Mansoura Journal of Chemistry Vol. 35 (1), June, 2008.

DETERMINATION OF TB INFECTION IN HEPATITIS C VIRUS-INFECTED INDIVIDUALS

Attallah A.M.[1]; Attia M.A.[1] and El-Waseef A.M.[2]
[1]R & D Dept., Biotechnology Research Center, New Damietta City, Egypt.
[2]Chemistry Dept., Faculty of Science, Mansoura University, Egypt.
(Received: 16 / 2 / 2008)

ABSTRACT

Hepatitis C virus (HCV) is a major etiological factor in chronic hepatitis, affecting up to 24% of blood donors, and about 75% of chronic liver diseases patients in Egypt. Tuberculosis (TB) is an air born disease, caused by *M. tuberculosis*. The two epidemics fuel each other, together making the leading infectious causes of mortality worldwide. The present study aimed at the detection of 55 kDa circulating TB antigen (TB-Ag) using Western blot and ELISA technique among chronic hepatitis C (CHC) patients. Serum samples from 264 CHC patients in addition to selected 100 healthy individuals were used as controls. All serum samples were screened for HCV NS4 antigen (HCV NS4-Ag), 176 CHC patients out of 264 were positive for HCV NS4-Ag, and total sera of 100 healthy controls were negative for HCV NS4-Ag. The 55 kDa TB-Ag was identified using Western blot technique. The 55 kDa TB-Ag was screened utilizing ELISA in 176 CHC patients and 100 healthy controls. Detection rate of TB infection was 46% (81 of 176 patients) among CHC patients, while it was 3% (3 of 100 individuals) in the healthy controls. No significant correlation ($p>0.05$) was shown between the serum TB-Ag levels and the HCV NS4-Ag levels. Our results explained that there is a relation between HCV and TB infection, so the high TB prevalence and recurrence in Egypt may be due to the elevated incidence of HCV throughout the country.

Correspondence:
Dr. Abdelfattah M. Attallah-Director, Biotechnology Research Center, New Damietta-Egypt, Tel. : 020572402889
Fax: 020572401889 E-mail: amattallah@hotmail.com.

INTRODUCTION

HCV was first identified in 1989, as the virus responsible for most transfusion-associated non-A non-B hepatitis **[WHO (1997)]**. The World Health Organization has declared HCV a global health problem, with approximately 170 million people (3%) of the world's population infected with HCV. Egypt has the highest prevalence of adult HCV infection in the world, averaging about 15% to 25% in the general population **[El-Raziky et al., (2007)]**. HCV is mainly transmitted through contact with infected blood. In Egypt, the major route of exposure to HCV appears to be the injection of antischistosomal treatment, although schistosomiasis was the major public health problem in the past, HCV has become the most significant problem in Egypt **[Frank et al., (2000)]**. Due to the lack of efficient prevention, such as therapy and vaccines, an accurate early diagnosis is essential for the preventing transmission of the disease **[Li et al., (2007)]**. HCV infection has been associated with a number of extra-hepatic manifestations, including hematological, dermatological and renal disorders, autoimmune manifestations and neurological disorders **[Hoofnagle (2002)]**. Lung involvement has also been integrated into this list. HCV infection is etiologically involved in the development of several pulmonary abnormalities **[Erturk et al., (2006)]**. *Mycobacterium tuberculosis* (*M. tuberculosis*), the cause of TB, spreads through the air, is the most effective pathogen on earth, infecting about one-third of the world's population, in large measure because of its latent state **[Paul & Stephen (2002)]**. About 2 billion humans with latent disease generally are not ill but serve as a pool from which about 8-10 million active cases of TB are drawn annually, and approximately 2 million people are died each year **[Gautam et al., (2007)]**. **Attallah et al., 2003*b* & 2005** identified the target 55 kDa TB-Ag in different body fluids of pulmonary and extra pulmonary TB patients. The aim of the present study was the investigation of TB infection using the target 55 kDa circulating TB-Ag among CHC patients.

MATERIALS AND METHODS

Samples:

Serum samples of 264 CHC patients (aged 20 to 65 years, mean age 40.39 ± 8.579 and median 39) were collected. They were 191 males

and 73 females. In addition to selected 100 serum samples from healthy individuals (74 males and 26 females) were included as controls. Each subject in the present study had negative antibody test results for HAV, HBV, and HIV. All patients with CHC were positive for anti-HCV antibodies (100%), and all of 100 healthy individuals were negative for anti-HCV antibodies.

SDS-PAGE and Western blot:

Serum samples were subjected to SDS-PAGE, at 50 µg/lane, using vertical slabs of 12% polyacrylamide **[Laemmli (1970)]**, according to **[Attallah et al., (2003*b*)]**.

Detection of HCV NS4-Ag using ELISA:

HCV NS4-Ag was detected according to **Attallah et al., (2003*a*)**. After optimization of the reaction conditions, flat-bottomed, polystyrene, microtiter plates (Costar, USA), were coated with diluted serum samples in coating buffer to bind overnight to wells of ELISA plates. After blocking, 50 µl (per well) of 1:200 dilution, in PBS with 0.5% (v/v) Tween 20 (PBS-T20), of a human serum samples were added to each well. Serum from healthy persons was used as negative controls. The plates were incubated at 37°C for 2 h, washed, and then incubated, at 37°C for 1 h, with Anti-rabbit IgG alkaline phosphatase (Whole molecule, Sigma), conjugate developed in goat was diluted (1:700) in PBS-T20 containing 0.2% BSA. After washing, the substrate (p-nitro-phenyl phosphate in 0.1 M glycine buffer; pH 10.4) was added and the plates incubated for 30 min at 37°C. Optical densities (OD) were read at 490 nm using a microplate autoreader (Metertech Inc, USA). The cut-off OD for ELISA positivity was set as mean OD plus three SD for the sera from healthy individuals (cut-off = 0.2).

Detection of TB-Ag using ELISA:

After optimization of the reaction conditions, flat-bottomed, polystyrene, microtiter plates (Costar, USA), were coated with diluted serum samples in coating buffer to bind overnight to wells of ELISA plates. After blocking, 50 µl (per well) of 1:75 dilution, in PBS with 0.2% (v/v) Tween 20 (PBS-T20), of human serum samples were added to each well. Serum from healthy individuals was used as negative controls. The plates were incubated at 37°C for 2 h, washed, and then incubated, at 37°C for 1 h, with Anti-rabbit IgG alkaline phosphatase (Whole molecule, Sigma), conjugate developed in goat was diluted

(1:500) in PBS-T20 containing 0.2% BSA. After washing, the substrate (p-nitro-phenyl phosphate in 0.1 M glycine buffer; pH 10.4) was added and the plates incubated for 30 min at 37°C. Optical densities were read at 490 nm using a microplate autoreader (Metertech Inc, USA). The cut-off was set as mean OD plus three SD for the sera from healthy individuals (cut-off = 0.3).

RESULTS

Identification of the TB-55 mAb target antigen in serum samples using SDS-PAGE and Western immunoblotting assays:

SDS-PAGE separates proteins by size alone. Because sodium dodecyl sulfate is a negatively charged ionic detergent binds to protein in direct proportion to molecular weight. Antigenic extracts from sera of TB infected individuals were subjected to SDS-PAGE, and Western blotting was carried out to determine the target epitope of TB-Ag. The coomassie blue stained separated polypeptides have a wide range of molecular weights ranged from 215 kDa to 18.3 kDa. The gel was visualized and photographed, see **Figure 1**. The target antigen of the TB-55 mAb was identified by the Western blot at 55 kDa in serum samples from CHC patients according to **[Attallah et al., (2003*b*)]**.

Detection of HCV NS4-Ag in serum samples of CHC patients using ELISA:

ELISA technique of **[Attallah et al., (2003*a*)]** as a sensitive and specific assay was used to detect HCV NS4-Ag in serum. Diluted serum samples in coating buffer were allowed to bind overnight to wells of ELISA plates. Specific antibodies to HCV NS4-Ag were added and then alkaline phosphatase conjugated goat anti-rabbit IgG were applied. All of 264 patients with CHC were tested by ELISA for the detection of HCV NS4-Ag against sera collected from 100 healthy individuals. Serum samples of 176 CHC patients (67%) out of 264 were positive for HCV NS4-Ag, and all of 100 healthy controls were negative for HCV NS4-Ag, see **Figure 2** and **Figure 3**.

Detection of TB-Ag in sera of CHC patients using ELISA:

It has been suggested that immunoblotting is not suitable for routine detection of TB-Ag. Certainly, for laboratories with limited resources, this could prove difficult. TB-55 mAb was used as a probe in ELISA to detect TB-Ag in serum samples. Detection of TB-Ag using

ELISA is a simple, rapid, and a qualitative assay technique. Serum samples of 176 patients with CHC were tested by ELISA for the detection of TB-Ag against sera collected from 100 control individuals. The detection rate of TB infection was 46% (81 of 176 patients) among CHC patients, while it was 3% (3 of 100 individuals) in healthy controls, see **Figure 4** and **Figure 5**.

Correlation between TB-Ag levels and HCV NS4-Ag levels in sera of CHC patients:

A total of 81 selected cases showing positive ELISA results for both TB-Ag and HCV NS4-Ag were included for the correlation. There was no significant correlation ($P>0.05$, $r = -0.093$) was shown between the serum TB-Ag levels (OD) and the HCV NS4-Ag levels (OD), see **Figure 6**.

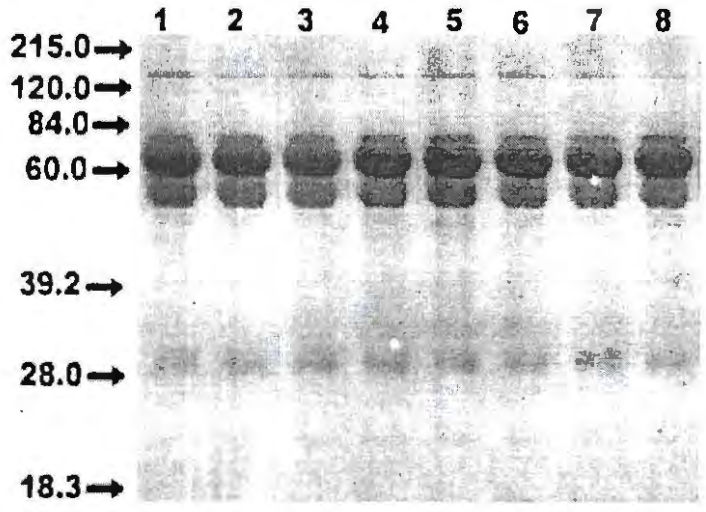

Fig. (1): Coomassie blue stained SDS-PAGE showing the polypeptide pattern of serum samples of CHC patients and selected healthy controls.

Lanes 1-2: Serum of healthy control individuals.

Lanes 3-8: Serum of patients with CHC.

Molecular weight marker includes: Myosin (215.0 kDa), phosphorylase B, (120.0 kDa), Bovine serum albumin (84.0 kDa), Ovalbumin (60.0 kDa), carbonic anhydrase (39.2 kDa), trypsin inhibitor (28.0 kDa), and lysozyme (18.3 kDa).

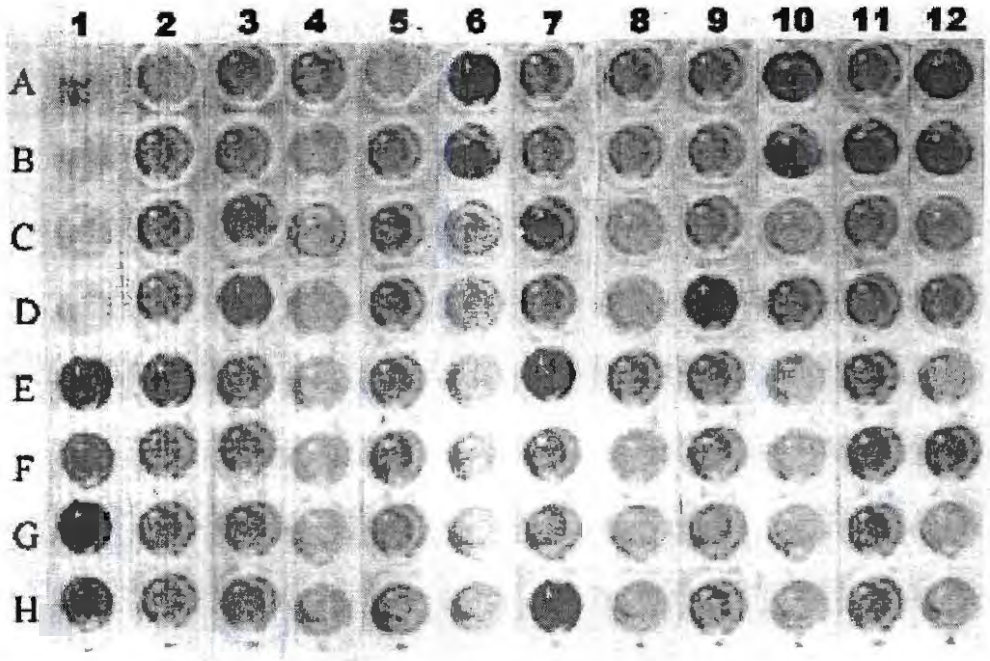

Fig. (2): The optical densities (at 490 nm) of serum samples tested for HCV NS4-Ag using ELISA.

A1-D1 wells: Negative control sera.
E1-H1 wells: Positive control sera.
A2 to H12 wells: Selected 88 out of 264 screened serum samples.

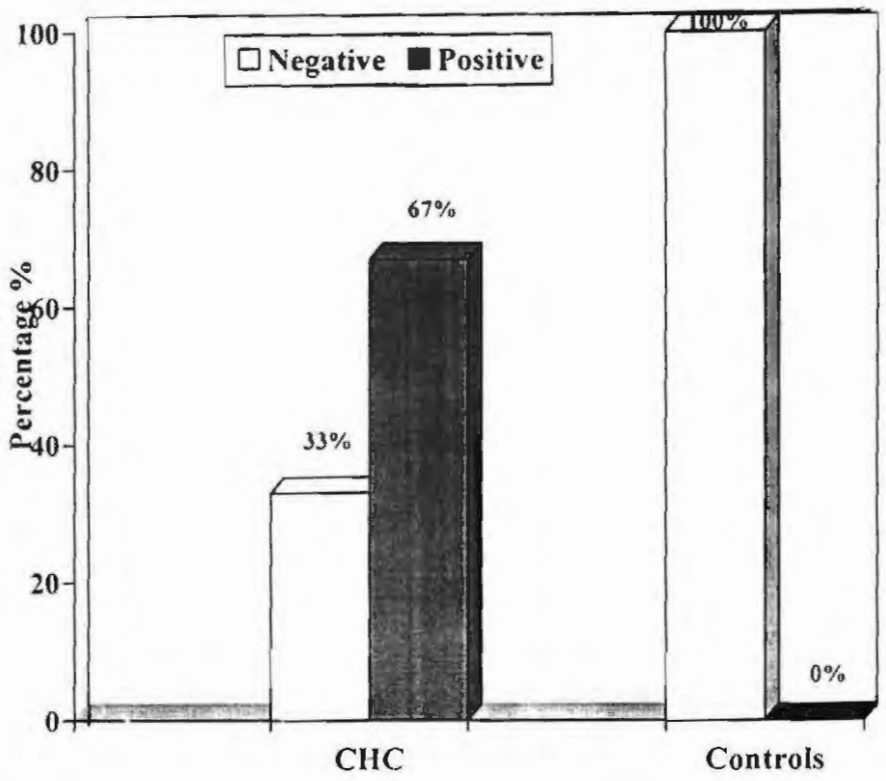

Fig. (3): Detection of HCV NS4-Ag in sera of CHC patients positive for anti-HCV antibody and controls negative for anti-HCV antibody using ELISA.

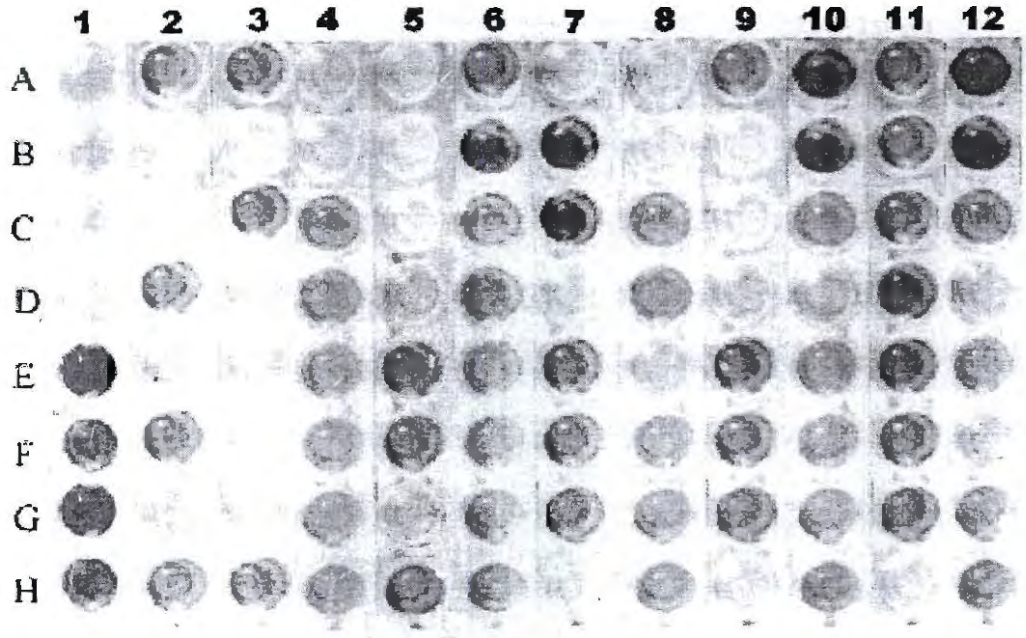

Fig. (4): The optical densities (at 490 nm) of serum samples tested for TB-Ag using ELISA.

A1-D1 wells: Negative controls.

E1-H1 wells: Positive controls.

A2 to H12 wells: 88 serum samples out of 176 HCV infected patients.

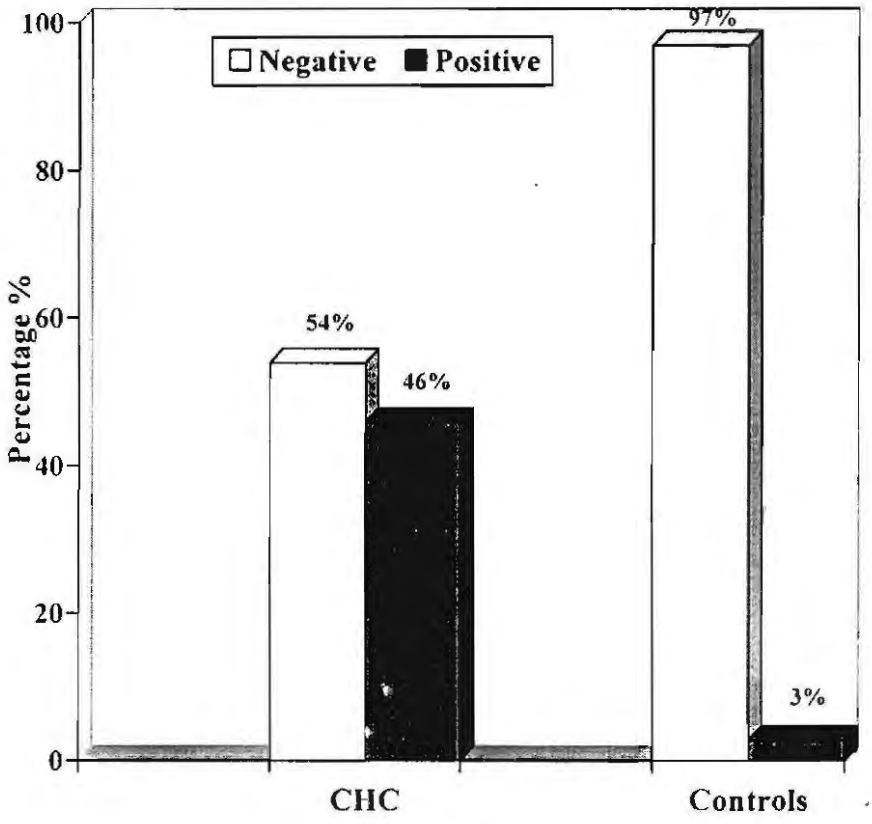

Fig. (5): Detection of the 55 kDa TB-Ag in sera of CHC patients infected with HCV (176 patients) and healthy controls (100 individuals) using ELISA.

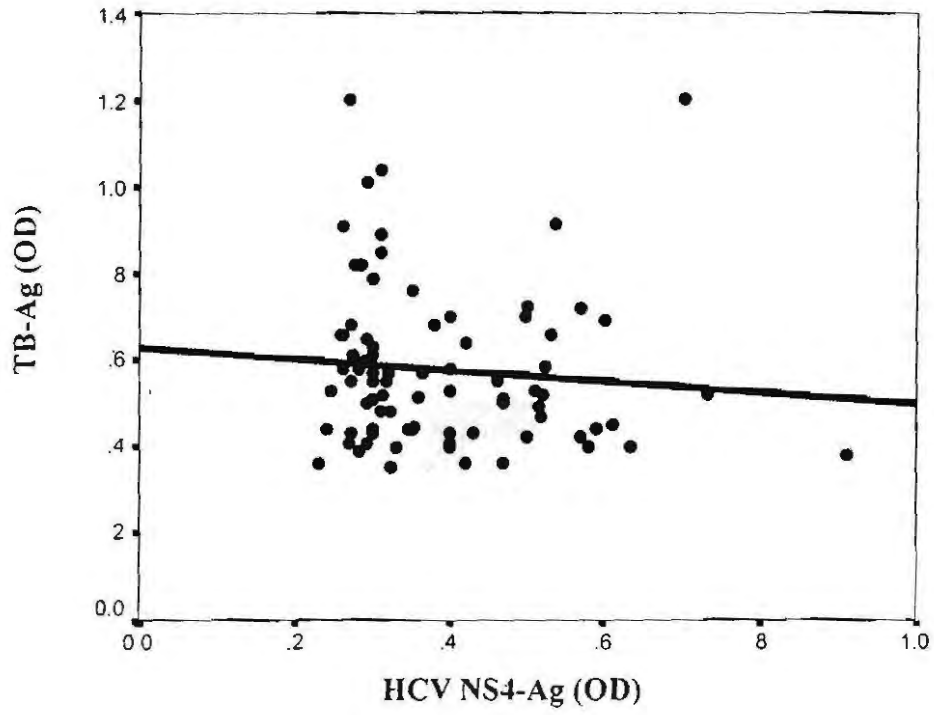

Fig. (6): Correlation between TB-Ag levels (OD) and HCV NS4-Ag levels (OD) in sera of CHC patients coinfected with both HCV and TB (n = 81, r = - 0.093, p>0.05).

DISCUSSION

HCV is a notorious virus for its ability to evade the host immune system and leads to persistent infection in the majority of the acutely infected patients [**Cerny & Chisari (1999)**]. Chronic HCV infection is associated with multiple extrahepatic manifestations as well, including recently recognized effects on the lung. Chronic viral infection may increase the risk for development of accelerated lung destruction **[Kanazawa et al., (2003)]**. Chronic HCV infection is also associated with both direct and indirect effects on pulmonary tissue **[Moorman et al., (2005)]**. However, further studies will be required to confirm the localization and replication of HCV in lung tissues **[Kanazawa et al.,**

(2003)]. TB and viral hepatitis are two of the commonest coinfections **[Padmapriyadarsini et al., (2006)]**. Studies concerning TB-HCV coinfection are limited, especially for detection of TB infection in HCV patients, but there were few studies described the diagnosis of HCV in TB patients. **Richards et al., (2006)** declared that HCV coinfection was common among patients infected with TB, 22% were found to be HCV seropositive among those infected with TB. **Kuniholm et al., (2007)** determined the prevalence of HCV antibodies among TB infected individuals to be 12%. In the present study, sera of 176 CHC patients out of 264 were positive for HCV NS4-Ag developed by **Attallah et al., 2003*a***; they had not received interferon treatment before sampling. All sera of selected 100 healthy individuals were negative for HCV NS4-Ag. Neither physicians nor patients had any information about past or recent history for infection with TB; they informed us just about HCV infection. For diagnosis of TB, antigen detection assays can give a more accurate indication of current infection rather than past infection **[Zheng et al., (1990)]**. **Attallah et al., 2003*b* & 2005** developed a simple and rapid immunoassay for the direct detection of a circulating mycobacterial antigen in sera of TB infected individuals using ELISA. TB-55 mAb was used as a probe in ELISA to detect TB antigen in serum samples. In the present study, we used the circulating 55 kDa TB-Ag for detection and screening of TB infection in HCV patients. **WHO, (2007)** estimated that TB incidence rate in the general population of Egypt, was 25/100000, using sputum smear microscopy for AFB detection. In the present study, the detection rate of TB infection was found to be 46% (81 of 176 patients) among CHC patients, while it was 3% (3 of 100 individuals) in the healthy control group. In addition, no significant correlation ($p>0.05$) was shown between the serum TB-Ag levels (OD) and the HCV NS4-Ag levels (OD) in sera of CHC patients infected with both HCV and TB (n=81). Since therapy for schistosomiasis was the major cause of the extensive distribution of HCV in Egypt, we suppose that HCV epidemic may be a possible reason for the emergence and recurrence of TB infection in HCV patients, due to its impact on the immune system, in addition to delay in TB diagnosis. Further studies are needed to assess the impact of the high prevalence of HCV infection on prevalence, diagnosis and treatment outcomes of TB, especially in high risk populations as Egypt where HCV is the major epidemic.

ACKNOWLEDGMENTS

The authors would like to thank Dr. H. Ismail, Dr. Mohamed Mustafa, and Mrs. Rania El-Sherbeny at R & D Dept., Biotechnology Research Center, New Damietta City, Egypt, for their kind help.

REFERENCES

Attallah AM, Ismail H, Tabll AA, Shiha GE, and El-Dosoky I. (2003*a*). A novel antigen detection immunoassay for field diagnosis of HCV infection. J. Immunoassay Immunochem. 24 (4): 395-407.

Attallah AM, Abdel-Malak CA, Ismail H, El-Saggan AH, Omran MM, and Tabll AA. (2003*b*). Rapid and simple detection of a *Mycobacterium tuberculosis* circulating antigen in serum using dot-ELISA for field diagnosis of pulmonary tuberculosis. J. Immunoassay Immunochem. 24: 73-87.

Attallah AM, Osman S, Saad A, Omran MM, Ismail H, Ibrahim G, and Abo-Naglla A. (2005). Application of a circulating antigen detection immunoassay for laboratory diagnosis of extra-pulmonary and pulmonary tuberculosis. Clinica. Chimica. Acta. 356 (1-2): 58-66.

Cerny A, and Chisari FV. (1999). Pathogenesis of chronic HCV: immunologic features of hepatic injury and viral clearance. Hepatology. 30: 595-601.

El-Raziky MS, El-Hawary M, Esmat G, Abouzied AM, El-Koofy N, Mohsen N, Mansour S, Shaheen A, Abdel-Hamid M, and El-Karaksy H. (2007). Prevalence and risk factors of asymptomatic HCV infection in Egyptian children. World J. Gastroenterol. 13 (12): 1828-1832.

Erturk A, Tokgonul AN, Capan N, Erturk H, Dursun AB, and Bozkaya H. (2006). Pulmonary alterations in patients with chronic HCV infection. Digestive and Liver Disease. 38: 673-676.

Frank C, Mohamed MK, and Strickland GT. (2000). The role of parenteral antischistosomal therapy in the spread of HCV in Egypt. Lancet. 355: 887-891.

Gautam R, Arvind S, and Sanjay MJ. (2007). Indian medicinal plants as a source of antimycobacterial agents. J. Ethnopharmacology. 110: 200-234.

Hoofnagle JH. (2002). Course and outcome of hepatitis C. Hepatology. 36: 21-29.

Kanazawa H, Hirata K, and Yoshikawa J. (2003). Accelerated Decline of Lung Function in COPD Patients With Chronic Hepatitis C Virus Infection. Chest. 123: 596-599.

Kew M, François G, Lavanchy D, Margolis H, Van Damme P, Grob P, Hallauer J, Shouval D, Leroux-Roels G, and Meheus A. (2004). Prevention of hepatitis C virus infection. J. Viral Hepatitis. 11 (11): 198-205.

Kuniholm MH, Mark J, Aladashvili M, Shubladze N, Khechinashvili G, Tsertsvadze T, Del Rio C, and Nelson KE. (2007). Risk factors and algorithms to identify HCV, HBV, and HIV among Georgian tuberculosis patients. Int. J. Infect. Dis. 20: 218-219.

Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

Li XIE, Xiao-dong WU, Huang De-zhuang, Chen Hai-lun, Li-xiang HE, Wang J, and Da-kang H. (2007). Clinical application and analysis of hepatitis C virus NS3 antigen detection by ELISA in human serum. Chinese Medical Journal. 120 (4): 294-299.

Lowry OH, Rosenbrough NJ, Farr AL, and Randall RJ. (1951). Protein measurement with folin-phenol reagent. J. Biol. Chem. 193: 265-275.

Moorman J, Mustafa S, Semaan K, and Guha K. (2005). Hepatitis C Virus and the Lung. Chest. 128: 2882-2892.

Padmapriyadarsini C, Chandrabose J, Victor L, Hanna LE, Arunkumar N, and Swaminathan S. (2006). Hepatitis B or hepatitis C co-infection in individuals infected with human immunodeficiency virus

and effect of anti-tuberculosis drugs on liver function. Journal of Postgraduate Medicine. 52 (2): 92-96.

Paul FR, and Stephen C. (2002). Latent tuberculosis: Models, mechanisms, and novel prospects for eradication. Seminars in Pediatric Infectious Dis. 13 (4): 263-272.

Richards DC, Mikiashvili T, Parris JJ, Kourbatova EV, Wilson JC, Shubladze N, Tsertvadze T, Khechinashvili G, Del Rio C, and Blumberg HM. (2006). High prevalence of HCV but not HIV co-infection among patients with tuberculosis in Georgia. Int. J. Tuberc. Lung. Dis. 10 (4): 396-401.

Towbin H, Staechlin T, and Gordan J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets, procedures and some application. National Academy of Sciences, USA. 76: 4350-4354.

World Health Organization. (1997). Hepatitis C: Global prevalence. Weekly Epidemiological Record, 72: 341-344.

World Health Organization. (2006). Tuberculosis Fact sheet No. (104): Global and regional incidence.

World Health Organization. (2007). International travel and health. Infectious diseases of potential risk for travelers. 5: 53-92.

Zheng HJ, Tao Zheng-Hou CW, and Pessens WF. (1990). Comparison of dot-ELISA with sandwich ELISA for the detection of circulating antigens in patients with Bancroftian filariasis. Am. J. Trop. Med. Hyg. 42: 546-549.

الكشف الكيميائى الحيوى عن العدوى بالدرن فى حالات الإصابة بفيروس التهاب الكبد الوبائـــى ســـى

عبد الفتاح عطا الله ومحمود عطية وأحمد الوصيف
قسم الكيمياء- كلية العلوم- جامعة المنصورة

إن فيروس التهاب الكبد الوبائـــى ســـى يعتبر أحد أخطر الفيروسات التى تـــصيب الكبد؛ وهناك ما يزيد عن ١٧٠ مليون شخص فى العالم مصابون بفيروس ســـى. وتعتبـــر عمليات نقل الدم أوالتعامل مع الدم المصاب بفيروس ســـى من مصادر العـــدوى الرئيـــسية بالمرض. كما أن معدل الإصابة بفيروس ســـى فى مصـــر يعتبر الأعلى عالمياً حيث يبلغ ١٥ إلى ٢٥%.

ويعد الدرن من الأمراض البكتيرية المعدية؛ وبالرغم من اختفاء مـــرض الـــسل الرئوى إلا أنه عاد للظهور بقوة فى السنوات الأخيرة. وتشير التقارير إلى أن ثلـــث ســـكان العالم تقريبا مصابون بالدرن ومعدل الوفيات سنوياً يقدر بحوالى ٢ مليون شـــخص. وقـــد تكون معدلات الإصابة العالية بفيروس ســـى فى مصر أحد أسباب عودة وظهور الدرن مرة أخرى ويرجع ذلك لتأثير فيروس ســـى على الرئتين وكذلك تأثيره على الجهـــاز المنـــاعى للإنسان.

لذلك فقد تم تعيين معدل الإصابة بالدرن في أمصال المرضى المصابين بفيـــروس ســـى بواسطة الإليزا وعمل علاقة إحصائية بين مستوى أنتيجين الدرن (الكثافة الـــضوئية) ومستوى أنتيجين فيروس ســـى؛ ومن خلال النتائج تبين وجود علاقة بين الإصابة بفيروس ســـى والدرن.