Impact of Varicella-Zoster Virus/ Hepatitis C Virus (VZV/HCV) co-infection on liver enzymes activity

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Abstract

Varicella is a common, benign and self-limiting disease. It is related to hepatitis and malfunction/failure of the liver. This study was designed to assess Varicella-Zoster Virus/ Hepatitis C Virus (VZV/HCV) co-infection on the levels of liver enzymes. A total of 88 blood samples regardless of Hepatitis C Virus positivity were collected. The age and sex of the sample population are included in the demographic statistics. HCV-RNA was detected by RT-nested PCR and VZV-DNA was identified by nested PCR in all the sera. Approximately 27 patients were positive for HCV and VZV co-infection. Liver function was also tested on the basis of parameters such as Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT).

Infection with HCV and VZV is more prevalent in the 34-55 age group tested by nested-PCR and liver function tests based on Alt and AST levels using qualitative test kits. The ALT and AST levels were high 102 and 99.6 IU/L in co-infected cases as compared to 35 and 32 IU/L in normal cases without HCV, HIV and VZV. The study results indicate the impact of VZV in the abnormality of liver function. This study concludes that coinfection with HCV/VZV can be seen as a high-risk category for the development of liver disease where long-term outcomes of the disease should be controlled.

Keywords: HCV, VZV, Liver function, Co-infection.

Introduction

The Varicella-Zoster Virus (VZV) is a member of the alpha-herpesvirus family and contains a double-stranded DNA genome. A very common and generally infantile disease (Primary Varicella) is caused by VZV.[14] A primary VZV infection is followed in a latent state by a persistent viral infection and reactivation can take place later in life[12]. Therefore, in times of decline in immune response such as disease-related stress and drug therapy or during continuous immune system stimulation due to co-infection with other microorganisms or any inflammatory diseases, viral reactivation has been seen.[13] Acute liver failure occurs as part of infection with disseminated VZV. It occurs predominantly in recipients of liver or kidney transplants or immunosuppressed individuals.

In these patients, mild to moderately raised levels of transaminases and numerous histopathological liver alterations were observed.[1,5] This study aimed to investigate the co-infection of Varicella-Zoster Virus (VZV) (antibodies and DNA) in sera samples from patients positive and negative for HCV infection to study the effect of VZV-HCV co-infection on Liver function.

Material and Methods

Sample population: Total 88 samples of males and females were collected from the different hospitals including El-Hosyen General Hospital, Aswan General Hospital, Mansoura University Hospital and Alexandria General Hospital. Ethical approval was taken from the ethical committee of Ain Shams University for this study. 88 cases included 51 females and 37 males with age range (17-58) means (39.51±11.21) years. The study population was divided into two groups: group 1 consisted of patients positive for HCV-RNA (n=48) and the group 2 (n=40) comprised of cases negative for HCV-RNA and VZV). The age, gender, liver enzyme levels (AST and ALT) and HCV-RNA, VZV-DNA were analyzed and registered.

Detection of HCV RNA and VZV DNA: Reverse transcription- PCR was carried out to confirm HCV viremia by using specific nested primers. A 200 µl of serum sample was used to extract RNA with method of acid guanidium thiocyanate phenol-chloroform.[6] For amplification, the unique nucleotide sequence for HCV RNA was as follows: P1: 5'-GGTGCACGGTCTACGAGACCTC-3'; P2: F-5'AACTACTGTCTTCACGCAGAA-3'; P3: R-5'-TGCT CATGGTGCACGGTCTA-3'; Nested reverse primer, P4: 5'-ACTCGGCTAGCAGTCTCGCG-3' and nested forward primer, P5: 5'-GTGCAGCCTCCAGGACCC-3'. The protocol for PCR amplification consisted of denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C and cycle extension for 1 minute at 72°C for 30 cycles. The amplification of nested primers was confirmed on 2% agarose gel and visualized by UV trans-illuminator.

To detect VZV-DNA in samples, nested PCR was performed according to a previously described protocol.[8] For extraction of viral DNA, mini-kit for DNA purification (Madison, USA) was used and extraction protocol was followed according to the manufacturer's instructions. To amplify 556

bp fragment, a PCR 25 µl reaction mixture contained 5 µl DNA template, 0.5 µl dNTPs mix (50mM), 2.5µl 10X PRC-buffer (10mM Tris-HCl pH 8.0, 50mM KCl, 25mM $MgCl_2$), 0.25µl of primers VZV1: 5'-AATGCCGTGACCAC CAAGTATAAT-3' and VZV2: 5'-TACGGGTCTTGCC GGAGCTGGTAT-3', 0.1µl of *Taq* polymerase and 16.4µl deionized water.

The cycling protocol for nested PCR was as follows: initial denaturation at 94 ºC for 5 minutes followed by 35 cycles of denaturation at 94 ºC each for 30 seconds, annealing at 57 ºC for 30 seconds, cycle extension at 72 ºC for 60 seconds and then final extension at 72 ºC for 4 minutes. For amplification of 191 bp fragment of *VZV* gene the second cycle of nested PCR was performed with 2µl of the PCR product of the first reaction but annealing was carried out with internal primers VZV3: 5'-TCCATCTGTCTT-TGTCTTTCA-C-3'and VZV4: 5'-ATTTTCTGGCTCTAA TCCAAGG-3' (Bioneer, Alameda, USA) for amplification of 191 bp fragment in VZV gene, under the same thermal cycling protocol. 2% agarose gel electrophoresis was performed to separate and visualize nested amplification products.

Liver function tests: The liver function was evaluated by the measurement of liver enzymes including the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by using the commercially available kits of Siemens Healthcare diagnostic Inc., USA.

Statistical analysis: Data was analyzed by using SPSS 19.0 version. Data were presented as mean and standard deviation. The comparison of variables like HCV, VZV and the liver function tests was carried out by using the Chi-Square test and a p-value <0.05 was considered statistically significant.

Results

Prevalence of HCV-RNA in studied subjects: Out of total 88 cases, forty-eight subjects were positive for HCV-RNA while 40 cases were documented as negative for HCV-RNA. The nested RT-PCR confirmed the levels of HCV viremia. The confirmation of HCV-RNA was carried out by detecting of 174 bp fragment on 2% agarose gel.

Prevalence of VZV-DNA in patients with HCV versus controls: Figure 1 showed the fragment of 191 bp of nested PCR product of the *VZV* gene in some subjects. Figure 2 showed that in group 1, about 19 (39.5%) HCV patients were positive for VZV-DNA as compared to 5 (12.5%) positive VZV-DNA cases in group 2 (cases without HCV-RNA). The difference between the prevalence of VZV-DNA in group 1 (HCV patients) and group 2 (cases without HCV, HBV) was statistically significant ($p < 0.05$).

Level of serum ALT and AST amongst study groups: Serum levels of liver enzymes (ALT and AST) were slightly decreased in the patient group (HCV patients) as compared to the negative control group (positive VZV-DNA and negative HCV-RNA cases) and to the normal control group (negative HCV, VZV cases). In HCV patients, serum ALT levels was 91.5±15.98 IU/L) and serum AST was 89.3±16.24 IU/L whereas, mean levels of ALT and AST in the negative control group were 48.76±8.4 IU/L and 45.34±10.28 IU/L respectively. Mean serum ALT levels in the normal control group were 35.7±10.4 IU/L) and mean AST levels were (32.47±9.4 IU/L).

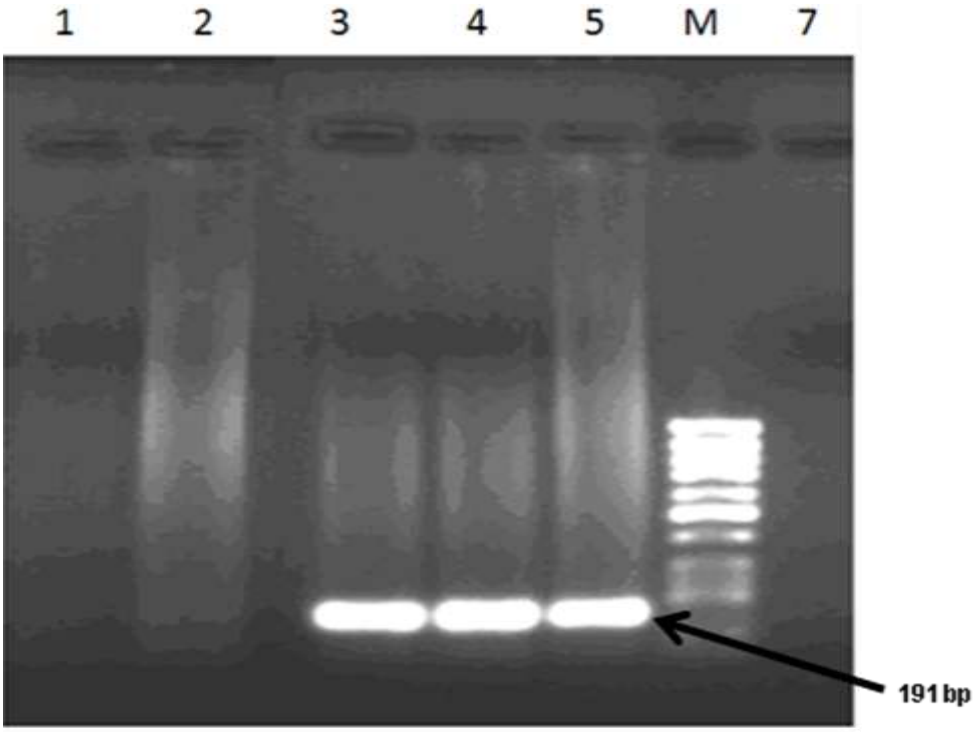

Figure 1: VZV-DNA results of nested PCR

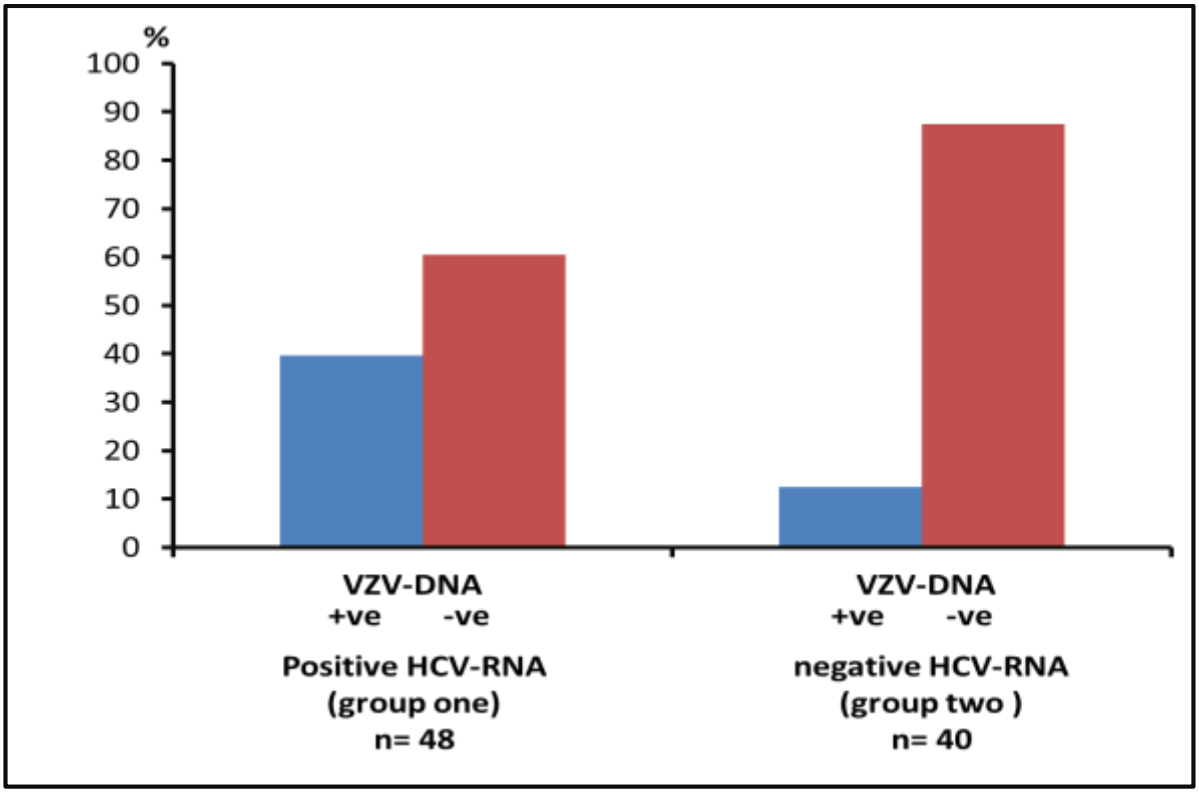

Figure 2: Detection of VZV-DNA in the study groups

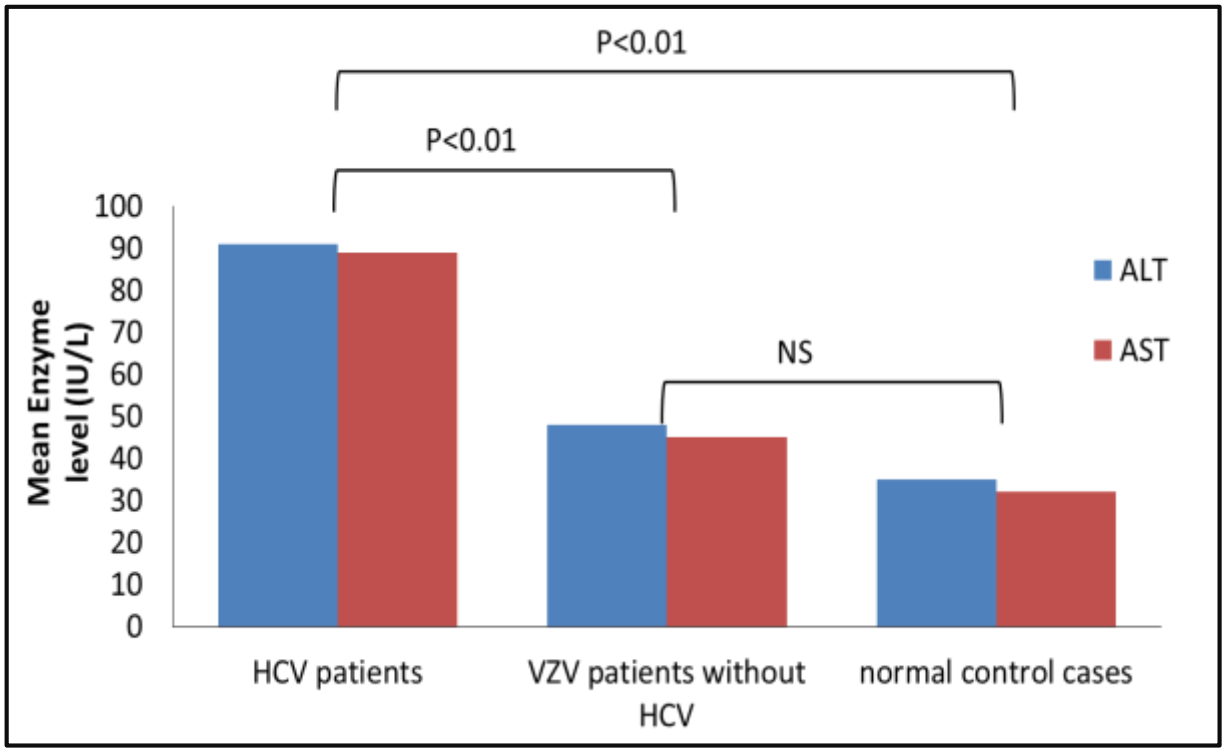

Figure 3: Levels of liver enzymes in study cases
ALT: Alanine Amino Transferase, **AST:** Aspartate Amino Transferase. **NS:** Non Significant

No statistically significant difference was observed in the serum levels of ALT and AST between the negative and normal control groups while serum levels of ALT and AST serum were significantly higher in the patient group than the negative control and normal control groups. Remarkably, in VZV-DNA positive HCV patients, ALT (mean: 102.5±21.8 IU/L) and AST (mean: 99.6±20.1 IU/L) were higher than that of VZV-DNA negative HCV patients in which ALT means were 81.41±14.2 IU/L and AST was 79.8±15.6 IU/L. The difference was highly significant (P<0.01) as shown in figures 3 and 4.

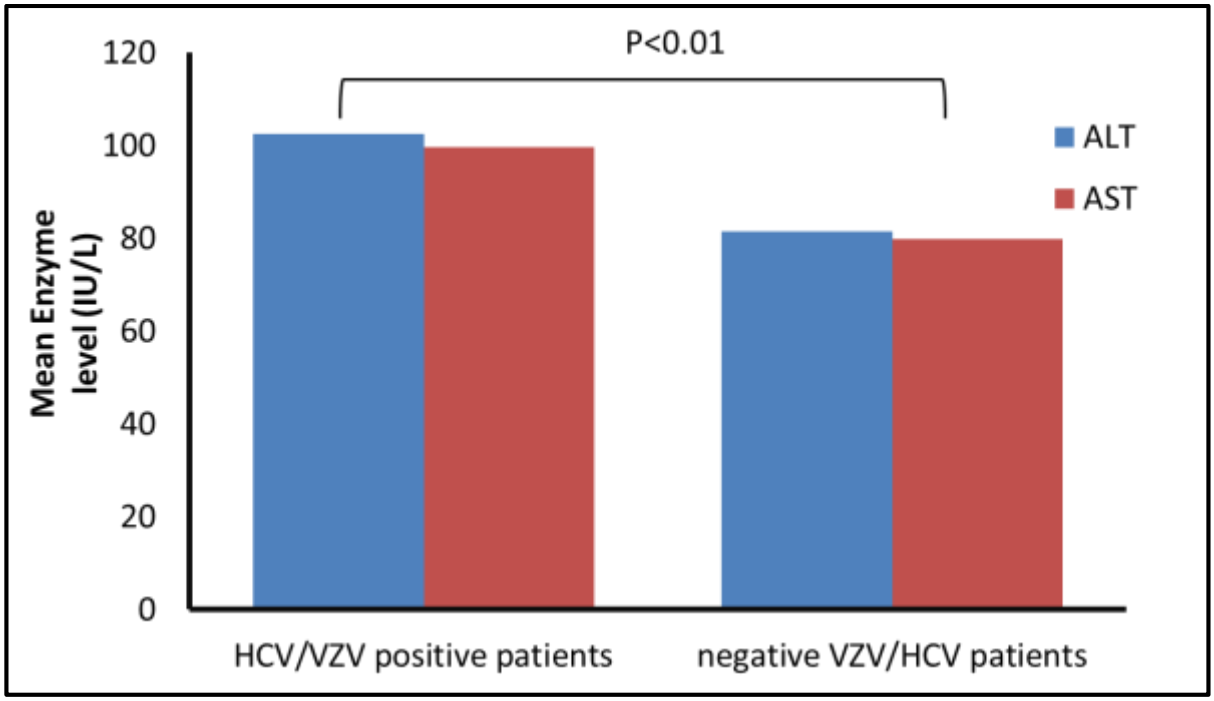

Figure 4: Liver enzymes levels (ALT & AST) in HCV patients positive and negative for VZV

Discussion

The family of herpes viruses is known for its ability to create lifelong infections. Adults are infected mostly with Cytomegalovirus (CMV), varicella-zoster virus (VZV) and Epstein–Barr virus[3]. VZV reactivation is central to the disease pathogenesis in the absence of an effective immune response.[7] Therefore, reactivation of the virus is manifested during periods of decline in immune system control such as drug treatment or during the persistent activation of the immune system such as inflammatory diseases, or simultaneous infections with other viruses.[2] Other studies suggested that VZV can be found in viral hepatitis cases mostly in the form of dual infection and can aggravate the course of the disease.[10] The VZV infection is diagnosed based on the detection of VZV-DNA in the blood. So VZV reactivation in immunosuppressed cases plays a crucial role in disease progress.[7]

This study was planned to determine the presence of VZV in sera of patients with hepatitis C and compared with subjects without hepatitis C (control group) and to study the effect of co-infection on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. To document the presence of VZV in sera of subjects under study, VZV DNA was tested in serum samples by nested PCR.

The present data revealed that the percentage of positive VZV-DNA is higher (P<0.01) in HCV patients compared to HCV-negative cases. Virus –virus interaction is classified by into three items: vial gene or gene product direct interaction, the impact of host environmental change that leads to indirect interaction and immunological interactions. Mean ALT and AST levels in VZV/HCV patients were higher than those obtained from HCV patients without VZV infection which was statistically significant (p<0.001). These findings were similar to Perelló et al[13] who found that all of the VZV reactivations that occurred in our HCV patients, happened during HCV treatment. Since VZV has immunomodulating properties[9] so in critically ill patients, it was thought that VZV infection could accelerate HCV pathogenesis. This suggestion was confirmed by the results of this study that in VZV-positive patients, both the mean ALT and AST levels were higher as compared to the VZV-negative cases.

Conclusion

Acute liver failure differential diagnosis involves several etiologies including medications, chronic hepatitis, toxins and physiological and infectious etiologies including mainly viruses (hepatitis A-E, CMV, VZV, EBV, HSV and adenovirus).[5] Interactions between viruses and viruses have been shown to affect the pathogenesis of viral infections in humans.[4,11]

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