



## Prevalence of Tuberculosis in Chronic Hepatitis C Patients

### KEYWORDS

Mycobacterium tuberculosis, Hepatitis C virus, Co-infection

#### Abdelfattah M. Attallah

Research & Development Department, Biotechnology Research Center, New Damietta City, Egypt.

#### Camelia A. Abdel Malak

Faculty of Science, Damietta University,, New Damietta, Egypt.

#### Mohamed M. Omran

Faculty of Science, Helwan University, Cairo, Egypt.

#### khaled Farid

Tropical Medicine Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

#### Mohamed S. Albannan

Research & Development Department, Biotechnology Research Center New Damietta City, Egypt.

#### Ahmed A. Attallah

Research & Development Department, Biotechnology Research center New Damietta City, Egypt.

#### Mahmoud A. Attia

Research & Development Department, Biotechnology Research Center New Damietta City, Egypt.

#### Ragab A. Ibrahim

Research & Development Department, Biotechnology Research Center, New Damietta City, Egypt.

#### Ahmed M. El-Waseef

Faculty of Science, Mansoura University, Mansoura, Egypt.

**ABSTRACT** Background: Little is known about the characteristics of tuberculosis (TB) in chronic hepatitis C (CHC). We aimed to investigate the co-infection between TB and HCV and estimating their common impact on progression rates of fibrosis. Methods: A total of 558 individuals constituted this study (TB=126; CHC=322; Healthy=110). Western-blot and ELISA were used for identifying TB-55kDa and HCV-NS4 antigens. Results: A single immunoreactive band was shown at 55-kDa and 27-kDa corresponding to TB-55kDa and HCV-NS4, respectively, due to binding with their respective antibodies. TB-55kDa provided area under ROC curve (AUC) of 0.90 for identifying TB patients with sensitivity=82% and specificity=100% while HCV-NS4 provided AUC=0.96 for identifying HCV with sensitivity=97% and specificity=90%. TB-55kDa significantly correlated with the progression of liver disease ( $r=0.50$ ,  $P<0.0001$ ) and its detection rate was 6%, 27%, 35% in patients with fibrosis, cirrhosis and hepatocellular carcinoma, respectively, with an increase in its level. Furthermore, HCV was detected in 66% of TB-infected patients indicating that TB-patients are susceptible to HCV. Conclusion: Detection rate of TB-55kDa was found to increase with the progression of liver pathology indicating that advanced liver stages are more likely to be susceptible to TB. Moreover, TB may be a potential risk factor on liver fibrosis progression.

### INTRODUCTION

Globally, tuberculosis (TB) remains a grave burden to public health(1) and is considered one of the most life threatening infectious diseases that is caused by Mycobacterium tuberculosis. In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease (2). According to a 1997 report from the Egyptian National TB Program, the annual risk of TB infection in this country is 0.32% (3). TB typically attacks the lungs, but can also affect other parts of the body. TB infection is transmitted by respirable droplets generated during forceful expiratory manoeuvres such as coughing (4). On the other hand, hepatitis C virus (HCV) is considered to be one of the leading causes of end-stage liver disease requiring liver transplantation (5). HCV infection remains a major global health burden affecting approximately 160–170 million people worldwide and causes significant liver-related morbidity and mortality due to hepatic decompensation and development of hepatocellular carcinoma (HCC)(6). HCV is estimated to be the cause of 27% of cirrhosis and 25% of hepatocellular carcinoma cases worldwide (7). Worldwide, the prevalence of HCV infection among patients with TB

has not been extensively investigated, and very limited data on rates of HCV co-infection among patients with TB exists. Moreover, little is known about the characteristics of TB in chronic hepatitis C (CHC) patients. For the majority of individuals with normal immune function, proliferation of Mycobacterium tuberculosis is arrested once cell-mediated immunity develops, even though small numbers of viable bacilli may remain within the granuloma (8). But, the ability of the host to respond to Mycobacterium tuberculosis may be reduced by certain diseases. Therefore, this work is concerned with the identification of both TB and HCV antigens. Then, we aim to estimate if there is a significant increase in the progression rates of fibrosis in the co-infected group compared to the HCV mono-infected group and deciding whether advanced stages of liver disease are more likely to be susceptible to TB or not.

### MATERIAL AND METHODS

#### Samples

A total of 558 consecutive Egyptian individuals composed of three different groups constituted the present study. The first group included serum samples of 126 pulmonary TB

patients that were obtained from the Abbassia Chest Hospital, Cairo, Egypt. This cohort comprised 106 men and 20 women with a mean ( $\pm$ SD) age of 40.81 ( $\pm$ 13.05) years. These patients were diagnosed as having active pulmonary tuberculosis by acid-fast bacilli using Ziehl-Neelsen staining and culture of sputum, in combination with confirmed clinical symptoms. The blood and sputum samples were obtained from all patients before initiation of TB treatment. The second group included serum samples of another 322 CHC patients [224 with liver fibrosis (F1-F3), 26 with liver cirrhosis (F4) and 72 with HCC] that were collected from the Tropical Medicine department, Mansoura University hospitals, Mansoura, Egypt. These patients were tested positive for the presence of HCV-RNA using quantitative polymerase chain reaction assay (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Pleasanton, USA). Histopathological classification for liver fibrosis and cirrhosis was performed according to the METAVIR score (9). Liver fibrosis was defined as a Metavir score of  $\leq 3$  (F1-F3) whereas cirrhosis was defined as a Metavir score of 4 (F4). The diagnosis of HCC in those patients was carried out according to the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines (10). The diagnosis of HCC relied on an elevated AFP value  $\geq 400$  U/L or the presence of a malignant liver nodule as established by imaging techniques (ultrasound, computed tomography and magnetic resonance). The final diagnosis was confirmed by histopathologic analysis on ultrasound assisted fine-needle biopsy. The third group included serum samples of another 110 healthy volunteers used as a control group. This cohort comprised 81 males and 29 females with a mean ( $\pm$ SD) age of 40.37 ( $\pm$ 13.25) years.

#### SDS-PAGE and western blot

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 0.75 mm-thick, 12% vertical slab gels according to the method of Laemmli (11). Serum samples separated on SDS-PAGE were electrotransferred onto nitrocellulose membrane (0.45 mm pore size, Sigma) in a protein transfer unit (12). Next, they were immunostained using monoclonal anti-TB-55 antibody and mono specific anti-HCV-NS4 antibody separately as previously described by Attallah et al. (13, 14).

#### Detection of TB and HCV antigens using ELISA

In brief, this procedure is a four step test. In step 1, diluted serum samples (1:250) in coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6) were tested (50  $\mu$ l/well) for TB antigen bound on a 96-well microtiter plate at 4°C overnight. At the end of this step, the plate was washed three times using 0.05% (v/v) PBS-T20 (pH 7.2) and the free active sites were blocked with PBS+0.5% Tween 20 (200  $\mu$ l/well). In step 2, 50  $\mu$ l/well of a monoclonal antibody to the TB 55- kDa antigen at a dilution 1:150 in PBS was added and incubated at 37°C for 2 h. In step 3, 50  $\mu$ l/well of anti-mouse IgG alkaline phosphatase-conjugated (Sigma), diluted 1:400 in 0.2% BSA in PBS-T20, was added. At the end of the third step, the wells were washed to remove any unbound material. The conjugate was bound to monoclonal antibody to TB antigen coating the micro-well surface. At the end of step 3, the micro-wells were washed to remove any unbound conjugate. In step 4, an enzyme detection system composed of nitrophenyl phosphate substrate (50  $\mu$ l/well) was added to the micro well. In the presence of bound conjugate, the p-nitrophenyl phosphate was hydrolyzed; resulting in a colored end product and the absorbance was read at 450 nm after 10 minutes using a microtiter plate reader ( $\Sigma$ 960, Metretech Inc, Germany). Color intensity was proportional to the

amount of bound conjugate and therefore is a function of the concentration of TB antigen present in the serum sample. Similarly, the aforementioned steps was performed in respect of HCV-NS4 antigen using the same quantities and intervals but in different concentrations as the following: sera dilution (1:250) in coating buffer, monospecific anti-HCV-NS4 antibody at dilution 1:200 in PBS and alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma) was diluted 1:450 in 0.2% BSA in PBS-T20.

#### Statistical analysis

All statistical calculations were done by SPSS software v.15.0 (SPSS Inc., Chicago, IL) and GraphPad Prism package; v.5.0 (GraphPad Software, San Diego, CA). Continuous variables were expressed as mean  $\pm$  standard deviation. A value of  $P < 0.05$  was considered statistically significant. Chi-square ( $\chi^2$ ) test was used to compare categorical data. The correlation was evaluated by Spearman's rank correlation coefficient. The diagnostic value of TB and HCV-NS4 antigens were assessed by calculating the area under the receiver operating characteristic (ROC) curves. An area under the curve (AUC) of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value. Based on the receiver-operating characteristic (ROC) analysis, the best cutoff points were selected and diagnostic performances (sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)) were determined.

## RESULTS

### Identification of TB antigen in patients with pulmonary TB

The target TB antigen was identified based on SDS-PAGE procedure followed by Western blotting. As a result, a single immunoreactive band was shown at 55-kDa molecular weight corresponding to TB in sera of patients with pulmonary TB. That's due to the binding with its respective monoclonal antibody. No specific reaction was observed in sera of healthy individuals that were used as a control group as depicted in Figure 1.A.

### Identification of HCV-NS4 antigen in patients with pulmonary TB and CHC

The serum samples of patients with pulmonary TB, patients with CHC and healthy individuals were separated by SDS-PAGE followed by Western blotting. As a consequence, a single band was identified at 27 kDa corresponding to HCV-NS4 antigen in serum samples of pulmonary TB and CHC patients. No specific reaction was observed in sera of healthy individuals as shown in Figure 1.B.

### Diagnostic accuracy of TB-55 kDa antigen

In order to estimate the diagnostic accuracy of TB-55 kDa antigen, ROC curve was used. As a result, The TB-55 kDa enabled the correct identification of patients with pulmonary TB with an AUC of 0.90 (Figure 2.A). Based on ROC analysis, the best cutoff point greater than 0.25 was chosen for the optimal prediction of TB infection. A cutoff of 0.25 gave 82% sensitivity and 90% accuracy for detecting TB infection. At this point, absolute specificity (100%) was obtained for discriminating patients with pulmonary TB from healthy individuals. Moreover, at this point, TB infection could be confirmed with an absolute PPV (100%) as presented in Figure 2.B. The difference in TB-55 kDa levels was statistically significant in patients with pulmonary TB versus healthy individuals as shown in Figure 2.C.

### Diagnostic accuracy of HCV-NS4 antigen

The diagnostic accuracy of HCV-NS4 antigen was then

estimated based on ROC analysis giving an AUC of 0.96 (Figure 3.A). HCV-NS4 antigen provided a superior sensitivity of 97%, specificity of 90% and accuracy of 95% for discriminating HCV-infected patients from healthy individuals. Based on this method, HCV-infection could be excluded with a high NPV of 92%, i.e. only 8% of HCV-infected patients would be classified falsely. In addition, HCV-infection could be confirmed with 96% PPV and only 4% of healthy individuals would be classified falsely (Figure 3.B).

#### Prevalence of TB-55 kDa antigen among CHC patients

Characteristics of CHC patients at the time of liver biopsy are summarized in Table 1. TB-55 kDa antigen was detected in 14 (6%) out of 224 patients with liver fibrosis but was detected in 7 (27%) out of 26 patients with cirrhosis, and 25 (35%) out of 72 patients with HCC (Figure 4.A). This may indicate that the detection rate of the TB-55 kDa antigen increased with the progression of liver pathology. In addition, Bivariate Spearman's rank correlation coefficient ( $r$ ) was calculated to measure the relationship of TB-55 kDa antigen to the progression of liver pathology. As a result, TB-55 kDa antigen significantly correlated with the progression of liver disease with a correlation coefficient ( $r$ ) of 0.50 ( $P<0.0001$ ). Next, the levels of TB-55 kDa antigen in relation to different groups of liver diseases is presented in Figure 4.B showing an increase in the level of TB-55 kDa antigen with the severity of liver disease. Moreover, Odds ratio (OR) was used as a measure of association between TB-55 kDa antigen and different groups of liver diseases. Patients with HCC were associated with higher odds ratio (OR=10.12) than those who developed liver cirrhosis (OR=5.98) or liver fibrosis (OR=2.11) as depicted in Figure 4.C. On the other hand, HCV-NS4 antigen was detected in 82 (66%) out of 124 patients infected with pulmonary TB indicating that patients with TB are more likely to be susceptible to HCV. Furthermore, HCV-NS4 antigen was detected in 66 (66%) out of 100 patients positive for Ziehl-Neelsen staining but was detected in 18 (69%) out of 26 patients negative for Ziehl-Neelsen staining without any significant difference ( $X^2=0.097$ ,  $P=0.819$ ).

**Table 1. Characteristics of healthy individuals and liver-diseased patients (N= 432)**

Variable <sup>a</sup>	Healthy N=110	Fibrosis (F1-F3) N=224	Cirrhosis (F4) N=26	HCC N=72	P value <sup>b</sup>
Age (years)	40.37±13.2	41.3±7.2	47.1±5.2	59.2±10.3	P<0.0001
AST (U/ml)	25.3±6.5	43.3 ±22.5	64±34	131 ±168	P<0.0001
ALT (U/ml)	28.2±4.9	45.6±36.1	64± 39	59 ±41	P<0.0001
ALP (U /L)	40.6±20.1	85.3 ±43.2	119±53	223±173	P<0.0001
Albumin (g/L)	45.2±2.5	41.3±3.5	37 ±7.3	28 ±5.2	P<0.0001
T. Bilirubin (mg/dl)	0.45±0.21	0.83±0.33	1.3 ±0.81	4.4 ±6.4	P<0.0001
PLT (×10 <sup>9</sup> /L)	310±30	190± 50	162 ±125	139±38	P<0.0001
AFP (U/L)	1.5±0.5	3.0 ±0.32	8.2±7.1	913±213	P<0.0001

Variables were expressed as mean ± SD. <sup>a</sup>Reference values: aspartate aminotransferase (AST) (male up to 37 U/L, female up to 31 U/L); alanine aminotransferase (ALT) (male up to 41 U/L, female up to 31 U/L); alkaline phosphatase (ALP) 22-92 U/L; albumin 3.8-5.4 g/dL; total bilirubin up to 1 mg/dL; platelet count (PLT) 150-400 ×10<sup>9</sup>/L; alpha feto-

protein (AFP) up to 10 U/L. <sup>b</sup>P>0.05 is considered non-significant; P<0.05 is considered significant.

#### DISCUSSION

TB is a dangerous disease and its death toll is increasing year by year(15). The occurrence of TB and viral hepatitis infections in the same patient poses unique clinical and public health challenges, because medications to treat TB are hepatotoxic(16). In spite of the importance of the co-infections of HCV and TB, the association of HCV infection and TB remains to be clarified and there have been few studies on TB infection among HCV patients. Several investigators had detected various Mycobacterium tuberculosis antigens in different body fluids of infected individuals, e.g., 30-kDa antigen and 31-kDa antigen in serum(17, 18), 43-kDa antigen in ascitic fluid(19), 43-kDa antigen, antigen 5, and 14-kDa antigen in cerebrospinal fluid(20-22). In this work, western blot analysis revealed that monoclonal antibody reacted against TB antigen at an apparent molecular weight of 55-kDa in sera. That's due to the binding of TB antigen with its respective monoclonal antibody. Our results showed that this antigen enabled the correct identification of patients with pulmonary TB with an AUC of 0.90 yielding 82% sensitivity, 90% accuracy and absolute specificity for discriminating patients with pulmonary TB from healthy individuals. This finding suggest that TB-55kDa antigen could be used as a potential marker for diagnosing TB. On the other hand, liver fibrosis is a common complication of chronic viral hepatitis leading to the progressive destruction of normal tissue architecture or the replacement of hepatocytic tissue with fibrous tissue (23). It is worthy noting that the major hepatological consequence of HCV infection is the progression to cirrhosis and its potential complications: haemorrhage, hepatic insufficiency and primary liver cancer (24). Several factors have been shown to be associated with fibrosis progression rate: duration of infection, age, male gender, heavy consumption of alcohol, HIV co-infection, low CD4 count and necrosis grade (25). But, little is known about the prevalence of TB in CHC patients as a risk factor associated with fibrosis progression rate. In this work, we aim to assess the prevalence of TB infection among patients with chronic hepatitis C. Our findings showed that the detection rate of the TB-55 kDa antigen increased with the severity of liver disease. Patients who had HCC and cirrhosis were more likely to be susceptible to TB. This may be explained by the fact that patients who develop liver cirrhosis show an acquired immune deficiency because of poor homeostasis and malnutrition. Furthermore, all host defense systems, antigen-specific as well as nonspecific functions, are compromised in cirrhotic patients (26). In addition, liver cancer has been shown to be a risk factor for the development of TB, either through immune suppression by the tumor or through the effects of chemotherapy(27). In multivariable analysis, TB infection (OR=10.12, 95% CI=4.42-23.12), (OR=5.98, 95% CI=2.13-16.78) and (OR=2.11, 95% CI=0.74-6.03) was found to be an independent risk factor for developing HCC, cirrhosis and fibrosis, respectively, among patients with CHC. This may give an indicator that TB possesses a threat on liver fibrosis progression. Globally, the prevalence of HCV infection among patients with TB has not been extensively investigated, and very limited data on rates of HCV co-infection among patients with TB exists (2). In this study, HCV-NS4 antigen was detected in 66% of patients infected with pulmonary TB. This percentage was higher than that obtained by Richards et al. (28) who reported a high prevalence (22%) of HCV infection among patients with TB. Torres et al.(29) also investigated the importance of HCV infection in the development of TB in a

cohort of kidney transplant recipients using multivariate analysis showing that the presence of HCV infection is associated with the development of TB ( $P = 0.003$ ). This may give clues that patients with HCV may be more likely to be susceptible to TB. In summary, a significant increase in the progression rates of fibrosis was observed in the co-infected group compared with the HCV mono-infection group and the advanced stages of liver disease are more likely to be susceptible to TB. Moreover, HCV infection seems to be involved in the pathogenesis of tuberculosis and further studies involving a greater number of patients are warranted to validate HCV infection as a risk factor predisposing for Mycobacterium tuberculosis infection and progression to active disease.

**CONFLICT OF INTEREST**

The authors declared that there is no conflict of interest.

**ACKNOWLEDGEMENTS**

This study has been completely supported financially and carried out at Biotechnology Research Center, New Damietta, Egypt

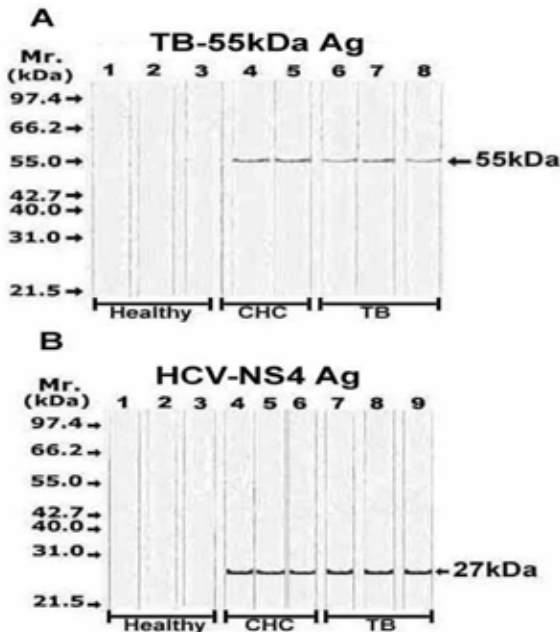


Figure 1. Identification of both TB-55kDa and HCV-NS4 antigens using Western blot analysis (A) Western blotting using monoclonal antibody corresponding to TB-55kDa antigen at 55-kDa; (B) Western blotting using mono-specific antibody corresponding to HCV-NS4 antigens at 27-kDa. The molecular mass ( $M_r$ ) protein markers (not shown but indicated by arrows) are phosphorylase B ( $M_r = 97.4$  kDa), bovine serum albumin ( $M_r = 66.2$  kDa), glutamate dehydrogenase ( $M_r = 55.0$  kDa), ovalbumin ( $M_r = 42.7$  kDa), aldolase ( $M_r = 40.0$  kDa), carbonic anhydrase ( $M_r = 31.0$  kDa), and soybean trypsin inhibitor ( $M_r = 21.5$  kDa).

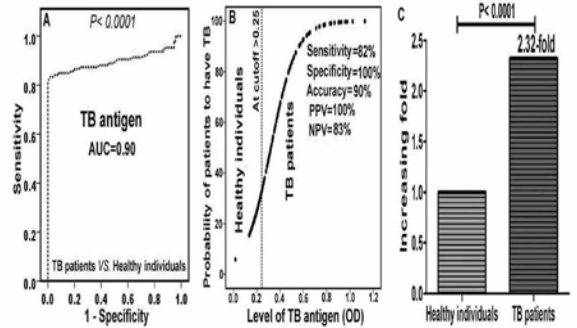


Figure 2. Diagnostic accuracy and distribution of TB-55 kDa antigen for detecting tuberculosis infection (A) Receiver-operating characteristic (ROC) curve with an area of 0.90 for TB-55 kDa antigen; (B) Diagnostic performances (Sn: sensitivity, Sp: specificity, PPV: positive predictive value and NPV: negative predictive value) for TB-55 kDa antigen for identifying tuberculosis infection; (C) Distribution of TB-55kDa antigen levels in healthy individuals versus patients with tuberculosis.

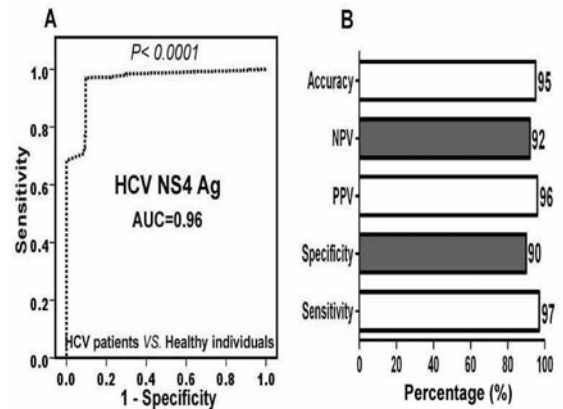


Figure 3. Diagnostic accuracy of HCV-NS4 antigen for detecting HCV infection (A) Receiver-operating characteristic (ROC) curve with an area of 0.96 for HCV-NS4; (B) Diagnostic performances (Sn: sensitivity, Sp: specificity, PPV: positive predictive value and NPV: negative predictive value) for HCV-NS4 identifying HCV infection.

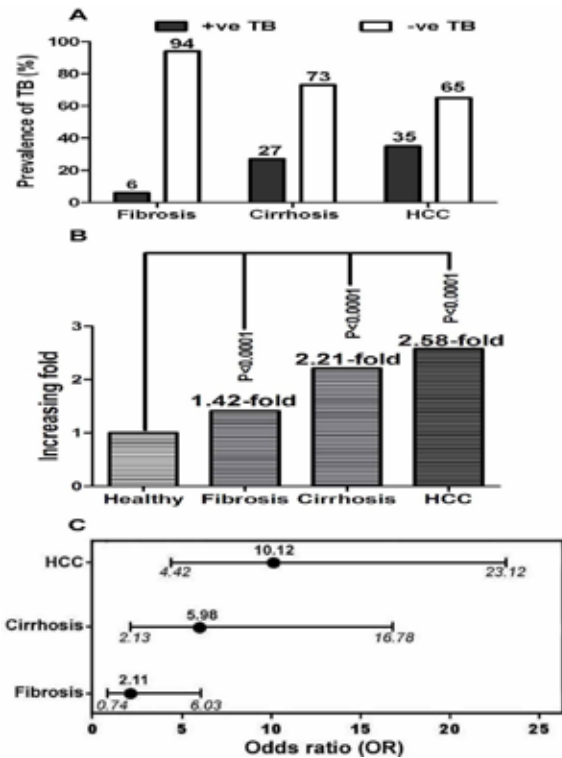


Figure 4. Distribution and odds ratio of TB-55 kDa antigen among patients with different liver pathology (A) Prevalence of TB-55 kDa antigen among patients with fibrosis, cirrhosis and hepatocellular carcinoma (HCC) showing an increase in its detection rate with the progression of liver pathology; (B) Distribution of observed fold changes for TB-55 kDa antigen between patients who had different liver pathology versus healthy individuals ( $P < 0.0001$  is considered extremely significant); (C) Odds ratio (OR) and 95% confidence intervals showing the risk of TB on liver fibrosis progression.

## REFERENCE

- Tasbiti AH, Bahramand A, Shokrgozar MA, et al. Evaluation of antigen detection test (chromatographic immunoassay): potential to replace the antibody assay using purified 45-kDa protein for rapid diagnosis of tuberculosis. *J Clin Lab Anal* 2014;28:70-76. | 2. Lomtadze N, Kupreishvili L, Salakaia A, et al. Hepatitis C virus co-infection increases the risk of anti-tuberculosis drug-induced hepatotoxicity among patients with pulmonary tuberculosis. *PLoS One* 2013;8:e83892. | 3. El Ibiary S, de Coster EJ, Tolba FM, et al. Trend in the annual risk of tuberculous infection in Egypt, 1950-1996. *Int J Tuberc Lung Dis* 1999;3:294-299. | 4. Konstantinos A. Testing for tuberculosis. *Australian Prescriber* 2010;33:12-18. | 5. Jang JY, Chung RT. Chronic hepatitis C. *Gut and liver* 2011;5:117-132. | 6. Maasoumy B, Wedemeyer H. Natural history of acute and chronic hepatitis C. *Best Pract Res Clin Gastroenterol* 2012;26:401-412. | 7. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006;45:529-538. | 8. Dannenberg AM, Jr. Immune mechanisms in the pathogenesis of pulmonary tuberculosis. *Rev Infect Dis* 1989;11 Suppl 2:S369-378. | 9. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-832. | 10. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005;42:1208-1236. | 11. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685. | 12. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1979;76:4350-4354. | 13. Attallah AM, Abdel Malak CA, Ismail H, El-Saggan AH, Omran MM, Tabll AA. Rapid and simple detection of a Mycobacterium tuberculosis circulating antigen in serum using dot-ELISA for field diagnosis of pulmonary tuberculosis. *J Immunoassay Immunochem* 2003;24:73-87. | 14. Attallah AM, Omran MM, Nasif WA, et al. Diagnostic performances of hepatitis C virus-NS4 antigen in patients with different liver pathologies. *Arch Med Res* 2012;43:555-562. | 15. Santhosh S, Sini TK, Anandan R, Mathew PT. Hepatoprotective activity of chitosan against isoniazid and rifampicin-induced toxicity in experimental rats. *European Journal of Pharmacology* 2007;572:69-73. | 16. Sirinak C, Kittikraisak W, Pijneesekikul A, et al. Viral hepatitis and HIV-associated tuberculosis: risk factors and TB treatment outcomes in Thailand. *BMC public health* 2008;8:245. | 17. Ng TT, Strang JI, Wilkins EG. Serodiagnosis of pericardial tuberculosis. *Cjm* 1995;88:317-320. | 18. Nair ER, Banerjee S, Kumar S, Reddy MV, Harinath BC. Purification and characterization of a 31 kDa mycobacterial excretory-secretory antigenic protein with a diagnostic potential in pulmonary tuberculosis. *Indian J Chest Dis Allied Sci* 2001;43:81-90. | 19. Wadde AA, Boting L, Reddy SG. Antigen capture assay for detection of a 43-kilodalton Mycobacterium tuberculosis antigen. *J Clin Microbiol* 1990;28:2786-2791. | 20. Aggarwal P, Wali JP, Singh S, Handa R, Wig N, Biswas A. A clinico-bacteriological study of peripheral tuberculous lymphadenitis. *J Assoc Physicians India* 2001;49:808-812. | 21. Radhakrishnan VV, Mathai A. A dot-immunobinding assay for the laboratory diagnosis of tuberculous meningitis and its comparison with enzyme-linked immunosorbent assay. *J Appl Bacteriol* 1991;71:428-433. | 22. Sumi MG, Mathai A, Sarada C, Radhakrishnan VV. Rapid diagnosis of tuberculous meningitis by a dot immunobinding assay To detect mycobacterial antigen in cerebrospinal fluid specimens. *J Clin Microbiol* 1999;37:3925-3927. | 23. Calvaruso V, Craxi A. Fibrosis in chronic viral hepatitis. *Best Pract Res Clin Gastroenterol* 2011;25:219-230. | 24. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003;362:2095-2100. | 25. Massard J, Ratziu V, Thabut D, et al. Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 2006;44:S19-24. | 26. Cho YJ, Lee SM, Yoo CG, et al. Clinical characteristics of tuberculosis in patients with liver cirrhosis. *Respirology* 2007;12:401-405. | 27. Kim HR, Hwang SS, Ro YK, et al. Solid-organ malignancy as a risk factor for tuberculosis. *Respirology* 2008;13:413-419. | 28. Richards DC, Mikiashvili T, Parris JJ, et al. High prevalence of hepatitis C virus but not HIV co-infection among patients with tuberculosis in Georgia. *Int J Tuberc Lung Dis* 2006;10:396-401. | 29. Torres J, Aguado JM, San Juan R, et al. Hepatitis C virus, an important risk factor for tuberculosis in immunocompromised: experience with kidney transplantation. *Transpl Int* 2008;21:873-878. |