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Prevalence of Epstein - Barr virus infection in Hepatitis C Patients

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ABSTRACT

Background: Epstein - Barr virus (EBV) may be an omnipresent, common infective agent of severe illness in patients with impaired immune functions. Reactivation of Epstein-Barr virus in immunocompetent host is typically symptomless, however could deteriorate the prognosis of patient with chronic illness.

Objectives: This study was conducted to detect EBV infection in patients with chronic hepatitis C virus (HCV) infections and to point out the effects of EBV-HCV co-infections on liver enzymes activity.

Study design: Expression of EBV-DNA was determined in Serum samples by nested polymerase chain reaction (nested-PCR) method. There were 79 chronic HCV within the study group. Control group consisted of 52 cases without viral hepatitis.

Results: EBV-DNA infection was demonstrated in 29% of chronic HCV patients. Although alanine aminotransferase (ALT) and (AST) levels of EBV-infected HCV patients were increased.

Conclusion: We conclude that EBV infection is common in chronic HCV patients, who can be regarded as patients at high risk for EBV disease.

INTRODUCTION

Epstein-Barr virus (EBV) is a ubiquitous, worldwide pathogen that is harbored persistently by virtually all adults, regardless of geographic location (Anne, 2005). EBV is a member of the *Gammaherpesvirinae* subfamily of herpes viruses. It infects more than 90% of the adult population all over the world (Henry *et al*., 2013). EBV shares the tendency of establishing latency in the host with other herpes viruses (Petrova *et al*., 2010). Primary infection leads to transitional viremia, followed by a strong T-cell adaptive immune response, which actually holds the infection latent in immunocompetent individuals (Cohen *et al*., 2009). Short episodes of spontaneous reactivation and consequent viral replication normally occur in healthy individuals (Paschale *et al*., 2012).

In the immunocompetent individual the occurrence of EBV reactivation leading to immortalization of B-lymphocytes is strongly regulated by cytotoxic T lymphocytes (CTLs) specific for lytic and latent antigens (Petrova *et al*., 2010). The role of EBV in the evolution of chronic hepatitis from hepatotropic viruses is considered. Chronic EBV associated hepatitis is suspected in immunocompetent adults with compatible serology, suggestive histology and detection of the viral genome in the liver and/or increase of specific circulating cytotoxic T-lymphocytes (Dejcinov *et al*., 2011). Co-infections with EBV or CMV in patients infected with HCV have been proven to accelerate the course of chronic hepatitis C thus leading to a more severe histological picture and facilitating the disease progression to fibrosis, cirrhosis and hepatocellular carcinoma (Julio *et al*., 2007).

The present study aimed to investigate the incidence of co-infection of Epstein - Barr virus (EBV) in sera samples from patients (cases with positive HCV infection) and controls (cases with negative HCV infection). Study the effect of EBV pathogenicity on the changes in the liver functions (like liver enzymatic activity of ALT and AST).

MATERIALS AND METHODS

Study population

The study consisted of a patient group (n = 79) with chronic viral hepatitis C and a control group (n = 52) without viral hepatitis. We evaluated 79 consecutive patients with chronic hepatitis C (mean age 44.6±11.3; range: 17–68; 44 males, 35 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 = 52; mean age 31.34±8.7 range: 18–58; 32 males, 20 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 EBV (anti-EBV-IgM, anti-EBV IgG), EBV-DNA of the serum samples of the cases were assessed and recorded.

Serological analysis of EBV infection

EBV-IgM and EBV-IgG antibodies were determined by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available EBV-IgM and IgG Kits. EBV-IgM antibodies were determined by commercially available Diagnostic Automation EBV-IgM Kit (Diagnostic Automation, INC 23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302, USA). EBV-IgG antibodies were determined by commercially available ATLAS Medical, William James House, Cowley Road, Cambridge, CB4 0WX, UK. Tests were done according to the instructions of the manufacturer.

Detection of HCV RNA

RNA was isolated from serum samples as described by Lohr *et al*., (1995) and El Awady *et al*., (2006). Reverse transcription-nested PCR was carried out according to Chomczynski and Sacchi (1992). Primers used for detecting HCV in clinical samples were purchased from Promega and their sequences were as following: P1: 5\ GGTGCACGGTCTACGAGACCTC 3\ – P2: 5\ AACTACTGTCTTCACGCAGAA 3\ - P3: 5\ TGCTCATGGTGCACG GTCTA 3\ -P4: 5\ ACTCGGCTAGCAGTCTCGCG 3\ - P5: 5\ GTGCAGCCTCCAGGACCC 3\ (Madison, WI, USA).

Detection of EBV-DNA

Viral nucleic acid DNA was extracted from serum sample using Wizard® DNA purification mini kit, Promega (Madison, USA). Nested PCR of serum samples for detection of EBV-DNA was carried according to (Kapranos *et al*., 2003). The reaction mixture of the qualitative PCR contained, in total volume of 25 µl, 5 µl 10x buffer (10mM Tris-HCl pH 8.0, 50mM KCl, 25mM $MgCl_2$), 0.5 µl 50mM dNTP mix, 0.25 µl of primers E2P1: 5\ ATCCTTGCACTTAGCCAAGC 3\ and E2P2: 5\ TCCAGATGTGTCTCCCTTCT 3\ (Bioneer, Atlantic Avenue, Alameda, USA)

for amplification of 556 bp. Except for internal primers Ap1: 5\ CCAGTAGCATCTCTGTCTGG 3\ and AP2: 5\ GAACCATCCTCGTCCTCATC 3\ (Bioneer, Atlantic Avenue, Alameda, USA) for amplification of 190 bp.

Agarose gel electrophoresis and analysis of nested-PCR product.

Analysis of nested-PCR products were performed according to Aaij and Borst, (1972); Ergazaki *et al*. (1994). Amplification products of both HCV RT-nested PCR and EBV nested-PCR 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). Tests were done according to the instructions of the manufacturer.

RESULTS

Detection of anti-EBV antibodies in study and control groups

From the study group, 45 out of 79 (56.9%) chronic HCV were positive for EBV-IgG antibodies (Table 1). Sera from 18 out of 52 (34.6%) individuals from the control group were positive for EBV-IgG antibodies (Table 2).

Three patients out of 79 (3.8%) chronic HCV were positive for EBV-IgM antibodies (Table 1). One case out of 52 (1.9%) from the control group was positive for EBV-IgM antibodies (Table 2).

Table 1: Detection of EBV-IgG and IgM antibodies among patient group

		Positive EBV-IgG samples				Positive EBV-IgM samples			
Sex		Male		Female		Male		Female	
		No.	%	No.	%	No.	%	No.	%
		27	60	18	40	2	66.7	1	33.3
Total		45/79 (56.9%)				3/79 (3.8%)			
Age	Range	19 - 61				19 - 61			
	Mean	42.3±19.5				42.3±19.5			

Table 2: Detection of EBV-IgG and IgM in Control group

		Positive EBV-IgG samples				Positive EBV-IgM samples			
sex		Male		Female		Male		Female	
		No.	%	No.	%	No.	%	No.	%
		9	50	9	50	1	100	-	-
Total		18/52 (34.6%)				1/52 (1.9%)			
Age	Range	19 - 58				50			
	Mean	32.4±9.75				50±1.0			

Detection of EBV-DNA by nested PCR

In chronic HCV patients, EBV-DNA was positive in 29% (23/79) of the serum samples. EBV-DNA was detected in 4 out of 52 (7.7%) samples obtained from the control group (Fig 1). The difference between the presence of EBV-DNA in patients with chronic HCV infection and the control group was statistically significant ($p < 0.01$).

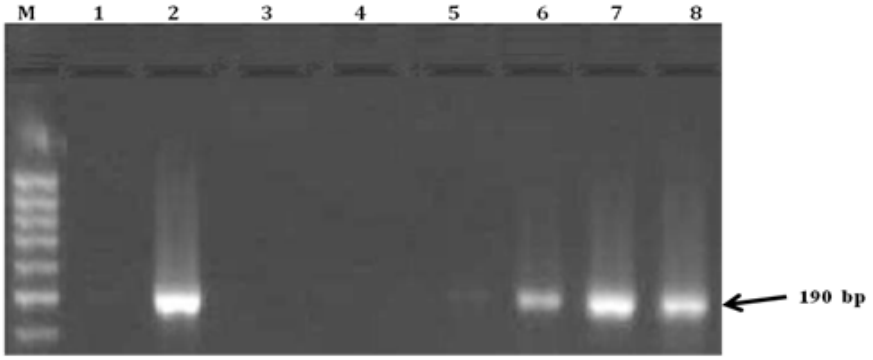

Figure 1: Nested PCR results of serum samples positive for EBV lane 2,6,7 and 8 while lane 1,3,4 and 5 were Negative for EBV.

Serum levels of the liver transaminases in the study groups

In positive HCV-RNA patient group, serum ALT activity levels was detected as 81.89±16.2 IU/L which means that, it was higher than that of control group 32.98±12.7 IU/L. Also serum AST activity level of HCV patient group was 80.18±15.8 IU/L and higher than that of control group 31.7±12.46 IU/L (Table 3 and Fig. 2).

Table 3: ALT& AST activity levels in study groups

Group / Variable	patients group	Normal control group	P-value
Mean ALT (IU/L)	81.89±16.2	32.98±12.7	<0.001
Mean AST (IU/L)	80.18±15.8	31.7±12.46	<0.001

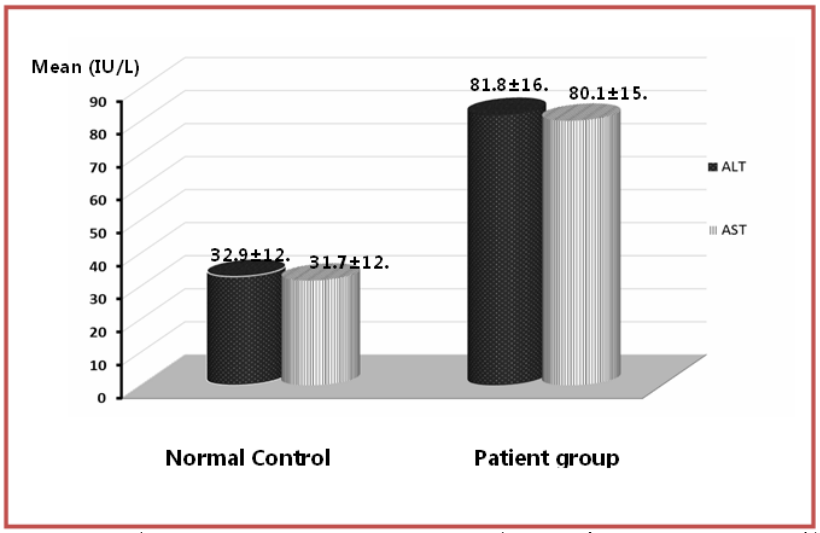

Fig. 2 roups

► In HCV-positive group (patient group), serum ALT levels (mean: 96.6±10.4 U/l) in EBV-positive patients were slightly higher than that of EBV-negative patients (mean: 75.3±15.2 IU/l). Serum AST activity level of EBV-positive patients (mean: 93.9±11.3 IU/L) was slightly higher than that of EBV negative patients (mean: 73.4±14.6 IU/L) (Table. 4 and Fig. 3).

Table 4: ALT& AST activity levels in EBV Patient group

Group / Variable	Positive EBV patients group	Negative EBV patients group	P-value
Mean ALT (IU/L)	96.6±10.4	75.3±15.2	<0.001
Mean AST (IU/L)	93.9±11.3	73.4±14.6	<0.001

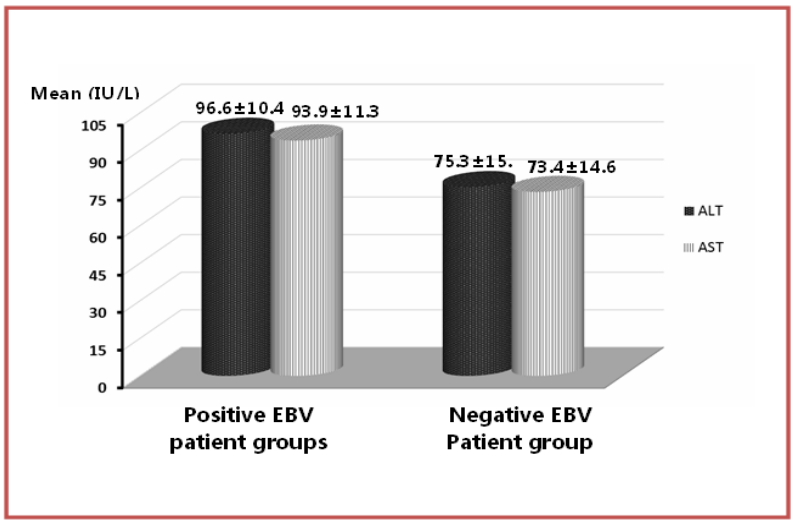

Fig. 3: The activity level of ALT and AST liver enzymes in EBV patient group

► In Control group, serum ALT levels (mean: 46.1±3.4 U/l) in EBV-positive patients were slightly higher than that of EBV-negative patients (mean: 31.8±12.6 U/l). Serum AST activity level of EBV-positive patients (mean: 44.2±2.9 IU/L) was slightly higher than that of EBV negative patients (mean: 30.6±12.4 IU/L) (Table. 5 and Fig. 4).

Table 5: ALT& AST activity levels in EBV Control group

Group / Variable	Positive EBV Control group	Negative EBV Control group	P-value
Mean ALT (IU/L)	46.1±3.4	31.8±12.6	>0.05
Mean AST (IU/L)	44.2±2.9	30.6±12.4	>0.05

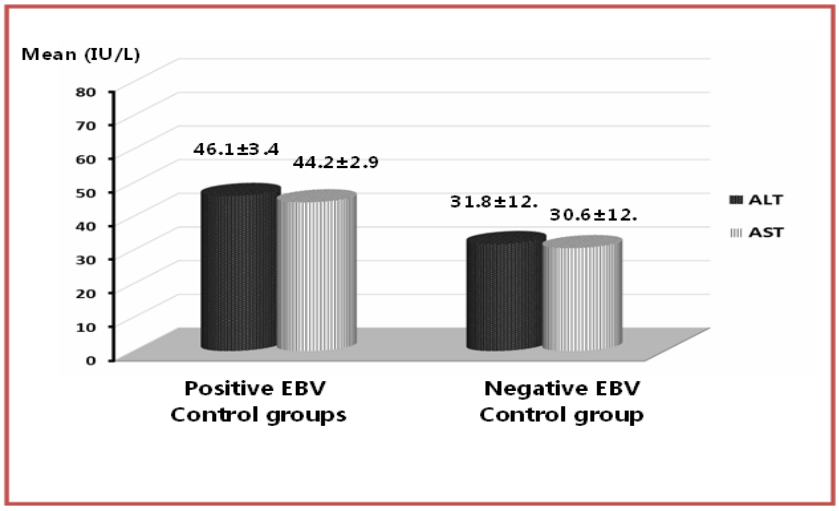

Fig. 4: The activity level of ALT and AST liver enzymes in EBV control group

DISCUSSION

Epstein - Barr virus infection is characterized by alternating periods of latency and reactivation. Replication of EBV in the absence of an effective immune response is central to the pathogenesis of the disease (Ning, 2011). 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 (IARC, 2011). Virus–virus interactions have been demonstrated to modify the pathogenesis of human viral infections (Palma *et al*., 2010).

In this study, we investigated the incidence of EBV infection in Egyptian HCV patients who progressed to chronic HCV infection and we examined the potential role that EBV plays in HCV progression. The present data showed that the percent of positive EBV Abs were significantly higher ($P < 0.001$) in chronic HCV patient than those in Control group. Also, the EBV DNA was detected in 29% of chronic HCV infected patients compared with 4 out of 52 (7.7%) Control group cases. The difference between the presence of EBV DNA among chronic HCV patients and the control group was statistically significant ($p < 0.05$). Moreover, the results confirmed that the detection of EBV DNA by PCR in peripheral blood leukocytes is a sensitive and reproducible procedure for detecting viral infection. As the serological methods reported to be insensitive and can't distinguish between EBV infection and EBV disease as IgM antibodies may persist for months or years and may be detected during reactivation of latent virus infections (Shibuya *et al*., 2003).

Due to the fact that EBV has immunomodulating properties, Gallegos-Orozca *et al*., (2010) it was presumed that reactivation of EBV could accelerate HCV pathogenesis in critically ill patients.

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 EBV-DNA than negative individuals. These findings indicated to the active EBV infection in chronic HCV patients that had high influence on activity of ALT and AST enzymes by increasing their levels in sera of EBV patients. These findings indicated to primary and reactivated EBV infections in chronic HCV patients had high effect on liver enzymes, which seems to be attributed to the immune system of the chronic HCV patient. The primary and reactivated EBV infections were interacted with HCV and raised the influence on the liver enzymes, as with other herpes viruses (Petrova and Kamburov, 2010).

Latency follows all primary infections and is considered to be lifelong, where EBV can reactivate periodically under the influence of exogenous and endogenous factors and cause immunosuppression (Hinedi TB *et al*., 2003). So there was high effect on serum activity levels of ALT and AST by EBV infection on HCV patients and immunosuppressed individuals, these findings are revealed with other studies, DNA of some types of HHVs (CMV, EBV and HHV-6) are more frequently encountered in specimens from patients with HCV hepatitis than from subjects without liver hepatitis (Claudio *et al*., 1999). HCV replication was promoted by EBV and that EBNA1 was responsible for supporting HCV replication (Sugawara *et al*., 1999; Cacopardo *et al*., 2003).

In HCV negative group (Control group), ALT and AST activity levels in positive EBV-DNA cases were slightly higher than that in negative cases. All the previous results indicated that the pathogenesis of HCV is strongly influenced by its interaction with EBV. These findings are in agreement with other studies donated that, Epstein- Barr virus infection can cause liver function test abnormalities without

pharyngitis or lymphadenopathy (Dogan *et al*., 2007). Liver involvement usually causes mild elevation of transaminases and this abnormality resolves spontaneously. Jaundice might develop rarely during the clinical course of Epstein- Barr virus infection. It reflects either more severe hepatitis or Epstein-Barr virus infection-associated hemolytic anemia (Hinedi and Koff, 2003).

The result of this study indicated to the infection with EBV was prevalent in HCV patients. This may be attributed to several reasons: Differences in exposure to EBV infection may be excluded because this is ubiquitous virus infecting almost the whole population since infancy (Claudio *et al*., 1999). EBV viruses may exert an immunomodulatory effect resulting in enhanced immunosuppression (Anne *et al*., 2007).

These pervious findings are in agreement with Other studies (Li *et al*., 2004; Petrova *et al*., 2010) 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 EBV serology. Petrova *et al*. (2010) reported that, The patients with reactivated EBV infection had lower levels of HBV DNA and higher mean values of serum hepatitis C virus (HCV) RNA respectively, compared to EBV-seropositive patients without reactivation.

REFERENCES

Aaij, C. and Borst, P. (1972). The gel electrophoresis of DNA. Biochem. Biophys. Acta, 269:192.

Anne, K. and Junker, MD. (2005). Epstein - Barr virus Pediatrics in Review Vol.26, No.3.

Cacopardo, B.; Nunnari,G.; Mughinil, M.; Tosto, S.; Benanti, F.; and Nigro, E.L. (2003). Fatal hepatitis during Epstein-Barr virus reactivation. European Review for Medical and Pharmacological Sciences; 7: 107-109.

Chomczynski, P.; and Sacchi, N. (1992): Single-step method of RNA isolation by acid guanidium- thiocyanatephenol-chloroform extraction. Anal. Biochem; 162, 156-159.

Claudio, C.; Mauro, C.; Paola, P.; Marisa, M.; Anna, M.; Teresa, S.; and Antonio, D. (1999). Herpesvirus DNA is frequently detected in liver tissue from hepatitis C patients, Journal of Clinical Virology; 14, 9–16.

Cohen, J.I.; Kimura, H.; Nakamura, S.; Ko, H.; and Jaffe, E. (2009). Epstein–Barr virus-associated lymphoproliferative disease in non-immunocompromised hosts: a status report and summary of an international meeting. Annals of Oncology; 20, 1472–1482. doi:10.1093/annonc/mdp064

Dogan, I.; Meltem, E.; Mehmet, C.; and Selahattin, U. (2007). Acute hepatitis induced by Epstein- Barr virus infection, Turk J Gastroenterol; 18 (2): 119-121.

Dojcinov, S.D.; Venkataraman, G.; Pittaluga, S.; Wlodarska, I.; Schrager, J.A.; Raffeld, M.; Hills, R.; and Jaffe, E.S. (2011). Age-related EBV-associated lymphoproliferative disorders in the Western population: a spectrum of reactive lymphoid hyperplasia and lymphoma. BLOOD, V: 117; 4726-4735. doi:10.1182/blood-2010-12-323238

El Awady, M.; Badr El Din, N.; El Garf, W.; Youssef, S.; Omran, M.; El Abd, J.; and Goueli, S. (2006): Antisense oligonucleotide inhibition of hepatitis C virus genotype 4 replication in HepG2 cells. Cancer Cell International; 6, 18

El Awady, M.; Tabll, A.; El-Abd, Y.; Bahgat, M.; Shoeb, H.; Youssef, S.; Bader El Din, N.; Redwan, M.; El-Demellawy, M.; Omran, M.; El-Garf, W.; and Goueli, S. (2006): HepG2 cells support viral replication and gene expression of hepatitis C virus genotype 4 in vitro. World J Gastroenterol.; 14, 4836-4842.

Ergazaki, M.; Xinarianos, G.; Giannoudis, A.; and Markomichelakis, N. (1994): Detection of HSV, CMV and EBV by the polymerase chain reaction technique in patients with inflammatory eye diseases. *Oncology Reports*; 1, 1207-1210.

Gallegos-Orozco, J.F.; and Jorge, R. (2010). Hepatitis viruses: Not always what it seems to be. *Rev Med Chile*; 138, 1302-1311

Henry, H. and Priya, V. (2013). Primary Epstein–Barr Virus Infection: Impact of Age at Acquisition, Co-infection, and Viral Load. *Journal of Infectious Diseases*. DOI: 10.1093/infdis/jit096

Hinedi TB, Koff RS. (2003). Cholestatic hepatitis induced by Epstein-Barr virus infection in an adult. Diges Dis Sci; 48, 539-41

IARC (2011). Epstein-barr virus. IARC Monogr Eval Carcinog Risks Hum, 70: 49 – 92

Julio, C.; Medina1; Graciela, P.; and José, M. (2007): Interactions between Cytomegalovirus and Other Viruses (HHV6, HHV7, HCV and EBV) in Transplantation. Trends in Transplantation; 1, 129-136.

Kapranos, N.; Petrakou, E.; Anastasiadou, C.; and Kotronias, D. (2003). Detection of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in the semen of men attending an infertility clinic. FERTILITY AND STERILITY_ VOL. 79, SUPPL. 3. doi:10.1016/S0015-0282(03)00370-4

Li, W.; Wu, B.; Zeng, Y.; Chen, G.; Li, X.; Chen, J.; Guo, Y.; Li, M.; and Zeng, Y. (2004). Epstein-Barr virus in hepatocellular carcinogenesis. World J Gastroenterol; 10(23):3409-3413

Lohr, HF.; Goergen, B.; Meyerzum, KH.; Gerken, G. (1995): HCV replication in mononuclear cells stimulates anti-HCVsecreting B cells and refl ects nonresponsiveness to interferonalpha. J Med Virol; 46, 314-320

Ning, S. (2011). Innate immune modulation in EBV infection. Ning Herpesvirida , 2:11.

Palma, T. Da.; Doonan, B.P.; Trager, N.N.M.; and Kasman, L.M. (2010). A systematic approach to virus-virus interactions VIRUS RESEARCH DOI: 10.1016/j.virusres.

Paschale, M.D.; and Clerici, P. (2012). Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. World J Virol; 1(1): 31-43. doi:10.5501/wjv.v1.i1.31

Petrova, M.; and Kamburov, V. (2010). Epstein-Barr virus: Silent companion or causative agent of chronic liver disease? World J Gastroenterol; 16(33): 4130-4134. doi:10.3748/wjg.v16.i33.4130

Petrova, M.; Kamburov, V.; Nikolovska, D.; Kosseva O, Nikolova, M.; and Krastev, Z. (2010). Epstein-Barr virus: is there any contribution to chronic hepatitis B and C? Liver Int; 30, 488-489

Sugawara, Y.; Makuuchi, M.; Kato, N.; Shimotohno, K.; and Takada, K. (1999). Enhancement of hepatitis C virus replication by Epstein-Barr virus-encoded nuclear antigen 1. EMBO J; 18: 5755-5760

ARABIC SUMMARY

مدي انتشار الإصابة بفيروس الإبشتاين بار في المرضي المصابين بالالتهاب الكبدي الوبائي سي

حسام غانم [1]- سحر شومان[1]- محمد نبيل[1]- أشرف طبل[2]

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2- قسم التكنولوجيا الطبية الحيوية - المركز القومي للبحوث - الجيزة - مصر.

يعتبر فيروس الابشتاين بار من الفيروسات واسعة الانتشار في اغلب البشر، وهو من الفيروسات التي تظل كامنة داخل خلايا الجسم الي ان يحدث خلل في الجهاز المناعي مما يسبب في نشاط هذا الفيروس مما يؤدي الي ظهور امراض خطيرة. و لقد صممت هذه الدراسة لتقصي ما مدي انتشار فيروس الابشتاين بار في المرضي المصابين بالتهاب الكبدي الوبائي (سي). وتضمنت هذه الدراسة مجموعتان المجموعة الاولي وهي الحالات المصابة بالالتهاب الكبدي الوبائي سي وعددهم حوالي 79 حالة اما المجموعة الثانية وتضمنت 52 حالة وهي الحالات الغير مصابه بفيروس الالتهاب الكبدي الوبائي سي. ومن خلال الدراسة تبين ان فيروس الابشتاين بار منتشر بين الحالات المصابة بالالتهاب الكبدي الوبائي سي وهذه الاصابة المزدوجة ادت الي ارتفاع معدل انزيمات الكبد في هؤلاء المرضي.