

## ANTITHROMBIN III AS MARKER OF FIBROSIS IN CHRONIC HEPATITIS C

BHALLI A.,<sup>1</sup> MOHSIN S.,<sup>2</sup> ASLAM M.,<sup>3</sup> SAEED T.,<sup>4</sup> HUSSAIN S.,<sup>5</sup> AND BASHIR N.,<sup>6</sup>

Department of Haematology, <sup>1,3,4</sup>Sharif Medical College, <sup>2,5,6</sup>University of Health Sciences, Lahore – Pakistan

### ABSTRACT

**Background and Objective:** Chronic hepatitis C is one of the most common causes of liver cirrhosis. Haemostasis profile derangement including decrease in anticoagulant factors is a regular feature in liver disease but degree of decrease in anticoagulant factors and its clinical significance is currently unclear. Liver biopsy is the gold standard for the assessment of cirrhosis in chronic hepatitis. However, biopsy is invasive and costly and is associated with patient discomfort and risk of major complications. Antithrombin – III (AT) is synthesized in liver and changes in its levels are associated with the extent of cirrhosis. This study aimed to establish the role of AT as a noninvasive marker of fibrosis in chronic hepatitis C patients.

**Methods:** Fifty chronic hepatitis patients were included in this study. They were further divided into two groups on the basis of their histological stages of fibrosis. Group A included stages 0-3 of fibrosis whereas group B included stages 4 – 7 of fibrosis. Each group comprised of 25 chronic hepatitis patients. AT, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and platelet count were performed on all subjects.

**Results:** Mean  $\pm$  SD of AT in patients with group A (fibrosis stage 0 – 3) was  $96.48 \pm 12.13\%$  whereas AT level in group B patients (fibrosis stage 4 – 6) was  $58.92 \pm 22.03\%$  (p value < 0.001).

**Conclusion:** These results showed level of AT was reduced in advanced stage of fibrosis when compared with early stage of fibrosis.

**Key words:** Antithrombin-III (AT), alanine aminotransferase (ALT), aspartate aminotransferase (AST).

### INTRODUCTION

Chronic hepatitis C is a major cause of morbidity and mortality and is often associated with complex defects in haemostasis.<sup>1</sup> The etiology of deranged homeostasis in liver disease is due to; impaired coagulation factors synthesis,<sup>2</sup> deficiencies of natural anticoagulants,<sup>3</sup> qualitative and quantitative platelets disorders.<sup>3,4</sup> About 20% of the chronic hepatitis C patients develop cirrhosis<sup>5</sup> which is a serious and irreversible disease characterized by replacement of liver tissue by fibrotic tissue and regenerative nodules resulting in blockage of portal flow of blood leading to progressive loss of liver function.<sup>6</sup> AT, a natural anticoagulant, is primarily synthesized in liver. It is the major inhibitor of thrombin, factor XIIa, XIa, Xa and IXa. It also inhibits complement enzyme C1, plasmin and kallikrein.<sup>8</sup> AT deficiency occurs in end stage liver disease, because of inadequate synthesis, diffuse intravascular coagulation and reduced transcapillary flux ratios.<sup>9,10</sup> Similarly in hepatitis C related chronic liver disease the level of AT is also markedly reduced.<sup>11</sup> Patients have prolonged circulation of activated coagulation factors due to lack of inhibition from AT. Stasis, alteration of coagulability of the blood and vessel wall damage increases the risk

of thrombus formation. The balance between the procoagulants and anticoagulants, which is crucial to avoid surplus thrombin generation under physiological conditions, is not maintained in liver disorders. So excess of thrombin, which is involved in tissue remodeling and increased clot formation, leads to blood flow obstruction and hypoxia that in turn favors fibrosis.<sup>12</sup>

Liver biopsy is the gold standard to determine the progression of fibrosis in chronic hepatitis C through necroinflammatory activity (referred to as grading) and fibrosis (often referred to as staging)<sup>13</sup> (Table 1). However, biopsy is associated with major discomforts and complications. It is invasive and costly as well.<sup>14</sup> The liver enzymes such as AST and ALT are elevated in chronic liver disorders. Evaluation of these enzymes is included in the screening tests for liver diseases and is an early indication of asymptomatic hepatic disorders. The ratio of ALT to AST is a predictor of fibrosis as ratio > 1 is proposed as a test for cirrhosis.<sup>15</sup> In addition thrombocytopenia is present, so platelet count is also a predictor of liver fibrosis and correlates with fibrotic stage.<sup>16</sup> In our study we measured the level of AT in patients of chronic liver disease and correlated it with the different stages of fibrosis. AT can be used as

noninvasive marker of fibrosis in advance stage of fibrosis. From clinical point of view the determination of level of AT may be valuable in monitoring fibrosis in chronic liver disease.<sup>17</sup>

## MATERIALS AND METHODS

Permission from ethical review committee of University of Health Sciences (UHS), Lahore, Pakistan was obtained prior to the start of the study. This cross sectional comparative study was conducted at Department of Haematology, UHS. Fifty diagnosed patients of hepatitis C at different stages of fibrosis, were evaluated by percutaneous liver biopsy. All biopsies were evaluated by the same histopathologist. The histological changes of chronic hepatitis were classified according to the modified histologic activity index (HAI). The chronic hepatitis grading score (1 – 8), which represents necroinflammatory activity as the sum of the piecemeal necrosis score (0 – 4), confluent necrosis score (0 – 6), focal lytic necrosis, apoptosis and focal inflammation score (0 – 4), and portal inflammation score (0 – 4). The fibrosis score (0 – 6) is based on the degree and extent of fibrosis, architectural alterations, and development of cirrhosis. Patients on anticoagulants, oral contraceptives, with decompensated cirrhosis, congenital deficiency of AT and DIC were excluded.

Subjects were divided into two groups according to early and advanced histological stages of fibrosis. Group A included stages 0-3 whereas group B included stages 4 – 7 of fibrosis. Each group contained 25 chronic hepatitis patients. Consecutive sampling technique was used. Blood samples were collected from each subject on the day of liver biopsy. Blood was withdrawn by means of a 10 ml disposable syringe. 3ml of blood was first transferred to EDTA vacutainers for platelet estimation, 3ml in 3.2% sodium citrate vacutainers for AT – III and rest was transferred to yellow topped gel vacutainers for ALT and AST estimation. Platelet count of all the samples were done on automated Hematology Analyzer Sysmex XT – 1800i. AST and ALT activity level in serum was determined by commercially available kit of FORTRESS diagnostics by using HUMALYZER 3500 analyser (Human diagnostica Germany).

The quantitative assay of AT is based on the determination of AT activity level in plasma by synthetic chromogenic substrate method by using STA Compact® auto analyser (Diagnostica Stago France). The detection of chromogenic assay was based on the absorbance (optical density O.D.) of monochromatic (405 nm) light. In the presence of heparin the plasma was incubated with known excess of thrombin. The residual thrombin was then quantitated by its amidolytic action on the synthetic chromogenic substrate [Para nitro aniline (pNA)] and was measured at 405 nm. Since the quantity of thrombin neutralized in first reaction step was proportional to the AT level present

in the plasma being tested, the residual thrombin in the second reaction step (as measured by pNA release) was inversely proportional to the AT level of the test plasma.

The data was analyzed using SPSS 16.0. Mean  $\pm$  S.D were given for quantitative variables. Two independent sample t – test was applied to observe mean differences in different groups. Pearson correlation was applied to observe correlation between quantitative variables. Two – tailed probability values of  $< 0.01$  were considered of statistical significance.

## RESULTS

Fifty diagnosed patients of hepatitis C with mean age of  $44.40 \pm 6.26$  (range 22 – 52 years) were included in the study. Among which 27 were male and 23 were female patients. Higher mean concentration of AT was observed in fibrosis stage (0 – 3). Mean value was  $96.48 \pm 12.13\%$  and range was 23 – 111%. A lower mean concentration of  $58.92 \pm 22.01\%$  was observed in advanced stages 4 – 6 with statistically significant difference ( $p < 0.001$ ). When level of AT was compared within groups (Table 2) (Figure 1). Mean value of AST and ALT in early fibrosis was  $142.56 \text{ IU/L} \pm 52.47 \text{ IU/L}$  (84 – 258 IU/L) and  $118.24 \pm 46.66 \text{ IU/L}$  (range 65 – 242 IU/L) respectively as compared to advanced fibrosis  $295.32 \text{ IU/L} \pm 89.99 \text{ IU/L}$  (range 92 – 428 IU/L) and  $245.71 \pm 81.81$  (range 78 – 388) respectively. A significant difference ( $p < 0.001$ ) was observed when mean values were compared between the groups (Table 3).

**Table 1:** Modified staging score.

Modified Staging: architectural changes, fibrosis and cirrhosis	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging portal to portal (P-P) as well as portal to central (P-C)	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6

*Ishak et al., 1995; Knodell et al., 1981*

Mean Platelet count was higher;  $254.72 \pm 71.68 \times 10^9/\text{L}$  (range 156 –  $395 \times 10^9/\text{L}$ ) when observed in fibrosis stage (0 – 3) while the advanced stage of fibrosis

4-6 revealed considerably lower mean platelet count of  $120.88 \pm 76.4 \times 10^9/L$  (range  $38 - 348 \times 10^9/L$ ). There was significant difference between the two groups ( $p$  value  $< 0.001$ ).

**DISCUSSION**

In chronic hepatitis C, cirrhosis can be predicted by combinations of clinical features, biochemical tests, an array of fibrosis markers and radiological studies. Liver biopsy is the gold standard for assessment of cirrhosis. However, biopsy is associated with patient discomfort and risk of major complications, including death. It is invasive and costly as well. Thus an inexpensive, noninvasive and accurate method for diagnosing cirrhosis should be established.

Our results demonstrated that the mean plasma AT levels were significantly higher in group A (fibrosis stage 0 - 3) as compared to the group B (fibrosis stage 4 - 7). In the present study we observed decrease in antithrombin levels with advancement of fibrosis. As in group A mean antithrombin level was found to be 96.48% and in group B its concentration was 58.92%. Considerably lower level of AT in advanced stage of fibrosis was reported by another study conducted by Papatheodoridis *et al* in 2003.<sup>18</sup> The levels of AT - III in plasma were low in patients with chronic cirrhosis ( $p < 0.05$ ). These low levels of AT - III in patients with chronic liver disease can be used as a non-invasive marker for the diagnosis of cirrhosis.<sup>19</sup> Several studies have inspected the reduction of AT - III in chronic liver disease as plasma concentration of AT - III is significantly reduced in cirrhosis.<sup>20,21</sup> Saray A *et al* in 2012 has shown that in chronic hepatitis the level of antithrombin is reduced and may be used as an early marker of hepatocellular damage.<sup>22</sup> In venous embolization there is a disturbance of microcirculation in liver that leads to the changed plasma concentration of AT - III in cirrhosis<sup>23</sup>. Thrombosis and phlebitis lead to obstruction of small portal or hepatic veins which leads to progression of chronic liver disease and ultimately death of hepatocytes and development of fibrosis. In liver cirrhosis, levels of AT III are very low due to its inadequate hepatic synthesis, reduced transcapillary flux ratios and diffuse intravascular coagulation.<sup>24</sup>

It is **concluded** that Level of AT was significantly reduced with advancing stages of fibrosis and it can be used as noninvasive marker of fibrosis in chronic hepatitis C.

**Limitations of Study**

Fifty patients were included in the study which is not sufficient to establish antithrombin III as noninvasive marker in hepatitis C patients in our population of

**Table 2:** Mean value of the Antithrombin III in stage 0 – 3 and 4 – 6.

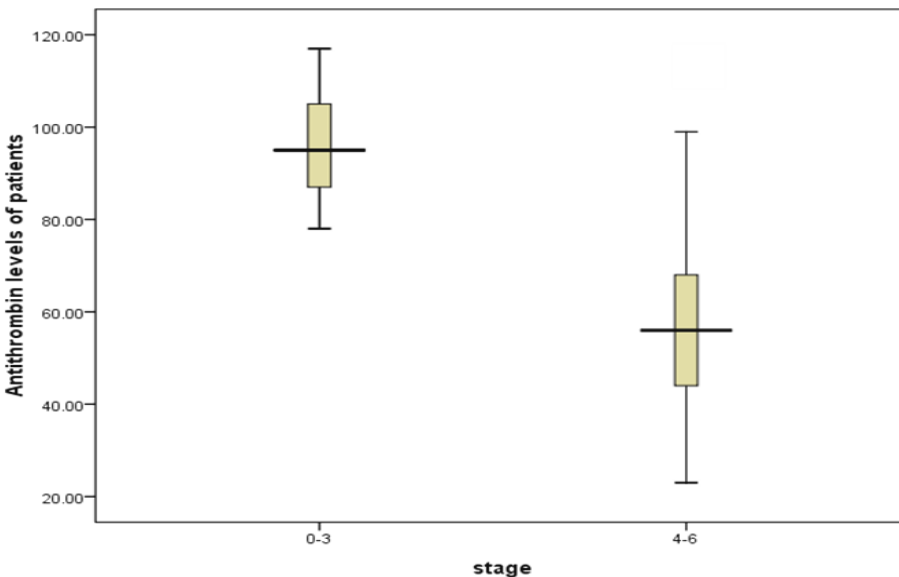
Study Variables	Stage (0 – 3) (n = 25)		Stage (4 – 6) (n = 25)		p-value
	Mean ± SD	Range	Mean ± SD	Range	
AT (%)	96.48 ± 12.13	78 – 117	58.92 ± 22.03	23 – 111	< 0.001

Legends: Antithrombin III (AT)

**Table 3:** Mean values of the AST and ALT in stage 0 – 3 and stage 4 – 6.

Study Variables	Stage (0 – 3) (n = 25)		Stage (4 – 6) (n = 25)		p-value
	Mean ± SD	Range	Mean ± SD	Range	
AST (IU/L)	142.56 ± 52.47	84 – 258	295.72 ± 89.9	92 – 428	< 0.001
ALT (IU/L)	118.24 ± 46.66	65 – 242	245.71 ± 81.81	78 – 388	< 0.001

Legends: Aspartate aminotransferase (AST), Alanine aminotrasferase (ALT)



**Fig. 1:** Box – plots for mean concentration of antithrombin III in early and advance stages of fibrosis. Boxes show range of antithrombin and horizontal line inside the boxes indicates the mean value. The decreased mean concentration of anti-thrombin III was observed in advance stages of fibrosis.

Pakistan. The samples of plasma were taken and stored at  $-70^{\circ}\text{C}$  prior to assay, the results might be slightly different from the where coagulation factors are measured on fresh samples.

#### ACKNOWLEDGEMENTS

We would like to acknowledge University of Health Sciences Lahore, Pakistan for financial and technical support. We are also grateful to all the patients for providing samples for this study.

#### REFERENCES

- Lisman T, Porte R. Rebalanced hemostasis in patients with liver disease: evidence and clinical consequences. *Blood*. 2010; 116 (6): 878-885.
- Gresele P, Binetti BM, Branca G, Clerici C, Ascitti S, Morelli A et al. TAFI deficiency in liver cirrhosis: Relation with plasma fibrinolysis and survival. *Thrombosis Research*, 2008; 121: 763-8.
- Sogaard KK, Puho EH, Gronbaek H, Jepsen P, Vilstrup H, Sorensen H T. Risk of venous thromboembolism in patients with liver disease: A Nation – wide population based case control study. *Am J Gastroenterol*. 2009; 104: 96-101.
- Cesarman G, Maus, Katherine A. Molecular mechanism of fibrinolysis. *British journal of hematology*, 2005; 129: 307-21.
- Davarpanaah MA, Saberi – Firouzi M, Basherilankarani K, Mehrabani D, Behzad – Behbahani A, Serati A et al. Hepatitis C virus genotype distribution in Shiraz, southern Iran. *Hepatitis*, 2009; 9 (2): 122-7.
- Khan H, Zarif M. Risk factors, complication and prognosis of cirrhosis in a tertiary care hospital in Peshawar. *Hepatitis*, 2006; 6 (1): 7-10.
- Greer JP, Wintrob MM. Blood coagulation and fibrinolysis. *Wintrob's Clinical Haematology*, 2008; 1 (5): 563.
- Dahlback B. Blood coagulation and its regulation by anticoagulant pathways: genetic pathogenesis of bleeding and thrombotic diseases. *J. Intern. Med*. 2005; 257: 209–23.
- Marchant K. K & Duncan A. Antithrombin Deficiency. *Arch. Pathol. Lab. Med*. 2004; 126: 1326-36.
- Muller G. Acquired antithrombin III deficiency. *Z. Gesamte. Inn. Med*. 1994; 47 (2):74-7.
- Ponziani FR, Zocco MA, Garcovich M, D'Aversa F, Roccarina D, Gasbarrini A, What we should know about portal vein thrombosis in cirrhotic patients: A changing perspective. *World J Gastroenterol*. 2012; 18 (36): 5014-5020.
- Binita S, Mehul C, Vandit T & Gaurang S. Anti-fibrotic effect of heparin, silymarin and its combination on liver fibrosis model in rats. *Journal of pharmaceutical research and opinion*, 2011; 1 (6): 180-186.
- Theises N. D. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. *Modern. Pathology*, 2007; 20: 3–14.
- Dienstag J. L. The Role of Liver Biopsy in Chronic Hepatitis C. *Hepatology*, 2002; 36: 152-60.
- Khokhar N. Serum aminotransferase levels and platelet count as predictive factor of fibrosis and cirrhosis in patients with chronic hepatitis C infection. *Journal of Pakistan Medical Association*, 2003; 53: 101-07.
- Hayashi H, Beppu T, Shirabe K, Maehara Y, Baba H. Management of thrombocytopenia due to liver cirrhosis: A review. *World J Gastroenterol*. 2014; 20 (10): 2595–2605.
- Kujovich J. L. Hemostatic defects in end stage liver disease. *Crit. Care. Clin*. 2005; 21: 563–87.
- Papatheodoridis G. V, Papakonstantinou E, Andrioti E, Cholongitas E, Petraki K, Kontopoulou I. et al. Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis. *Gut*. 2003; 52: 404–09.
- Sajjadih S. R and Viunytska V. L. Antithrombin III as non-invasive marker in Chronic Liver Disease. *Hepatitis. Monthly*, 2009; 9 (2): 128-132.
- Gursoy S, Baskol M, Torun E, Yutci A, Soyuer I, Eser B, Guven K, Ozbakir O, Yucesoy M. Importance of anticoagulant proteins in chronic liver diseases. *Turk J Gastroenterol*. 2005; 16 (3): 129-33.
- Ghumlas A. K & Abelgader A. G. The liver and the hemostatic system. *The Saudi Journal of Gastroenterology*, 2003; 9 (2): 9-68.
- Saray A, Mesihovic R, Vanis N, Gornjakovic S & Prohic D. Clinical significance of haemostatic tests in chronic liver disease. *Med. Arh*. 2012; 66 (4): 231-5.
- Makson M. S.M, Ryschich E, Ulger Z, Gebhard M. M, Schmidt J. Disturbance of hepatic and intestinal microcirculation in experimental liver cirrhosis. *World. J. Gastroenterol*. 2004; 11 (6): 846-49.
- Muller G. Acquired antithrombin III deficiency. *Z. Gesamte. Inn. Med*. 1994; 47 (2): 74-7.