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Pathogenicity of HSV-2 Co-infection on Liver function of HCV patients

Mohamed Nabil[1, 2], Ezeldine K Abdalhabib[1],Abdelbaset Mohamed Elasbali[1], Omar Alfaruok[2]

[1]Department of clinical laboratories, College of applied medical sciences, Jouf University, KSA.
[2] Department of Microbiology, Ain Shams University, Cairo, Egypt.

Corresponding author: *Dr. Mohamed Nabil Mohamed, Department of Clinical Laboratory Science, College of Applied Medical Sciences, Jouf University, KSA.

Abstract

Herpes simplex virus type-2 (HSV-2) infection is the most common cause of genital ulcer disease worldwide**.** Epidemiologic links between herpes simplex virus type-2 (HSV-2) and hepatitis C virus (HCV) exist but are poorly characterized. Seroprevalence studies of HSV-2 in populations with chronic HCV infection are lacking.This study attempts to determine the assessment of HSV-2 Co-infection in HCV infected patients and to point out the effects of HSV-2/HCV co-infections on liver Function. The study population includes 49 chronic HCV (patient group) and 44 cases without viral hepatitis (control group). Nucleic acid of HSV-2 was detected in Serum samples by nested polymerase chain reaction (nested-PCR) method.

HSV-2 infection was demonstrated in 39% of chronic HCV patients. Alanine aminotransferase (ALT) levels and Aspartate aminotransferase (AST) of HSV-2 infected HCV patients were increased.We conclude that HSV-2 infection is common in HCV patients, who can beregarded as patients at high risk for HSV-2 disease.

Keywords: HCV, HSV-2, Pathogenicity and Liver function.

Introduction

Herpes Simples Virus type 2 (HSV-2) is a member of the alpha herpes virus family, contains a large double stranded, linear DNA genome. Prevalence of HSV-2 is increasing worldwide (Smith *et al*., 2001). HSV-2causes genital ulcer disease (GUD) in the developed world (Bernstein *et al*., 2013). A primary HSV-2 infection is followed by a life-long persistence of the virus in a latent state, and reactivation may occur later in life (WHO, 2017).Therefore, reactivation of the virus is seen during periods of down-regulation of the immune system, such as drug treatment and illness-related stress, or during on-going activation of the immune system such as inflammatory diseases, or co-infection with other pathogens (Severson*et al*., 1999). HSV-2 hepatitis occurs as part of disseminated HSV infection. It occurs mainly among liver or kidney transplant recipients or immunosuppressed persons (Smith *et al*., 2001and Bernstein *et al*., 2013). Mild-moderately elevated levels of transaminases and various histopathological changes of the liver were encountered in these patients. This study aimed to investigate the co-

infection of Herpes Simplex Virus type 2 (HSV-2) (antibodies and DNA) in sera samples from patients positive and negative for HCV infection to study the effect of HSV2-HCV co-infection on Liver function.

Materials and methods

Study population:

This study (approved by the Ethical Committee of Ain Shams University) consisted of a patient group (*n* =49) with chronic viral hepatitis C and a control group (*n* = 44) without viral hepatitis, all samples were collected from Cairo (El-Demerdash Hospital), EL-Menia (El-Menia general hospital), Mansoura (Mansoura University hospital) and Alexandria General Hospital. We evaluated 49 consecutive patients with chronic hepatitis C (range 29:66, 12males,and 37 females). All of the chronic HCV patients were positive for antibodies against hepatitis C virus (anti-HCV) and serum HCV-RNA. The control group (*n* = 44; range: 16: 54; 19 males,25 females) consisted of individuals without viral hepatitis. All of the control patients were negative for anti-HCV and HCV-RNA. The age, sex, alanine aminotransferase (ALT) levels, antibodies against HSV2 (anti-HSV2-IgM, anti-HSV2-IgG), HSV2-DNA of the serum samples of the cases were assessed and recorded.

Detection of HCV RNA:

RNA was extracted from 200µl of serum specimen using the acid guanidiumthiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1992).

Primer sets used in the detection of HCV RNA were selected from the highly conserved 5'- untranslated region (UTR) of the HCV genome. p: 5’ GGTGCACGGTCT ACGAGACCTC 3’ - P2 forward primer: 5’ AACTACTGTCTTCACGCAGAA 3’ - P3 reverse primer: 5’ TGCTCATG GTGCACGGTCTA 3’- nested reverse primer P4: 5’ACTCGGCTAGCAGTCTCGCG 3’ and forward primer P5: 5’ GTGCAGCCTCCAGGACCC 3’ (Promega, USA).

The nested PCR amplification was done in a volume of 50µl; and the PCR protocol consisted of a reverse transcription step at 59 ◦C for 60 min by using 20 U of cloned Avian Myloblastosis Virus (AMV) reverse transcriptase and 1µl primer (p1). First round amplification was done by using (P2) forward primer and (P3) reverse primer. The second round amplification was done similar to the first round, except for use of the nested reverse primer (P4) and forward primer (P5), the products of nested PCR were analyzed on 2% agarose gel electrophoresis.

Serological analysis of HSV-2 infection:

HSV-2 IgM and HSV-2 IgG antibodies were estimated by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available HSV-2 IgM and IgG Kits, (Pishtaz TEB Diagnostic HSV-2 IgM ELISA Kit "PT-HSV-2 IgM-24", Tehran, Iran, and BioCheck HSV-2 IgGELISA Kit(USA). Tests were done according to the manufacturer instructions.

Detection of HSV-2 DNA:

DNA was extracted from 300 µl serum sample using Wizard® DNA purification mini kit, Promega (Madison, USA), following the instructions of the manufacturer.3 µl of the DNA extract from the sample was added to 20 µl of PCR mixture 1 µl of each primer 5` GGACGAGGCCCGAAAGCACA 3` and 5` GGACGAGGCCCGAAAGCACA 3` (Bioneer, USA). 2µl of the 1st PCR product were used in a nested-PCR containing the same conditions as mentioned above , using internal primers 5`CGGTGCTCCAGGATAAA3` and 5` TCTCCGTCCAGTCGTTTATCTTC 3` (Bioneer, USA). Amplification products

(nested-PCR products) were visualized after electrophoresis on 2% agarose gel stained with ethidium bromide.

Biochemical Analysis:

Biochemical tests, including Alanine amino transferase (normal range, 40 U/L) and Aspartate amino transferase (normal range, 38 U/L) levels were done on all collected samples with commercially available Flex ALAT (GPT) and ASAT (GOT) Kits (Siemens Healthcare Diagnostic Inc., USA). Levels of liver enzymes were measured as described by the manufacturer.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software program. The statistical difference was considered significant when $p \leq 0.05$.

Detection of HCV-2DNA by nested PCR

In chronic HCV patients, HCV2 -DNA was positive in 39% (19/49) of the serum samples. HSV 2 -DNA was detected in 8 out of 44 (18.2%) samples obtained from the control group. The difference between the presence of HSV 2 -DNA in patients with chronic HCV infection and the control group was statistically significant ($p< 0.01$).

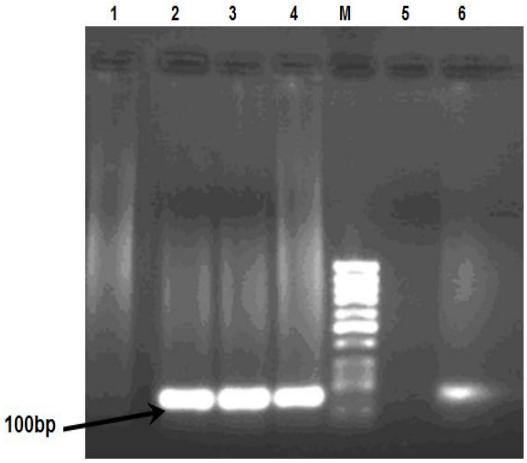
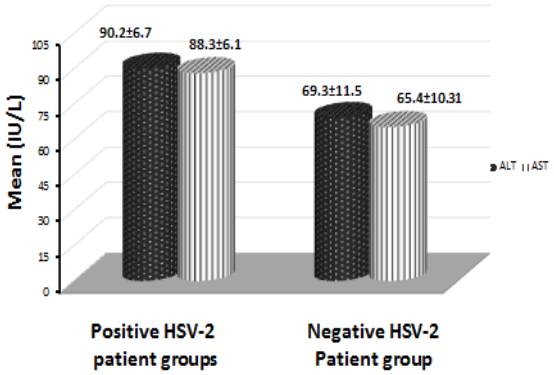


Figure 1: Nested PCR results of serum samples lane 2, 3, 4 and 6 were positive for HSV2 DNA while lanes 1 and 5 were negative for HSV.

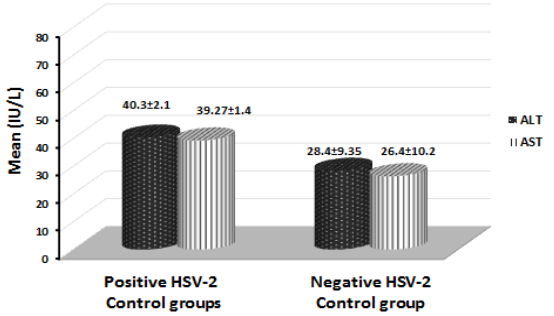
Serum levels of the liver transaminases in the study groups

In HCV-positive group (patient group), serum ALT levels (mean: 90.21±6.71 U/l) in HSV-2 positive patients were slightly higher than that of HSV-2 negative patients (mean: 69.33±11.54 IU/l). Serum AST activity level of HSV-2 positive patients (mean:88.2±6.1IU/L) was slightly higher than that of HSV-2 negative patients (mean:65.4±10.31 IU/L).



The activity level of ALT and AST liver enzymes in HSV-2 patient group

In Control group, serum ALT levels (mean: 40.3±2.1 U/l) in HSV-2 positive patients were slightly higher than that of HSV-2 negative patients (mean: 28.4±9.35 U/l). Serum AST activity level of HSV-2 positive patients (mean:39.27±1.3IU/L) was slightly higher than that of HSV-2 negative patients (mean:26.4±10.51 IU/L)



The activity level of ALT and AST liver enzymes in HSV-2 patient group

Discussion

Herpes simplex virus (HSV) is common throughout the world. Herpes simplex virus is estimated that up to 80% of adults contract HSV throughout their lifetime (Levitsky *et al*., 2008). Adults are infected with HSV-2, with rates of infection increasing with age. The majority of HSV-2 infections are asymptomatic and most infected persons remain unaware of this lifelong infection (Chase *et al*., 1987). The data of this study showed that the percentage of positive HSV-2 Abs were significantly higher ($P < 0.001$) in patient group (HCV patients) than those in Control group. Also, HSV-2 DNA was detected in 39% of HCV infected patients compared with 8 out of 44 (18.2%) Control group cases. Our findings were in agreement with those of other studies which emphasize the presence of HSV-2 infection in patients with HCV (Wald *et al*., 2002).The difference between the presence of HSV-2 DNA among patients and the control group was statistically significant ($p < 0.05$). In the two study groups (Patient group and Control), we study the activity levels of ALT and AST liver enzymes. In HCV-RNA positive cases, serum activity levels of ALT and AST enzymes illustrated in this study showed a highly significant ($p<0.001$) elevation in positive HSV-2 DNA than negative cases. These findings indicated that active HSV-2 infection in HCV patients had high influence on activity of ALT and AST enzymes by increasing their levels in sera of HSV-2patients. This data was in agreement with the report of Wald *et al*., (2016), who showed thatHSV-2 causes elevation of fulminant liver failure and chronic hepatitis.

There was high effect of HSV-2infection on the serum activity levels of ALT and AST in HCV patients and immunosuppressed individuals. These findings are revealed in other studies as DNA of HSV-2and other viruses are more frequently encountered in specimens from patients with HCV hepatitis than from subjects without hepatitis (Xu *et al*., 2006)

In HCV negative group (Control group), ALT and AST activity levels in positive HSV-2cases were slightly higher than that in negative cases. All the previous results indicated that the pathogenesis of HCV is influenced by its interaction with HSV-2.These data are in agreement with other studies donated that, HSV-2 infection can cause liver function test abnormalities (Severson *et al.*, 1999). Liver involvement usually causes mild elevation of transaminases and this abnormality resolves spontaneously (Xu *et al*., 2006).

The data of this study showed that infection with HSV-2was prevalent in HCV patients. HSV-1 and HSV-2may exert an immunomodulatory effect resulting in enhanced immunosuppression (Wald *et al*., 2002). The data of this study is in agreement with other studies (Levitsky *et al*., 2008) who reported that, in some patients with chronic liver disease caused by a major hepatotropic virus, an infection with other viral agents may be discovered. We previously evaluated patients with chronic hepatitis B and C regarding their herpes serology.

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