Ghweil and Helal, 2019). Other reports have revealed that HSV2 increases the susceptibility to HCV infection by sexual transmission (Shev *et al*., 1995), with the well-established epidemiological link between HSV2 and HCV infection (Burton *et al*., 2012). On the other hand, the data regarding the incidence and effect of HSV1 in chronic HCV infection are lacking. So, the aim of the present study was to evaluate HSV1 seroprevalence in chronic HCV patients and healthy controls as well as to demonstrate the clinical outcomes of HCV/HSV1 co-infection, focusing on the relationship between HSV1 antibodies and liver fibrosis and cirrhosis progression.

MATERIALS AND METHODS

Study Population: A total of 120 participants were included in this current study; all of them were Egyptian. Participants were divided into two groups: 40 healthy controls and 80 patients with chronic HCV infection who were diagnosed at the Medical Center of Excellence, National Research Centre. For each participant, written informed consent was obtained. The study was conducted in accordance with the World Medical Association's Declaration of Helsinki guidelines published in 1964 and its later revisions. Inclusion criteria: The group of patients included both sexes of adult ages who were proven to have HCV infection (positive HCV antibodies and HCV RNA), whereas the group of control individuals included both sexes of adult healthy subjects who were free from HCV infection (negative HCV antibodies and HCV RNA), and any other infectious or non-infectious disease. Exclusion criteria: Immunocompromised patients and subjects with the positivity for the hepatitis B surface antigen (HBsAg)

marker or with the positivity for the human immunodeficiency virus (HIV) antibodies were excluded from the study. Pediatric subjects were completely excluded from the study population.

Clinical biochemical measures of liver function, including levels of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST),gamma-glutamyl transferase (GGT), and alkaline phosphate (ALP) were used to assess the studied cohort (chronic HCV patients and healthy controls). Additionally, clinical hematological indicators were taken into account, such as prothrombin concentration (PC) and platelet count (PLC). All subjects were assessed for the presence of HSV1 IgG and HSV1 IgM antibodies serologically by ELISA. The APRI score was calculated to determine liver fibrosis and cirrhosis.

Determination of HCV RNA: The detection of HCV RNA was carried out by real-time PCR. Briefly, the extraction of HCV-RNA from sera samples was assessed using the QIAamp Viral RNA kit (Qiagen, Santa Clarita, CA) according to standard manufacturer's instructions, and then the detection of HCV was confirmed by one-step, real-time RT-PCR by using the Artus HCV QS RGQ Kit (Qiagen, Santa Clarita, CA) following the manufacturer's protocols. The amplification thermal profile was as follows: A first step included an initial incubation for 30 min at 51°C, followed by a second step of 10 min at 95°C, then 50 cycles of 30s at 95°C and 1 min at 60°C, followed by 40 cycles at 95°C for 15 s, 60°C for 1 min, and 72°C for 30 s. At the annealing/extension step of each cycle, fluorescence signal detection was performed. HCV RNA amplification and quantification were performed using Rotor Gene real-time PCR (Qiagen, Santa Clarita, CA) (Dawood *et al*., 2022).

Serological diagnosis of HSV1:

Determination of HSV1 IgG antibodies: The seropositivity of HSV1 IgG antibodies was assessed in serum samples of recruited subjects by using a commercial enzyme-linked immunosorbent assay (ELISA) kit, DRG HSV1 IgG (DRG International, Inc., USA), following instructions provided by the manufacturer. Briefly, solid-phase microtiter wells were coated with recombinant gG1 protein of HSV-1. Prepared patient samples and controls were pipetted into the wells. During incubation, the positive sample of HSV1 specific antibodies and controls were attached to immobilized antigens. Upon the addition of horseradish peroxidase-conjugated anti-human IgG antibodies into the wells. This anti-IgG conjugate can specifically bind to IgG antibodies, leading to the enzyme-linked immune complex formation during the second incubation. Upon incubation with TMB as a substrate, the immune complex resulted in the development of a blue color that turned yellow by stopping an enzymatic reaction with sulfuric acid. The color intensity was directly correlated to the total amount of HSV1 IgG antibodies in the patient's sample. The color intensity of the enzymatic reaction was measured at 450 nm by using a multi-well plate reader (TECAN; SUNRISE, Austria GmbH). The results were represented in DRG units [DU] using the following equation: patient's sample absorbance value ×10 / CO [CO referred to mean absorbance value of cut-off control]. Patients were classified based on their DU value as positive (> 11 DU), grey zone (9-11), or negative (< 9 DU).

Determination of HSV1 IgM antibodies:

The seropositivity of HSV1 IgM antibodies was assessed in serum samples of recruited subjects by using a commercial enzyme-linked immunosorbent assay (ELISA) kit, DRG HSV1 IgM (DRG International, Inc., USA), following instructions provided by the manufacturer. Despite the positive reaction referring to the presence of IgM to HSV1, the procedures showed common similarities with those of HSV1 IgG antibody detection, except that horseradish peroxidase-conjugated anti-human IgM antibodies were added into the wells. This anti-IgM conjugate binds specifically to IgM antibodies, resulting in the formation of enzyme-linked immune complexes during the second incubation, and then the color intensity was measured at 450 nm by using a multi-well plate reader

(TECAN; SUNRISE, Austria GmbH). The results were interpreted the same as in HSV1 IgG antibody detection and expressed in DU units.

Calculation of APRI Score: The estimation of liver fibrosis and cirrhosis was done using a non-invasive serum biomarker, the APRI score, that was calculated according to the formula originally reported by Wai *et al*. (2003) [42]. APRI = [(AST/upper limit of normal) × 100]/platelet count 10^9/L (Wai *et al*., 2003; Wadhva *et al*., 2018). The upper limit of normal for AST was 40 U/L, whereas the range of normal platelet count was 150,000 to 450,000/mm^3. At higher WHO cutoff values of APRI score (1.5 and 2), patients with significant fibrosis (late fibrosis, ≥ F2) were diagnosed at an APRI score ≥ cutoff of value 1.5, whereas below this cutoff value patients were identified with non-significant fibrosis (early fibrosis, < F2). Furthermore, patients with cirrhosis (F4 stage of liver fibrosis) were diagnosed at an APRI score ≥ cutoff of value 2, whereas below this cutoff value, patients were identified without cirrhosis (non-F4). Moreover, another two APRI cutoff values, lower WHO cutoff values (0.5 and 1), were applied to rule out late fibrosis and cirrhosis. An APRI score < cutoff of value 0.5 was used to rule out late fibrosis patients, while an APRI score < cutoff of value 1 was used to rule out cirrhosis cases (Baranova *et al*., 2011; Sripongpun *et al*., 2019 ; Catanzaro *et al*., 2021).

Statistical Analysis:

Data were collected, introduced into a Microsoft Excel worksheet and transferred to SPSS software version 20 (SPSS; Chicago, IL, USA) for analysis. Quantitative data was analyzed using t-test, qualitative data was analyzed using chi-square test, and the association of independent variables with one dichotomous dependent variable was detected by the logistic regression test. All statistical analyses were referred to by a *P*-value of ≤ 0.05, indicating significant results.

RESULTS

Demographic and Clinical Characteristics of The Study Population:

The demographic and clinical characteristics of the HCV patients and non-HCV control were reported in Table 1. The results of t-test analysis referred to a significant increase in age, ALT, AST, GGT, ALP, HSV IgG antibody titre, and APRI score among HCV patients (n = 80) compared with controls (n = 40). Similarly, compared with controls, a significant decrease in PLC, and PC was observed in chronic HCV patients (p < 0.05).

Table 1. Demographic and clinical features of chronic HCV patients and controls.

Parameter	Cohort of study			*P*-value
	Controls (n = 40)	Chronic HCV Patients (n = 80)		
Gender (M/F)	32(80%)/8(20%)	60(75%)/20(25%)		0.541
Age (years)	39.500 ± 10.281	49.510 ± 6.689		<0.001*
HCV RNA (IU/ml)	-	171,417.115 ± 441,191.238		-
ALT (U/L)	21.333 ± 8.752	37.962 ± 20.012		0.004*
AST (U/L)	21.750 ± 8.888	53.291 ± 31.532		0.006*
GGT (U/L)	34.400 ± 19.880	57.524 ± 52.943		0.030*
ALP (U/L)	89.000 ± 36.064	133.394 ± 66.246		0.003*
PC (%)	103.22 ± 12.050	51.066 ± 15.516		<0.001*
PLC (10^3/cmm)	186.600 ± 57.170	101.177 ± 58.904		<0.001*
HSV IgG titre (DU)	24.588 ± 21.745	161.891 ± 123.040		<0.001*
APRI Score	0.216 ± 0.052	1.693 ± 1.191		0.002*

Where: M: male, F: female, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamate transferase, ALP: alkaline phosphatase, PLC: platelet count, PC: prothrombin concentration, APRI: AST-to-platelet ratio index, *n*: number of subjects, and *: significant value. Normal ranges were as follows: AST: (0-40) U/L, ALT: (0-40) U/L, GGT: (10-55) U/L, ALP: (30-120) U/L, PC from (70-120) %, PLC: (150-450) 10^3/cmm, positive HCV RNA > 34 IU/ml, positive HSV IgG antibodies > 11 DU, HSV IgG antibodies were positive for (36/40) controls and (80/80) of HCV patients, age ranges (27-60) years for controls and (35-62) years for HCV patients. All data except gender were represented in mean and standard deviation values (M ± SD) and analyzed by t-test. A chi-square test was used in analyzing gender only.

Seroprevalence of HSV1 among the Study Population:

Data on the seroprevalence of HSV-1 IgG and HSV-1 IgM antibodies were analyzed and reported in Table 2. A significant increase in seropositivity of HSV-1 IgG antibodies was observed in 100%, 80/80 of chronic HCV patients compared to 90%, 4/40 of controls (P = 0.004). However, seropositivity of HSV-1 IgM antibodies varied in this respect. The positive cases of HSV-1 IgM antibodies were observed in 25%, 20/80 of chronic HCV patients compared to 10 %, 4/40 of controls (P = 0.052). Although a non-significant difference was recorded by chi-square analysis, the high tendency to an increase in the seropositivity of HSV1 IgM antibodies among HCV patients compared to controls was noted. The variation in the seroprevalence of HSV-1 IgG and HSV-1 IgM antibodies among HCV patients and controls is shown in Fig1 (Fig. 1a and Fig. 1b). Table 2b. Binary logistic regression for the effect of HCV infection on incidence of HSV IgG antibodies.

Table 2. Seroprevalence of HSV1 IgM among chronic HCV patients and controls.

Markers of HSV1 Seroprevalence		Subjects of Study				Chi-Square	
		Controls, n = 40		Chronic HCV Patients, n = 80			
		n	%	n	%	X^2	P-value
HSV1 IgG antibodies	Positive	36	90	80	100	8.276	0.004*
	Negative	4	10	0	0		
HSV1 IgM antibodies	Positive	4	10	20	25	3.750	0.052
	Negative	36	90	60	75		

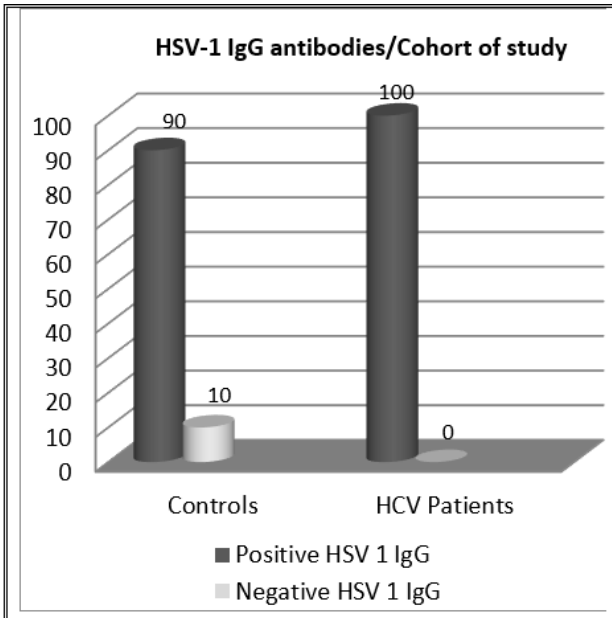

Fig. 1a. Comparison of seroprevalence of HSV IgG antibodies between HCV patients and controls.

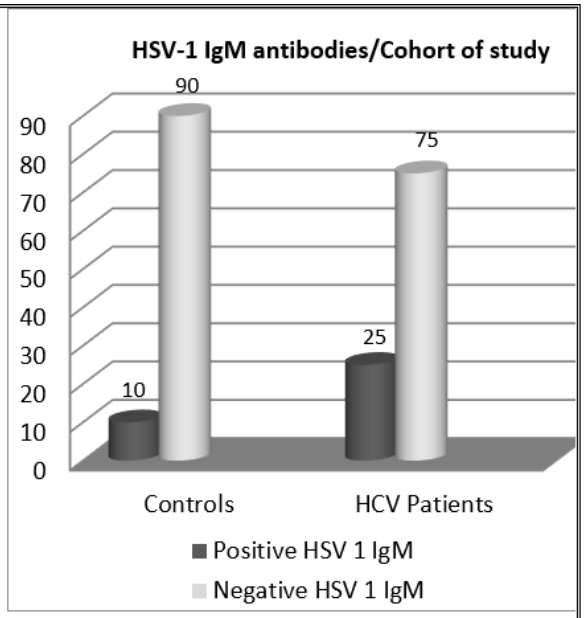

Fig. 1b. Comparison of seroprevalence of HSV IgM antibodies between HCV patients and controls.

Characteristic Features of HCV Monoinfected Patients and HCV/HSV1 Coinfected Others:

Chronic HCV patients were divided into two groups: group 1 with seronegative HSV1 IgM antibodies (HCV monoinfection) and group 2 with seropositive HSV1 IgM antibodies (HCV/ HSV1 coinfection). In Table 3, the results of t-test revealed a significant increase in GGT, and ALK was

observed in HCV/ HSV1 coinfected patients compared to HCV monoinfected others (*P* 0.005 and 0.004, respectively). Likewise, compared to HCV monoinfeted patients, a significant decrease in age, AST, albumin, T Bil, D Bil, and PLC was noted in HCV/ HSV1 coinfected patients (*P* <0.001, 0.001, 0.031, 0.018, 0.004 and 0.001, respectively).

Table 3. Comparison between chronic HCV patients with respect to seropositive HSV1 IgM antibodies.

Parameter	HSV1 IgM antibodies		*P*-value
	Negative, *n* = 60	Positive, *n* = 20	
Gender (M/F)	44(73.33%) /16(26.67%)	16(80%) /4(20%)	0.550
Age (years)	51.333 ± 5.805	43.789 ± 5.868	<0.001*
HCV RNA (IU/ml)	179,906.268 497,379.503	139,775.727 ± 420,86.427	0.396
ALT (U/L)	36.800 ± 17.848	41.632 ± 29.163	0.193
AST (U/L)	59.200 ± 33.337	34.632 ± 11.519	0.001*
GGT (U/L)	48.833 ± 88.653	87.733 ± 89.675	0.005*
ALP (U/L)	122.714 ± 52.442	173.267 ± 99.695	0.004*
PC (%)	52.364 ± 16.918	46.525 ± 7.857	0.093
PLC (10^3/cmm)	112.535 ± 61.385	67.842 ± 14.845	0.001*
HSV IgG titre (DU)	157.515 ± 136.052	173.02 ± 76.932	0.322
APRI Score	1.778 ± 1.290	1.441 ± 0.752	0.137

Where: M: male, F: female, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamate transferase, ALP: alkaline phosphatase, PLC: platelet count, PC: prothrombin concentration, APRI: AST-to-platelet ratio index, *n*: number of subjects, and *: significant value. Normal ranges were as follows: AST: (0-40) U/L, ALT: (0-40) U/L, GGT: (10-55) U/L, ALP: (30-120) U/L, PC from (70-120) %, PLC: (150-450) 10^3/cmm, positive HCV RNA > 34 IU/ml, positive HSV IgG antibodies > 11 DU. All data except gender were represented in mean and standard deviation values (M ± SD) and analyzed by t-test. A chi-square test was used in analyzing gender only.

Relationship Between Seroprevalence of HSV1 IgM Antibodies and Liver Fibrosis as Well as Liver Cirrhosis in Chronic HCV Patients:

The calculated APRI score in chronic HCV patients was a discriminating key at high and low cutoff values to distinguish chronic HCV patients into groups with regard to liver fibrosis and cirrhosis, the data were tabulated in Table 4.

At an APRI score cutoff of value 1.5, chronic HCV patients were discriminated for having liver fibrosis into two groups: group one of an APRI score ≥ 1.5, identified with late fibrosis (≥ F2, *n* = 40), and group two of an APRI score < 1.5, identified with early fibrosis (< F2, *n* = 40). Furthermore, at APRI score cutoff of value 2, chronic HCV patients were discriminated for cirrhosis into two groups: group one of an APRI score ≥ 2, identified with cirrhosis (F4, *n* = 24), and group two of an APRI score < 2, identified with non-cirrhosis (*n* = 56). In Table 4a, Chi-square results demonstrated a non-significant change in the seroprevalence of HSV1 IgM antibodies with regard to liver fibrosis in chronic HCV patients. Similarly, Chi-square results revealed a non-significant change in the seroprevalence of HSV1 IgM antibodies with respect to the liver cirrhosis in chronic HCV patients (*P* > 0.05). Moreover, logistic regression data revealed no association of the seroprevalence of HSV1 IgM antibodies and liver fibrosis (Odd's ratio 0.583, 95% C.I. 0.2086 to 1.6313, and *P* 0.304), and liver cirrhosis (Odd's ratio 0.500, 95% C.I. 0.1476 to 1.6937, and *P* 0.265).

Additionally, at the APRI score cutoff value of 0.5, chronic HCV patients were discriminated into two groups: group one with an APRI score < 0.5, identified with a high probability in order to rule out significant or late fibrosis (*n* = 4), and group two with an APRI score > 0.5, identified with

a low probability in order to rule out late fibrosis (n = 76). In Table 4b, Chi square results referred to a non-significant change in seroprevalence of HSV1 IgM antibodies and the probability of ruling out late fibrosis in chronic HCV patients. Finally, at APRI score cutoff of value 1, chronic HCV patients discriminated into two groups, a group with APRI score < 1, identified with high probability to rule out liver cirrhosis (n = 24) and another group with APRI score > 1, identified with low probability to rule out liver cirrhosis (n = 56). Also, in Table 4b, Chi square results referred to a non-significant change in seroprevalence of HSV1 IgM antibodies and the probability of ruling out cirrhosis in chronic HCV patients. Generally, in Table 4, logistic regression analyses referred to the absence of association between the seroprevalence of HSV1 IgM antibodies and the probability of ruling out late fibrosis and cirrhosis in chronic HCV patients.

Table 4a. Data analysis of the incidence of HSV1 IgM in relation to liver fibrosis and cirrhosis according to APRI score high cutoff values in HCV patients

APRI score high cutoff values			**HSV-1 IgM Antibodies**		**Chi-Square**		**Logistic regression**		
			Negative	**Positive**					
			***n* (%)**	***n* (%)**	**X^2**	***P*-value**	Odd's ratio	95% C.I.	*P* - value
Liver Fibrosis	APRI < 1.5	Early fibrosis	28 (46.67%)	12 (60%)	1.067	0.302	0.583	0.2086 to 1.6313	0.304
	APRI ≥ 1.5	Late fibrosis	32 (53.33%)	8 (40%)					
Liver Cirrhosis	APRI < 2	Non-Cirrhosis	40 (66.67%)	16 (80%)	1.270	0.260	0.500	0.1476 to 1.6937	0.265
	APRI ≥ 2	Cirrhosis	20 (33.333%)	4 (20%)					

Table 4b. Data analysis of the incidence of HSV1 IgM antibodies in relation to liver fibrosis and cirrhosis according to APRI score low cutoff values in HCV patients

APRI score low cutoff values			**HSV-1 IgM Antibodies**		**Chi-Square**		**Logistic regression**		
			Negative	**Positive**					
			***n* (%)**	***n* (%)**	**X^2**	***P*-value**	Odd's ratio	95% C.I.	*P* - value
Rule out Late fibrosis	APRI < 0.5	High probability	4 (6.67%)	0 (0 %)	2.504	0.114	0.172	0.00901 to 3.2904	0.242
	APRI > 0.5	Low probability	56 (93.33%)	36 (100%)					
Rule out Cirrhosis	APRI < 1	High probability	16 (26.67%)	8 (40%)	1.269	0.259	1.833	0.6339 to 5.3020	0.263
	APRI > 1	Low probability	44 (73.33%)	12 (60%)					

Relationship Between the Incidence of High Titre of HSV1 IgG Antibody and Liver Fibrosis as Well as Liver Cirrhosis in Chronic HCV Patients:

Based on the mean value (161.891) of HSV1 IgG antibody titre among the studied cohort of chronic hepatitis C patients, those patients were divided into 2 groups: group 1, which included patients with a higher HSV1 IgG antibody titre > 161.891 (n = 32), and group 2, which included patients with a lower HSV1 IgG antibody titre < 161.891 (n = 48). The data on the relationship between the

incidence of higher antibody titre and liver fibrosis as well as cirrhosis in chronic HCV patients were recorded in Table 5. In Table 5a, at high APRI score cutoff values, chi-square results revealed a non-significant change in the incidence of the higher and lower HSV-1 IgG antibody titre in HCV patients in relation to liver fibrosis and cirrhosis. Also, logistic regression results revealed no association of the incidence of higher HSV1 IgG antibody titre with liver fibrosis and cirrhosis. However, the results in Table 5b, refereed to the association between the incidence of higher HSV1 IgG antibody titre and the lower probability of ruling out cirrhosis in chronic HCV patients (Odd's ratio 1.833, 95% C.I. 0.0606 to 0.6604, and *P* 0.008).

Table 5a. Data analysis of the incidence of the higher and lower titres of HSV1 IgG in relation to liver fibrosis and cirrhosis according to APRI score high cutoff values in HCV patients

APRI score high cutoff values			**HSV-1 IgG Antibody Titre**		**Chi-Square**		**Logistic regression**		
			> 161.891, *n* =32	**< 161.891 *n* = 48**					
			***n* (%)**	***n* (%)**	**X^2**	***P*-value**	**Odd's ratio**	**95% C.I.**	***P* - value**
Liver Fibrosis	APRI < 1.5	Early fibrosis	16 (50%)	24 (50%)	0	1	1.0000	0.4088 to 2.4464	1
	APRI ≥ 1.5	Late fibrosis	16 (50%)	24 (50%)					
Liver Cirrhosis	APRI < 2	Non-Cirrhosis	20 (62.50%)	36 (75%)	1.429	0.232	1.8000	0.6830 to 4.7438	0.234
	APRI ≥ 2	Cirrhosis	12 (37.50%)	12 (25%)					

Table 5b. Data analysis of the incidence of the higher and lower titres of HSV1 IgG in relation to liver fibrosis and cirrhosis according to APRI score low cutoff values in HCV patients

APRI score low cutoff values			**HSV-1 IgG Antibody Titre**		**Chi-Square**		**Logistic regression**		
			>161.891, *n* =32	**< 161.891 *n* = 48**					
			***n* (%)**	***n* (%)**	**X^2**	***P*-value**	**Odd's ratio**	**95% C.I.**	***P* - value**
Rule out Late fibrosis	APRI < 0.5	High probability	0 (0%)	8 (16.67 %)	5.926	0.014*	0.0733	0.0041 to 1.3181	0.076
	APRI > 0.5	Low probability	32 (100%)	40 (83.33%)					
Rule out Cirrhosis	APRI < 1	High probability	4 (12.5%)	20 (41.67%)	7.778	0.005*	1.833	0.0606 to 0.6604	0.008*
	APRI > 1	Low probability	28 (87.5%)	28 (58.33%)					

DISCUSSION

The outcome of hepatitis infection may vary dramatically, depending on both host and viral factors (Zhang *et al*., 2019; Dawood *et al*., 2022). In nature, viral co-infection is as widespread as viral infection alone. Viral co-infections can cause mixed-up clinical symptoms, disrupted host immune defense mechanisms, and altered viral pathogenicity, leading to more difficult issues regarding disease diagnosis and treatment (Du *et al*., 2022). In this regard, several studies were designed to estimate the prevalence of other viral pathogens in chronic HCV patients and to report the clinical observations in co-infected cases as well as to examine their effect on the disease severity (Yurlov *et al*., 2021). Among those studies, there was a limited number conducted on herpes viruses in HCV patients. However, the reports on the seroprevalence and effect of HSV-1 infection in patients with chronic HCV infection are lacking.

In our study, seroprevalence of HSV-1 IgG antibodies was 90% among chronic HCV patients and 100% among controls. This finding referred to the fact that the entire study cohort except 10% of controls was subjected to HSV-1 infection. In fact, our finding is consistent with the previous reports, which revealed that the seroprevalence of HSV1 was 97.5% among Egyptians (Nasrallah *et al*., 2018; El-Ansary, 2022). Generally, in the Middle East, the increased seroprevalence of HSV1 was estimated as 80.5% among Palestinians, 81.4% among Lebanese, 82.3% among Qataris, 88.5% among Syrians, and 92.6% among Yemenis (Nasrallah *et al*., 2018 ;El-Ansary, 2022). On the other hand, due to ongoing improvements in hygiene and living conditions, seroprevalence of HSV-1 antibodies appears to be declining in western countries and around 30% among adolescents in the United States over the last three decades (Xu *et al*., 2007; Bernstein *et al*., 2013; El-Ansary, 2022). In wealthy countries, seroprevalence was found to be lower, showing a global link between HSV-1 infection and socioeconomic position (Ryder *et al*., 2009; El-Ansary, 2022). Compared to those wealthy countries in MENA, such as Iran, Jordan, and Qatar, low- to middle-income countries like Egypt, Sudan, and Yemen had the highest seroprevalence (Bradley *et al*., 2014; El-Ansary, 2022). Also, our finding disagreed with those reported by Taha *et al*. (2023), who observed low seroprevalence of HSV1 IgG antibodies in healthy blood donors in a study cohort from Saudi Arabia. In their study, HSV1 IgG antibody positivity was reported in 60/300 of the study cohort (Taha *et al*., 2023). Indeed, like other herpes viruses, IgG antibodies against HSV1 remain in the body throughout the lifetime. During viral latency, HSV1 IgG antibodies are detected at a lower level that is gradually increased upon reactivation.

Unlike IgG antibodies, IgM antibodies are not extremely long-lasting. They are generally only detectable during a reactivated or recent infection. In our study, seroprevalence of HSV1 IgM antibodies showed a variation among HCV patients and controls (25%, 60/80, and 10%, 4/40, respectively). Unfortunately, the previous studies do not provide sufficient data on the seroprevalence of HSV1 among HCV patients. Aggarwal and Kaur, (2004) observed the seroprevalence of HSV1 IgM antibodies among patients attending the sexually transmitted disease (STD) clinic and controls [17.6%, 44/250 and 24%, 12/50, respectively] (Aggarwal and Kaur, 2004).

Despite their work being referring to a disease other than hepatitis C, Aggarwal and Kaur, (2004) studied the seroprevalence of HSV1 and HSV2 among patients attending sexually transmitted disease clinic. Our findings agreed to some extent with those reported by Aggarwal and Kaur, (2004) regarding seroprevalence of HSV1 IgM antibodies among the patient group However, our findings disagreed with the same study regarding the seroprevalence report of HSV1 IgM antibodies among the control group. Indeed, our findings regarding seroprevalence of HSV1 IgG (100%) and HSV1 IgM (25%) among HCV patients referred to serological evidence of reactivation of the latent virus due to the predominance of HSV1 IgG in all

positive HSV1 IgM cases. Furthermore, our finding revealed a tendency of an increase in HSV1 reactivation among HCV patients than controls, due to the predominance of HSV1 IgG in all of the positive HSV1 IgM control individuals (10%). Additionally, the tendency of HSV1 reactivation was evidenced by an increase in HSV1 IgG antibody titre among HCV patients than controls. The viral reactivation might be attributed to specific immune disturbances that have been described to downregulate the immune system in chronic infection with HCV. In general, HSV1 infection of susceptible non-neuronal cells causes lytic replication. However, the existence of latency in a proportion of non-neuronal cells is suggested by a recent in vitro study (Cohen *et al*., 2020; Zhu and Viejo-Borbolla, 2021). HSV1 latency typically happens in neurons, especially the neuronal nuclei found inside the trigeminal ganglion's ophthalmic branch. Moreover, HSV1 latency occurs as a result of an immunological mechanism wherein there is selective retention and activation of the cytotoxic T cells, which are specific for gB498-505 HSV1 as an immune dominant epitope in the infected gangalion to generate interferon-gamma (IFN-) that is required for latency (Khanna *et al*., 2003; Taha *et al*., 2023).

Perelló *et al*. (2016), observed the reactivation of herpesvirus in a case-series analysis of HCV patients from Spain (Perelló *et al*., 2016). Their study was conducted during treatment with direct-acting antiviral agents (DAAs). Virus reactivation was identified in 10/576 cases (2%) in 2 cohorts: immunocompromised patients and non-immunocompromised patients. All cases were diagnosed for Herpes Zoster (HZ) reactivation except one case was diagnosed with severe herpes labialis, and the other 3 cases were diagnosed with ophthalmology: one case with ocular herpes, another case with severe ophthalmic herpes, and a case with periorbital herpes (Perelló *et al*., 2016). They concluded that the immune changes that follow HCV clearance might result in reactivation of herpesvirus (Perelló *et al*., 2016). HCV patients who were having herpesvirus infection should be managed for immediate treatment. However, the treatment with DAAs theoretically would restore these disturbances related to an exhausted immune system, especially regarding cellular immunity (Hartling *et al.*, 2013; Martin *et al.*, 2014; Serti *et al*., 2015; Perelló *et al*., 2016). Thus, the mechanism of these reactivations could even seem paradoxic (Perelló *et al.*, 2016). Another study conducted by Ghweil and Helel, (2019) was concerned with the reactivation of herpesvirus in HCV patients from Egypt during DAA therapy (Ghweil and Helal, 2019). Virus reactivation was identified in 8/100 cases, who were diagnosed for HZ reactivated infection. They explained the reactivation of HZ as a consequence of HCV infection and due to a potential role of DAAs treatment (El Kassas *et al.*, 2017; Ghweil and Helal, 2019).

Upon the seroprevalence of HSV1 IgM antibodies, the outcome of HCV/HSV-1 co-infection was studied in chronic HCV patients. In this regard, HCV patients were divided into 2 groups. first group with HCV monoinfection (n = 60, 75%) and second group with HCV/HSV1 co-infection (n = 20, 25%). Compared to HCV monoinfected patients, observed clinical features among HCV/HSV1 co-infected patients referred to a significant increase in GGT and ALP, as well as a tendency of elevation of ALT. In addition to a significant decrease in platelet count. Those findings referred to more deterrence of liver disease in HCV/HSV1 co-infection. In general, the majority of persistently increased ALP levels may be associated with liver disease or bone problems. Moreover, elevated serum ALP levels can indicate a range of hepatic disorders, which might be primary and secondary disorders. The presence of hepatic ALP on the sinusoid membrane implies a role in transport function (Vroon and Israili, 1990). The elevated level of GGT usually refers to the liver damage but does not specify the cause of the damage. Generally, the higher the liver enzymes, referring to more damaged liver cells and indicating worsening conditions.

Indeed, HSV1 hepatitis is characterized by fulminant hepatic necrosis with serum aminotransferase levels 100 to 1,000-fold above normal (Norvell *et al*., 2007). Furthermore, our finding referred to a decreased level of platelet count among HCV/HSV1 coinfected cases compared to HCV monoinfected others. Consequently, there was an increased level of thrombocytopenia among HCV/HSV1 coinfected patients, with an increased possibility of coagulation disturbance and bleeding among these patients. Khedr *et al*. (2022) observed the coagulation disturbances in the presence of thrombocytopenia as a consequence of hepatitis C in chronic HCV patients (Khedr *et al*., 2022). Additionally, a case report conducted by Shionoya *et al*. (2023) referred to a decrease in platelet count in HSV hepatitis and acute liver failure in normal immune function (Shionoya *et al*., 2023). So, it is supposed that HSV1 co-infection might worsen the thrombocytopenia and contribute to a greater decrease in the platelet count level in HCV patients.

Further investigations in our study involved the assessment of the relationship between HCV/HSV1 coinfection and liver fibrosis and cirrhosis. In this respect, the APRI score biomarker for liver fibrosis and cirrhosis was applied at different higher and lower WHO cutoff values. Regarding higher WHO cutoff values, at the APRI score cutoff $\geq$ 1.5, chronic HCV patients were divided into a group of patients with a late fibrosis $\geq$ F2 stage, n = 40) and another group of patients with early fibrosis < F2 stage, n = 40). Our results revealed no association between the incidence of HSV1 IgM antibodies or HCV/HSV1 co-infection and liver fibrosis. Likewise, at the APRI cutoff value $\geq$ 2, chronic HCV patients were divided into a group of patients with liver cirrhosis (F4 stage, n = 24) and another group of patients without liver cirrhosis (non-F4 stage, n = 56). Also, our results revealed no association between the incidence of HSV1 IgM antibodies and liver cirrhosis. Additionally, at the lower APRI score cutoff values of 0.5 and 1, no association between the incidence of HSV1 IgM antibodies and the probability of ruling out late fibrosis and cirrhosis among chronic HCV patients.

Additionally, with respect to the relationship between the incidence of higher HSV1 IgG antibody titre (> 161.385, as a mean value) and liver fibrosis as well as cirrhosis, the studied cohort of HCV patients were divided into 2 groups; group 1 included HCV patients having HSV1 IgG antibody titre > 161.385 (n = 32), and group 2 included HCV patients having HSV1 IgG antibody titre < 161.385 (n = 48). At a lower APRI score cutoff of 1, our findings referred to the association between the increased incidence of the higher HSV1 IgG antibody titre in HCV patients and the lower probability of ruling out cirrhosis (Odd's ratio 1.833, 95% C.I. 0.0606 to 0.6604, and P 0.008). However, at high APRI score cutoff values, there was no observable association between the increased incidence of the higher HSV1 IgG antibody titre and liver fibrosis as well as cirrhosis in chronic HCV patients in the current study.

Generally, it was confirmed that all of the human herpesviruses can produce at least mild liver disease in the course of systemic illness. However, primary hepatic involvement is uncommon. Disease caused by the alpha herpes viruses (HSV1, HSV2, and varicella-zoster virus [VZV]) in particular can result in fulminant hepatic necrosis and death (Fingeroth, 2000). The data that described the status of liver fibrosis and cirrhosis in HSV1 reactivation in either HSV1 monoinfected or in HCV/HSV1 coinfected patients were rare. In fact, there were a few studies concerned with the studding of the effect on liver fibrosis progression due to viral co-infection among HCV patients. The majority of them are concerned with HCV coinfection with viruses other than HSV1. A recent study conducted by Khedr and Mokhles (2023) referred to the association between increased titre of Epestien Bar Virus (EBV)-related viral capsid (VCA) IgG antibodies and liver fibrosis progression in HCV patients (Khedr and Mokhles, 2023). In fact, EBV is another type of herpes virus, which shares strong

similarities with HSV1 in the latency and reactivation during the course of the viral pathogenicity. Aso, Ibrahim *et al*. (2017) identified the association of increased incidence and reactivation of human herpes virus 5 (cytomegalovirus [CMV]) with late fibrosis stages in chronic HCV patients (Ibrahim *et al*., 2017). Furthermore, recent reports indicate that HCV patients with hepatitis B virus (HBV) coinfection have a higher risk of developing liver disease complications, including liver fibrosis and cirrhosis (Maqsood *et al*., 2023). As a limitation in our study, the determination of HSV1 DNA determination and dermatologic clinical pictures of HSV1 reactivation were not available. To our knowledge, as far as we know, this is the first report referring to the coinfection of HCV/HSV1 based on the serological evidence of the presence of HSV-1 IgM antibodies in the predominant HSV1 IgG antibodies among HCV patients and to reveal the clinical outcome of this co-infection.

Conclusion

Our results shed light on the establishment of HSV1 reactivation among chronic HCV patients. An increased incidence of HSV1 IgG antibodies is observed in chronic HCV infection. In addition, in HCV/HSV1 co-infection, liver disease deterioration is represented by the worsening increase in GGT and ALP enzymes as well as an increased level of thrombocytopenia. In the studied cohort of HCV patients, the seroprevalence of HSV1 IgM antibodies is not associated with liver fibrosis and cirrhosis progression. However, the increased incidence of higher HSV1 IgG titre is associated with a lower probability of ruling out cirrhosis. Future studies are recommended to correlate the HSV1 infection on the level of DNAemia with clinical features and liver disease pathogenicity in chronic HCV patients.

Abbreviations:

HCV: Hepatitis C Virus;
HSV1: Type 1 Herpes Simplex Virus;
APRI: Alanine Aminotransferase to Platelet Ratio Index

Author Contributions: A. K.: Conception, methodology, collection of data, analysis and interpretations of data, writing of the manuscript, and revision of key contents. M. M.: Recruitment of patients, resources and validation. All authors approved the manuscript.

Conflict of Interest: The authors have no conflict of interest.

REFERENCES

Aggarwal, A., and Kaur, R. (2004). Seroprevalence of herpes simplex virus-1 and 2 antibodies in STD clinic patients. *Indian journal of medical microbiology*, *22*(4), 244–246.

Al-Shuwaikh, A., Hanna, D., and Ali, Z. (2019). Seroprevalence of herpes simplex virus type-1 igg antibody in healthy blood donor from Baghdad-Iraq. *Journal of Pure and Applied Microbiology*, *13*(2),1017–23.

Ashley R. L. (2002). Performance and use of HSV type-specific serology test kits. *Herpes: the journal of the IHMF*, *9*(2), 38–45.

Azak, A., Huddam, B., Koçak, G., and Duranay, M. (2011). Rare cause of acute hepatitis in a hemodialysis patient: herpes simplex. *Therapeutic apheresis and dialysis*, *15*(6), 593–594. https://doi.org/10.1111/j.1744-9987.2011.00988.x

Baranova, A., Lal, P., Birerdinc, A., and Younossi, Z. M. (2011). Non-invasive markers for hepatic fibrosis. *BMC gastroenterology*, *11*, 91. https://doi.org/10.1186/1471-230X-11-91

Bernstein, D. I., Bellamy, A. R., Hook, E. W., 3rd, Levin, M. J., Wald, A., Ewell, M. G., Wolff, P. A., Deal, C. D., Heineman, T. C., Dubin, G., and Belshe, R. B. (2013). Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. *Clinical*

infectious diseases: an official publication of the Infectious Diseases Society of America, *56*(3), 344–351. https://doi.org/10.1093/cid/cis891

Bradley, H., Markowitz, L. E., Gibson, T., and McQuillan, G. M. (2014). Seroprevalence of herpes simplex virus types 1 and 2--United States, 1999-2010. *The Journal of infectious diseases*, *209*(3), 325–333. https://doi.org/10.1093/infdis/jit458

Burton, M. J., Penman, A., Adams-McAlpine, G., Overman, T. L., and Hook, E. W., 3rd (2012). Prevalence and characteristics of herpes simplex virus type-2 coinfection in veterans with hepatitis C. *The American journal of the medical sciences*, *344*(6), 436–440. https://doi.org/10.1097/MAJ.0b013e31824585bd

Catanzaro, R., Aleo, A., Sciuto, M., Zanoli, L., Balakrishnan, B., and Marotta, F. (2021). FIB-4 and APRI scores for predicting severe liver fibrosis in chronic hepatitis HCV patients: a monocentric retrospective study. *Clinical and experimental hepatology*, *7*(1), 111–116. https://doi.org/10.5114/ceh.2021.104543

Christensen, M. H., and Paludan, S. R. (2017). Viral evasion of DNA-stimulated innate immune responses. *Cellular & molecular immunology*, *14*(1), 4–13. https://doi.org/10.1038/cmi.2016.06

Cohen, E. M., Avital, N., Shamay, M., and Kobiler, O. (2020). Abortive herpes simplex virus infection of nonneuronal cells results in quiescent viral genomes that can reactivate. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(1), 635–640. https://doi.org/10.1073/pnas.1910537117

Cooke, G. S., Andrieux-Meyer, I., Applegate, T. L., Atun, R., Burry, J. R., Cheinquer, H., Dusheiko, G., Feld, J. J., Gore, C., Griswold, M. G., Hamid, S., *et al*. (2019). Accelerating the elimination of viral hepatitis: a Lancet Gastroenterology & Hepatology Commission. *The lancet. Gastroenterology &hepatology*, *4*(2), 135–184. https://doi.org/10.1016/S2468-1253(18)30270-X

Dawood, R. M., Salum, G. M., El-Meguid, M. A., Elsayed, A., Yosry, A., Abdelaziz, A., Shousha, H. I., Nabeel, M. M., and El Awady, M. K. (2021). Development of a gene signature for predicting cirrhosis risk score of chronic liver disease associated with HCV infection in Egyptians. *Microbial pathogenesis*, *153*, 104805. https:// doi.org/10.1016/j.micpath.2021.104805

Dawood, R. M., Gomaa, A. A., Abd El Meguid, M., Hassan, E. A., Salum, G. M., Fares, H. M., El Awady, M. K., Fares, E. M., and Esmat, G. (2022). The Impact of Direct-Acting Antiviral Agents on Cytomegalovirus Reactivation in Chronic Hepatitis C Infection. *Asian Pacific journal of cancer prevention: APJCP*, *23*(4), 1365–1372. https://doi.org/10.31557/APJCP.2022.23.4.1365

Dawood, R. M., El-Meguid, M. A., Shousha, H. I., Elsayed, A., Nabeel, M. M., Yosry, A., Abdelaziz, A., and Salum, G. M. (2022). Seven gene signature explores the impact of DAAs on the appearance of hepatocellular carcinoma in HCV infected patients. *Heliyon*, *8*(8), e10119. https://doi.org/10.1016/j.heliyon.2022.e10119

Du, Y., Wang, C., and Zhang, Y. (2022). Viral Coinfections. *Viruses*, *14*(12), 2645. https://doi.org/10.3390/v14122645

El-Ansary, M.R. (2022). Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 among Egyptian Childern Aged from One to 15 Years old: A Comparative Study. *Afro-Egyptian Journal of Infectious and Endemic*

Diseases, 12(4), 383-398. doi: 10. 21608/AEJI.2022.168020.1260

El Kassas, M., Wifi, M. N., Mahdy, R., Afify, S., Hafez, E., El Latif, Y. A., Ezzat, M., El Tahan, A., Youssef, N., and Esmat, G. (2017). Herpes Zoster reactivation in patients with chronic hepatitis C under treatment with directly acting antiviral agents: A case series. *Arab journal of gastroenterology: the official publication of the Pan-Arab Association of Gastroenterology, 18*(1), 39–41. https://doi.org/10. 1016/j.ajg.2017.02.003

El Kassas, M., Funk, A. L., Salaheldin, M., Shimakawa, Y., Eltabbakh, M., Jean, K., El Tahan, A., Sweedy, A. T., Afify, S., Youssef, N. F., Esmat, G., and Fontanet, A. (2018). Increased recurrence rates of hepatocellular carcinoma after DAA therapy in a hepatitis C-infected Egyptian cohort: A comparative analysis. *Journal of viral hepatitis*, *25*(6), 623–630. https://doi.org/10.1111/jvh.12854

Eslam, M., Hashem, A. M., Romero-Gomez, M., Berg, T., Dore, G. J., Mangia, A., Chan, H. L. Y., Irving, W. L., Sheridan, D., Abate, M. L., *et al*. (2016). FibroGENE: A gene-based model for staging liver fibrosis. *Journal of hepatology*, *64*(2), 390–398. https://doi.org/10. 1016/j.jhep.2015.11.008

Fatahzadeh, M., and Schwartz, R. A. (2007). Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. *Journal of the American Academy of Dermatology*, *57*(5), 737–766. https: //doi.org/10.1016/j.jaad.2007.06.027

Fingeroth J. D. (2000). Herpesvirus infection of the liver. *Infectious disease clinics of North America*, *14*(3), 689–719. https://doi.org/10.1016/s0891-5520 (05)70127-6

Ghweil, A. A., and Helal, M. M. (2019). Reactivation of herpesvirus in patients with hepatitis C treated with direct- acting antiviral agents *Infection and drug resistance*, *12*, 759–762. https://doi.org/10.2147/ IDR.S184598

Glas, M., Smola, S., Pfuhl, T., Pokorny, J., Bohle, R. M., Bücker, A., Kamradt, J., and Volk, T. (2012). Fatal multiorgan failure associated with disseminated herpes simplex virus-1 infection: a case report. *Case reports in critical care*, *2012*, 359360. https: //doi.org/10.1155/2012/359360

Global Hepatitis Report. 2017. World Health Organization (WHO): Geneva, Switzerland.

Hartling, H. J., Gaardbo, J. C., Ronit, A., Salem, M., Laye, M., Clausen, M. R., Skogstrand, K., Gerstoft, J., Ullum, H., and Nielsen, S. D. (2013). Impaired thymic output in patients with chronic hepatitis C virus infection. *Scandinavian journal of immunology*, *78*(4), 378–386. https: //doi.org/10.1111/sji.12096

Heldwein, E. E., and Krummenacher, C. (2008). Entry of herpesviruses into mammalian cells. *Cellular and molecular life sciences: CMLS*, *65*(11), 1653–1668. https:// doi.org/10.1007/s00018-008-7570-z

Hull, H. F., Blumhagen, J. D., Benjamin, D., and Corey, L. (1984). Herpes simplex viral pneumonitis in childhood. *The Journal of pediatrics*, *104*(2), 211–215. https:// doi.org/10.1016/s0022-3476 (84) 80994-4

Ibrahim, M. K., Khedr, A., Bader El Din, N. G., Khairy, A., and El Awady, M. K. (2017). Increased incidence of cytomegalovirus coinfection in HCV-infected patients with late liver fibrosis is associated with dysregulation of JAK-STAT pathway. *Scientific reports*, *7*(1), 10364. https://doi.org/10.1038/ s41598-017-10604-7

James, C., Harfouche, M., Welton, N. J., Turner, K. M., Abu-Raddad, L. J.,

Gottlieb, S. L., and Looker, K. J. (2020). Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bulletin of the World Health Organization*, *98*(5), 315–329. https://doi.org/10.2471/BLT.19.237149

Kaufman, B., Gandhi, S. A., Louie, E., Rizzi, R., and Illei, P. (1997). Herpes simplex virus hepatitis: case report and review. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, *24*(3), 334–338. https://doi.org/10.1093/clinids/24.3.334

Khanna, K. M., Bonneau, R. H., Kinchington, P. R., and Hendricks, R. L. (2003). Herpes simplex virus-specific memory CD8+ T cells are selectively activated and retained in latently infected sensory ganglia. Immunity, *18*(5), 593–603. https://doi.org/10.1016/s1074-7613(03)00112-2

Khedr, A., Barakat, A.B., and Salama, M.S. (2022). Assessment of Factor V Gene G1691A Mutation (Factor V Leiden) among Chronic Hepatitis C Patients with Thrombocytopenia. *Journal of Biomedical Research and Environmental Science*, *3*(12), 1589-1599. doi: 10.37871/jbres1640

Khedr, A., and Mokhles, M. (2023). Association Between Seroprevalence of Epstein–Barr Virus (EBV) and FIB4 Score-Based Liver Fibrosis in Chronic Hepatitis C patients. *Egyptian Academic Journal of Biological Sciences C Physiology & Molecular Biology, 15*(1),603-618. doi:10.21608/EAJBSC.2023.392985.

Kusne, S., Schwartz, M., Breinig, M. K., Dummer, J. S., Lee, R. E., Selby, R., Starzl, T. E., Simmons, R. L., and Ho, M. (1991). Herpes simplex virus hepatitis after solid organ transplantation in adults. *The Journal of infectious diseases*, *163*(5), 1001–1007. https://doi.org/10.1093/infdis/163.5.1001

LeGoff, J., Péré, H., and Bélec, L. (2014). Diagnosis of genital herpes simplex virus infection in the clinical laboratory. *Virology journal*, *11*, 83. https://doi.org/10.1186/1743-422X-11-83

Martin, B., Hennecke, N., Lohmann, V., Kayser, A., Neumann-Haefelin, C., Kukolj, G., Böcher, W. O., and Thimme, R. (2014). Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. *Journal of hepatology*, *61*(3), 538–543. https://doi.org/10.1016/j.jhep.2014.05.043

Maqsood, Q., Sumrin, A., Iqbal, M., Younas, S., Hussain, N., Mahnoor, M., and Wajid, A. (2023). Hepatitis C virus/Hepatitis B virus coinfection: Current prospectives. *Antiviral therapy*, *28*(4), 13596535231189643. https://doi.org/10.1177/13596535231189643

Nasrallah, G. K., Dargham, S. R., Mohammed, L. I., Abu-Raddad, L. J. (2018). Estimating seroprevalence of herpes simplex virus type 1 among different Middle East and North African male populations residing in Qatar . *Journal of medical virology* , *90*(1), 184–190. https://doi.org/10.1002/jmv.24916

Norvell, J. P., Blei, A. T., Jovanovic, B. D., and Levitsky, J. (2007). Herpes simplex virus hepatitis: an analysis of the published literature and institutional cases. *Liver transplantation: official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *13*(10), 1428–1434. https://doi.org/10.1002/lt.21250

Perelló M, C., Fernández-Carrillo, C., Londoño, M. C., Arias-Loste, T., Hernández-Conde, M., Llerena, S., Crespo, J., Forns, X., and Calleja, J. L. (2016). Reactivation of Herpesvirus in Patients with Hepatitis C Treated With Direct-

Acting Antiviral Agents. *Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association*, *14*(11), 1662–1666.e1. https://doi.org/10.1016/j.cgh.2016.05.016

Ravi, S., Axley, P., Jones, D., Kodali, S., Simpson, H., McGuire, B. M., and Singal, A. K. (2017). Unusually High Rates of Hepatocellular Carcinoma After Treatment With Direct-Acting Antiviral Therapy for Hepatitis C Related Cirrhosis. *Gastroenterology*, *152*(4), 911–912. https://doi.org/10.1053/j.gastro.2016.12.021

Roche, B., Coilly, A., Duclos-Vallee, J. C., and Samuel, D. (2018). The impact of treatment of hepatitis C with DAAs on the occurrence of HCC. *Liver international: official journal of the International Association for the Study of the Liver*, *38 Suppl 1*, 139–145. https://doi.org/10.1111/liv.13659

Ryder, N., Jin, F., McNulty, A. M., Grulich, A. E., and Donovan, B. (2009). Increasing role of herpes simplex virus type 1 in first-episode anogenital herpes in heterosexual women and younger men who have sex with men, 1992-2006. *Sexually transmitted infections*, *85*(6), 416–419. https://doi.org/10.1136/sti.2008.033902

Salum, G. M., El Meguid, M. A., Abelhafez, T. H., Medhat, E., Abdel Aziz, A. O., and Dawood, R. (2021). Evaluation of seven gene signature for predicting HCV recurrence post-liver transplantation. *Journal, genetic engineering & biotechnology*, *19*(1), 174. https://doi.org/10.1186/s43141-021-00266-4

Schulz, K. S., and Mossman, K. L. (2016). Viral Evasion Strategies in Type I IFN Signaling - A Summary of Recent Developments. *Frontiers in immunology*, *7*, 498. https://doi.org/10.3389/fimmu.2016.00498

Serti, E., Chepa-Lotrea, X., Kim, Y. J., Keane, M., Fryzek, N., Liang, T. J., Ghany, M., and Rehermann, B. (2015). Successful Interferon-Free Therapy of Chronic Hepatitis C Virus Infection Normalizes Natural Killer Cell Function. *Gastroenterology*, *149*(1), 190–200.e2. https://doi.org/10.1053/j.gastro.2015.03.004

Shen, J. H., Huang, K. Y., Chao-Yu, C., Chen, C. J., Lin, T. Y., and Huang, Y. C. (2015). Seroprevalence of Herpes Simplex Virus Type 1 and 2 in Taiwan and Risk Factor Analysis, 2007. *PloS one*, *10*(8), e0134178. https://doi.org/10.1371/journal.pone.0134178

Shev, S., Widell, A., Bergström, T., Hermodsson, S., Lindholm, A., and Norkrans, G. (1995). Herpes simplex virus-2 may increase susceptibility of the sexual transmission of hepatitis C. *Sexually transmitted diseases*, *22*(4), 210–216. https://doi.org/10.1097/00007435-199507000-00002

Shionoya, K., Kako, M., Masuda, S., Makazu, M., and Koizumi, K. (2023). Acute liver failure due to herpes simplex viral hepatitis diagnosed by skin lesions and blood tests: a case report. *Journal of medical case reports*, *17*(1), 338. https://doi. org/10.1186/s13256-023-04083-w

Sripongpun, P., Tangkijvanich, P., Chotiyaputta, W., Charatcharoenwitthaya, P., Chaiteerakij, R., Treeprasertsuk, S., Bunchorntavakul, C., Sobhonslidsuk, A., Leerapun, A., Khemnark, S., *et al*. (2019). Evaluation of aspartate aminotransferase to platelet ratio index and fibrosis 4 scores for hepatic fibrosis assessment compared with transient elastography in chronic hepatitis C patients. *JGH open : an open access journal of gastroenterology and*

hepatology, *4*(1), 69–74. https://doi.org/10.1002/jgh3.12219

Taha, A.E., Ghazy, A.A., Almaeen, A., Taher, I., El-Metwally, T., Alayyaf, M.A., Alrayes, F., Alinad, A., Albulayhid, S., and Aldakhil, A. (2023). Estimation of Seroprevalence of Anti-Herpes Simplex Virus Type-1 IgG Among Healthy Blood Donors in Sakaka City, Aljouf, Saudi Arabia. *Jundishapur Journal of Microbiology*, *16*(7), e136606. https://doi.org/10.5812/jjm-136606

Velasco, M., Llamas, E., Guijarro-Rojas, M., and Ruiz-Yagüe, M. (1999). Fulminant herpes hepatitis in a healthy adult: a treatable disorder?. *Journal of clinical gastroenterology*, *28*(4), 386–389. https://doi.org/10. 1097/00004836-199906000-00025

Vroon, D. H., and Israili, Z. (1990). Alkaline Phosphatase and Gamma Glutamyltransferase. In H. K. Walker (Eds.) *et. al*., *Clinical Methods: The History, Physical, and Laboratory Examinations*. (3rd ed.). Butterworths.

Wadhva, R. K., Haque, M. M., Luck, N. H., Tasneem, A. A., Abbas, Z., and Mubarak, M. (2018). Diagnostic Accuracy of Aspartate Aminotransferase to Platelet Ratio Index and Fibrosis 4 Scores in Predicting Advanced Liver Fibrosis in Patients with End-stage Renal Disease and Chronic Viral Hepatitis: Experience from Pakistan. *Journal of translational internal Medicine*, *6*(1), 38–42. https://doi.org/10. 2478/jtim-2018-0008

Wai, C. T., Greenson, J. K., Fontana, R. J., Kalbfleisch, J. D., Marrero, J. A., Conjeevaram, H. S., and Lok, A. S. (2003). A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology (Baltimore, Md.)*, *38*(2), 518–526. https://doi.org/10.1053/jhep.2003.50346

Wind, L., van Herwaarden, M., Sebens, F., and Gerding, M. (2012). Severe hepatitis with coagulopathy due to HSV-1 in an immunocompetent man. *The Netherlands journal of medicine*, *70*(5), 227–229.

Xu, F., Lee, F. K., Morrow, R. A., Sternberg, M. R., Luther, K. E., Dubin, G., and Markowitz, L. E. (2007). Seroprevalence of herpes simplex virus type 1 in children in the United States. *The Journal of pediatrics*, *151*(4), 374–377. https://doi.org/10. 1016/j.jpeds.2007.04.065

Yurlov, K.I., Masalova, O.V., Kisteneva, L.B., Khlopova, I.N., Samokhvalov, E.I., Malinovskaya, V.V., Parfyonov, V.V., Shuvalov, A. N., and Kushch, A. A. (2021). Human Herpesviruses Increase the Severity of Hepatitis. *Biology*, *10*(6), 483.

Zhang, Z., Wang, C., Liu, Z., Zou, G., Li, J., and Lu, M. (2019). Host Genetic Determinants of Hepatitis B Virus Infection. *Frontiers in genetics*, *10*, 696. https://doi.org/10.3389/fgene. 2019.00696

Zhang S. Y. (2020). Herpes simplex virus encephalitis of childhood: inborn errors of central nervous system cell-intrinsic immunity. *Human genetics* *139*(6-7), 911–918. https://doi.org/ 10.1007/s00439-020-02127-5

Zhu, S., andViejo-Borbolla, A. (2021). Pathogenesis and virulence of herpes simplex virus. *Virulence*, *12*(1), 2670 –2702. https://doi.org/10. 1080/21505594.2021.1982373

.