ejpmr, 2018,5(6), 731-734

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CAN ANGIOPOIETIN-1 DIFFERENTIATE BETWEEN SIGNIFICANT AND NON-SIGNIFICANT FIBROSIS IN CHRONIC HEPATITIS C PATIENTS?

Gamal E. Shiha*[1,4], EL-Shahat A. Toson[2], Ahmed E. Amin[3], Reham E. Soliman[4,5] and Mohamed T. Ali[3]

[1]Internal Medicine Department, Faculty of Medicine, Specialized, Mansoura University, Mansoura, Egypt.
[2]Chemistry Departments, Faculty of Science, Damietta University, Damietta, Egypt.
[3]Chemistry Departments, Faculty of Science, Cairo University, Cairo, Egypt.
[4]Egyptian Liver Research Institute and Hospital (ELRIAH) Mansoura, Egypt.
[5]Tropical Medicine Department, Port Said University, Port Said, Egypt.

***Corresponding Author: Gamal E. Shiha**

Internal Medicine Department, Faculty of Medicine, Specialized, Mansoura University, Mansoura, Egypt.

Article Received on 19/04/2018 **Article Revised on 09/05/2018** **Article Accepted on 29/05/2018**

ABSTRACT

Background: The assessment of liver fibrosis by non-invasive methods is clinically important where hepatitis C virus (HCV) is common in Egypt. **The aim** is to assess the ability of Angiopoietin-1(Ang-1) as a direct marker to differentiate between significant and non-significant fibrosis **Method**: Blood samples were collected from 120 CHC patients (F0-F4). Ang-1 serum levels were assayed using an enzyme-linked immunosorbent assay, HCV RNA and HCV antibodies, liver function tests and platelet counts; beside, liver biopsy were evaluated. The numerical value of the AAR was calculated. Then, the diagnostic performance of Ang-1 was compared with the latter score; at their original cut-off. **Results:** Ang-1 can efficiently differentiate patients with non-significant (F0, F1) from those with significant fibrosis (F2–F4) with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and of AUC of 89, 91, 89, 91 and 0.919, respectively. **Conclusions:** Ang-1 can help in assessing hepatic fibrosis and superior to AAR score in discriminating the stages of the liver disorders in CHC Egyptian patients.

KEYWORDS: Angiopoietin-1, diagnostic performance, Liver fibrosis, Hepatitis C virus.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem[1] Liver fibrosis is defined as the building up of excessive amount of extracellular matrix (ECM causing hepatic fibrogenesis that distorts the normal parenchymal structure of the liver and impairs its function.[2, 3, 4]

The accumulation of inflammatory cells mediates the development of hepatic fibrosis. The latter may decrease blood flow and oxygen supply which mediate angiogenesis and angiogenic biomarkers including angiopoietin-1(Ang-1), leptin and transforming growth factor (TGF)-β[5, 6]. The Angiopoietin and its receptor system; namely, Tie2 plays a vital role during the late phase of angiogenesis because it is responsible for the maturation of newly formed vascular structures[7]. Therefore, this study was designed to measure the ability of serum Ang-1 level to assess hepatic fibrosis in patients with CHC.

PATIENTS AND METHODS

1) Patients

This study was conducted on 120 patients with hepatitis C virus infection attending from the Egyptian liver institute and Hospital (ELRIAH) in Dakahlia, Egypt. All patients confirmed to be chronic hepatitis C using HCV RNA by quantitative polymerase chain reaction RT-PCR None of the patients had received antiviral treatment before liver biopsies and blood sampling

Patients' consent

Informed written consent from each patient and local ethical committee approval were obtained before starting the data collection. With respect to patients' confidentiality, patients were represented in the study by code numbers. All personal data were concealed. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Exclusion criteria

Patients who had received any previous courses of antiviral or immunosuppressive therapy, those who had HBV or HIV co-infection, and those with any type of liver cancer were excluded from the study. Patients who refused to have a liver biopsy or for whom it was contra-indicated, i.e. low platelet count, prolonged prothrombin

time or decompensated cirrhosis, were also excluded from the study.

Samples and blood markers
Whole blood samples were withdrawn and were freshly used from all cases. On the other hand serum samples were kept frozen at -80° C until their use.

Biochemical investigations
Human Angiopoietin-1 (Ang-1)
Ang-1 was evaluated by solid-phase enzyme-linked immunosorbent assay (Boster Biological Technology Co., Ltd., Catalog No; EK0559, USA) according to the manufacturer's instruction. A standard curve was constructed using multiple dilutions of recombinant protein for Ang-1. The color development was stopped with stop solution, and the optical density was measured at 450 nm and 620 nm as a reference filter (Stat Fax, USA).

Baseline assessment
The patients were subjected to thorough history taking, clinical examination, routine laboratory work-up including Complete blood picture (hemoglobin, Red blood cells, Platelets and White blood cells) were done using D-cell 60 automated Hematology analyzer (Sysmex Xi 1800 incorporation, Japan), international normalized ratio (INR) was performed using (Sysmex® CA-1500, Japan) auto analyzer, and serum AST, ALT, ALP, albumin, bilirubin using automated Biochemistry analyzer (Cobas Integra 400, Roch, Switzerland).Also, abdominal ultrasonography and transient elastography (Fibroscan) were done.

Serological and molecular markers
Serological markers for detecting HCV infection [hepatitis C antibodies (HCV Abs)] were estimated by ELISA (Merieux anti-HCV, version 4.0, Diasorin S.P.A. via Crescent no 13040 Saluggia (VC) -Italy). HCV RNA was quantized by quantitative RT-PCR by using (fully automated Cobas amplified, Taqman48 analyzer, Roch Switzerland)

Liver biopsy
Liver biopsy was performed as a part of the routine clinical care of these patients to decide on antiviral therapy. Needle liver biopsy specimens (n = 220) were obtained with an 18 G or larger needle. Tissue specimens obtained by liver biopsy were fixed immediately in 10% formalin solution and sent to the pathologist at the same day and were routinely stained with hematoxyline–eosin stain. The liver biopsies had to measure at least 15 mm and/or contain five portal tracks. Liver pathologist was blinded to patients' clinical and laboratory features. The stage of fibrosis and grade of inflammatory activity in liver were determined according to the METAVIR scoring system[8] F0, no fibrosis; F1, portal fibrosis alone; F2, portal fibrosis with rare septa; F3, portal fibrosis with many septa; and F4, cirrhosis. In this study, the stages of liver fibrosis were classified into three groups; namely, significant fibrosis [METAVIR higher than 1 (F2- F4)], advanced fibrosis [METAVIR higher than 2 (F3-F4)], and cirrhosis [METAVIR higher than 3 (F4)].

Transient Elastography
Liver stiffness was also measured by transient elastography (Fibroscan; Echo sense SA, Paris, France). Ten successful acquisitions were performed on each patient. The results that obtained ten valid measurements with a success rate of at least 60% and an interquartile rang fewer than 30% were considered successful. A median of 10 valid measurements was regarded as the liver stiffness for a given subject, and expressed in kilopascals (kPa).

Formulas of the selected Scores
1-AAR: [AST/ (ALT) ratio (AAR)][9]

Statistical analysis
Statistical analysis was performed by Medcalc software version 15.0 (Medcalc 15.0, Mariakerke and Belgum). Continuous variables were expressed as mean ±standard deviation (SD). Comparisons of fibrogenic and angiogenic markers as well as routine laboratory tests and fibrosis stages were analyzed using a two-sided P-value. A value of $P < 0.05$ was considered statically significant. The diagnostic power of the developed model was measured by estimating its AUCs ROC curve was done to determine the cut-off point, AUC. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

RESULTS

Patient's characteristics
Comparison of the baseline characteristics of CHC patients with hepatic fibrosis in regard the two main classifications of hepatic fibrosis are outlined in Table1. The results showed that ALT and AST activities were increased in sera of patients with significant fibrosis (F2-F4) compared with those of non-significant (F0-F1) ($p<0.0001$). On the other hand, serum albumin levels and platelets count were decreased in patients with significant fibrosis compared with those of patients with non-significant ($p<0.0007$ and $p<0.0181$, respectively).

Performance characteristics and AUCs for the candidate biomarker; Ang-1, versus those of AAR as non-invasive scores in assessing hepatic fibrosis
Ang-1 value was found to be 133% higher in sera of patients with significant fibrosis compared with those of the non-significant one. Also, it was found that, at a cutoff point of more than 2186 pg/ml for Ang-1 was able to differentiate patients with significant from those with non-significant fibrosis with sensitivity of 89%, specificity 91%, positive predictive value (PPV) 89%, negative predictive value (NPV) 91% and with an area under curve of 0.919 ($p<0.0001$ and Table 2). Also, the performance characteristics of the AAR discriminate patients with significant and those with non-significant

fibrosis but with AUCs values of 0.700, with a sensitivity of 60.0%, specificity 47.5%, positive predictive value (PPV) 36.4%, negative predictive value (NPV) 70.4% at its original cutoff values (table 2). On contract, the performance characteristics of Ang-1 were higher than those of AAR score (Figure1).

Table 1: Baseline characteristics in patients with non-significant (F0–F1), significant (F2–F4) liver fibrosis.

	Control n=20	Non-sign. Fibrosis (F0, F1) (n=66)	Significant fibrosis (F2-F4) (n= 54)	P-value
Age(years)	30.6 ± 7.5	51.9 ± 11.24	36.46 ±10.3	<0.0001
ALT(U/L)	21.1 ± 5.6	35.46±20.73	42.08±24.46	0.111
AST(U/L)	18.6 ± 4.5	29.72±17.29	31.57±20.06	0.589
ALP(U/L)	74.5 ± 19.1	73.91±24.86	71.55±25.19	0.608
Alb(g/dl)	4.7 ± 0.18	4.49±0.25	4.49±0.27	0.958
T.Bili (mg/dl)	0.65 ± 0.15	0.61±0.21	0.57±0.17	0.251
Plt ($\times 10^9$/l)	273.2 ± 46.9	247.11±59.75	224.83±58.06	0.042
INR	0.99 ± 0.04	1.06±0.06	1.08±0.09	0.088
Ang-1(pg/ml)	1356.6 ± 85.2	2565.5 ± 374.0	1919.1 ± 339.5	<0.0001

Variables were expressed as mean ± SD. p values >0.05 were not statistically significant but those with p<0.05 were considered significant, and P<0.01 were considered very significant and P<0.001 or less were highly significant. Alanine aminotransferase (**ALT**); aspartate aminotransferase (**AST**); alkaline phosphatase (ALP); albumin (**Alb)**; total bilirubin (**T.Bili)**; platelets count (**plt**); international normalized ratio (**INR)** ;and Angiopoietin-1 (**Ang-1)**.

Table 2: Diagnostic powers of angiopoietin-1(Ang-1) versus those of AAR in discriminating the stages of liver fibrosis.

Patients with significant fibrosis versus those of non- significant fibrosis							
Parameter	**Cut off**	**AUC**	**Sn**	**Sp**	**PPV**	**NPV**	**P**
Ang-1	>2186	0.919	91	89	89	91	<0.0001
AAR	>0.91	0.700	60.0	47.5	36.4	70.4	<0.0065

AUC: area under the receiver-operating characteristic curve; PPV: positive predictive value; NPV: negative predictive value p values >0.05 were not statistically significant but those with p<0.05 were considered significant, and P<0.01 were considered very significant and P<0.001 or less were highly significant.

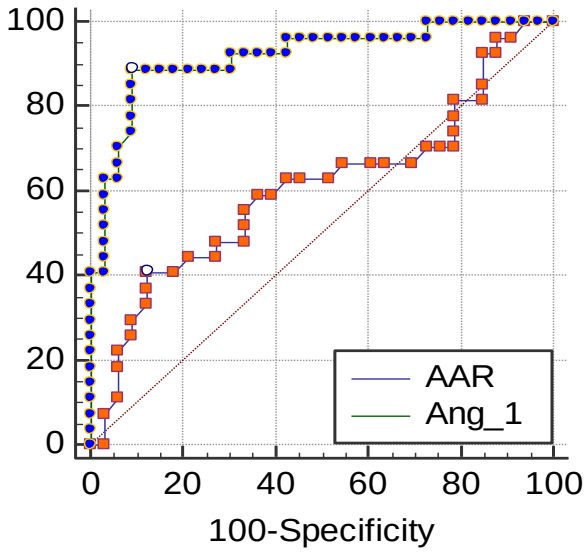

Figure1: **ROC curve of Angiopoitin-1 (Ang-1) versus those of AAR score.**

DISCUSSION

In present study circulating levels of angiopoietin-1 at a cutoff point more than 2186 pg/ml was able to differentiate between CHC patients with significant from those with non-significant fibrosis with sensitivity of 89%, specificity of 91%, PPV of 89% and NPV of 91% with an area under curve of 0.919 (p<0.0001).

The AAR is also a non-invasive method for the evaluation of liver fibrosis; this is because the sore is based on the simple variables of AST and ALT[9, 10].

Such score was re-evaluated in our patients. In this regard the AUROC of AAR to differentiate significant (F2–4) from non-significant fibrosis (F0-1) was 0.700, with a sensitivity of 60.0% and a specificity of 47.5% and a cutoff of > 0.91. The NPV was 70.4% and the PPV was 36.4%. Similar results were obtained by other authors who evaluated the performance of AAR in HCV monoinfected patients.

Our previous study[11] observed an AUROC of AAR 0.62, to be differentiating patients with significant fibrosis from those with non-significant one with sensitivity of 54% and a specificity of 78% at a cutoff value of >0.91. The NPV was 53% and the PPV was 78%.

Thus, the superiority of the Ang-1 in differentiation between significant and non-significant fibrosis in our study lead us to conclude that Ang-1, as a direct and angiogenic marker of hepatic fibrosis, is strongly related to hepatic disorders. This is because, vascular remodeling has repeatedly been observed during the evaluation of chronic liver disease and the levels of the main related factors; including Ang-1 might help to evaluate the progression of such diseases[12].

Also, Ang2/Ang1 ratio might constitute a useful tool for monitoring the progression of chronic liver disease towards cirrhosis and play an important role as therapeutic target as was described by[13]. In conclusion, the simple, sheep and easy to perform enzyme linked immune-sorbent assay for Ang-1 was superior to AAR in the diagnosis of non-significant fibrosis in Egyptian patients with chronic HCV infection.

REFERENCES

1. Lehman EM, Wilson ML. Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. J Viral Hepatology 2009; 16(9): 650–8.
2. Schiff ER, Lee SS, Chao YC, Kew-Yoon S, Bessone F and Wu SS. Long-term treatment with entecavir induces reversal of advanced fibrosis or cirrhosis in patients with chronic hepatitis B. *Clinical Gastroenterology Hepatology* 2011; 9: 274-276.
3. Chang TT, Liaw YF, Wu SS, Schiff E, Han KH and Lai CL. Long-term entecavir therapy results in the reversal of fibrosis/ cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology 2010*; 52: 886-893.
4. Attallah A M; Toson E A; Shiha G E; Omran MM ; Abdel-Aziz M and El- Dosoky I. Evaluation of serum Procollagen amino terminal peptide III laminin and Hydroxyproline as predictors of severe fibrosis in patients with chronic hepatitis C. Journal of Immunoassay and Immunochemistry 2007; 28: 199- 211.
5. Sakugawa H, Nakayoshi T and Kobashigawa K: Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. *World Journal of Gastroenterology* 2005; 11: 255–259.
6. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HI system. *Nature Medicine* 2003; 9: 677-844.
7. Thabut D, Shah V. Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? *J Hepatol* 2010; 53: 976-980.
8. Baranova A, La LP, Birerdinc A and Z. M. Younossi. Non-invasive markerfor hepatic fibrosis.*BMC Gastroenterology* 2011; 11: article 91
9. Williams AL and Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis Relationship to cirrhosis *Gastroenterology* 1988; 95: 734-9
10. Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998; 93: 44–48.
11. El-Shahat A. Toson, Gamal E. Shihab, Hatem A. El-mezayen, Waleed Samir, and Mohamed M. El-khininy. Noninvasive estimation of liver fibrosis in biopsy-proven hepatitis C virus-infected patients: angiogenic fibrogenic link. Eur J Gastroenterol Hepatol.2017 Feb; 29(2): 199-207.
12. Hernández-Bartolomé A, López-Rodríguez R, Rodríguez-Muñoz Y, Martín-Vílchez S, Borque MJ, García-Buey L, González-Moreno L, Real Y, Moreno-Otero R, Sanz-Cameno P. Angiopoietin-2 Serum Levels Improve Noninvasive Fibrosis Staging in Chronic Hepatitis C: A Fibrogenic-Angiogenic Link. PLoS One 2013; 8: e66143 [PMID: 23823085 DOI: 10.1371/journal.pone.0066143]
13. Hernández-Bartolomé Á, López-Rodríguez R, Jesús Borque M, González- Moreno L, Real-Martínez Y, García-Buey L, Moreno-Otero R and Sanz-Cameno P. Angiopoietin-2/angiopoietin-1 as non-invasive biomarker of cirrhosis in chronic hepatitis C. World J of Gastroenterology 2016; 22(44): 9744-9751.