

# Additional Benefit of $\alpha$ -Fetoprotein in Patients with Chronic Hepatitis B and C without Evidence of Hepatoma

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**Abstract:** ***Background:** Alpha fetoprotein is a substance produced by a fetus' liver that can be found in the amniotic fluid and in the mother's blood. But sometimes appear in abnormally increased level in certain diseases of adults, such as liver cancer, and its level in amniotic fluid can be used to detect certain fetal abnormalities, including Down syndrome and spina bifida. The importance of  $\alpha$ -fetoprotein level elevation in patients with chronic viral hepatitis B and C and its clinical significance in steatosis associated with HBV and HCV infection remain to be clarified. In this study assessed the clinical significance of  $\alpha$ -fetoprotein in patients with chronic hepatitis B and C with and without steatosis. **Methods:** Alpha fetoprotein was measures in fifty patients with chronic viral hepatitis which were divided into 25 patients with chronic hepatitis B (CHB) and 25 patients with chronic hepatitis C (CHC). Both groups further subdivided based on liver biopsy into chronic hepatitis B with, without steatosis and chronic hepatitis C with, without steatosis. **Results.** There was statistically significant increment ( $p < 0.05$ ) of the number and percentage of patients with steatosis in CHC (14, 56%) in comparison to CHB patients (9, 36%).  $\alpha$ -fetoprotein was significantly increased in chronic hepatitis B and C patients with steatosis ( $12.82 \pm 5.3$ ,  $13.01 \pm 7.2$ ) than patients without steatosis ( $6.29 \pm 4.9$ ,  $5.94 \pm 5.9$ ) respectively ( $P < 0.001$ ). Significant positive correlation was found between serum  $\alpha$ -fetoprotein and the severity of fibrosis in patients with CHC with steatosis ( $r = 0.49$ ,  $p < 0.05$ ). While no significant correlation in CHB patients with steatosis. In all patients with steatosis whatever CHC or CHB, there was significant positive correlation between AFP and grade of steatosis ( $p < 0.05$ ,  $r = 0.52$ ). **Conclusion.** Alpha fetoprotein levels significantly increased in patients with chronic hepatitis B and C with steatosis than patients without steatosis. The percentage of patients with steatosis was significantly higher in CHC than CHB. AFP levels had significant positive correlation with the severity of steatosis in patients with chronic hepatitis C and B and severity of fibrosis in chronic hepatitis C only. So in the absence of traditional causes of elevated serum AFP, steatosis should be among the differential diagnoses of elevated serum AFP levels. In other hand elevated AFP level could be considered as a marker of steatosis in chronic hepatitis B patients and marker of steatosis and fibrosis in chronic hepatitis C patients.*

**Keywords:** fibrosis, hepatoma, hepatitis, fetoprotein, steatosis

## 1. Introduction

Alpha-fetoprotein (AFP,  $\alpha$ -fetoprotein; also sometimes called alpha-1-fetoprotein, alpha-fetoglobulin, or alpha fetal protein) is a protein that in humans is encoded by the AFP gene. The AFP gene is located on the q arm of chromosome 4 (4q25) [1]. AFP is the most abundant plasma protein found in the human fetus. Plasma levels decrease rapidly after birth but begin decreasing prenatally starting at the end of the first trimester. Normal adult levels are usually achieved by the age of 8 to 12 months. The function of AFP in adults is unknown; however, in rodent fetuses it binds estradiol to prevent the transport of this hormone across the placenta. The main function of this is to prevent the masculinization of female fetuses. As human AFP does not bind estrogen, its function in human fetuses is less clear [2]. AFP is measured in pregnant women through the analysis of maternal blood or amniotic fluid, as a screening test for the early diagnosis of fetal neural tube defects, such as spina bifida and anencephaly [3]. But may also be found at an elevated level in the sera of adults having, ataxia-telangiectasia syndrome, hereditary tyrosinemia, cirrhosis, alcoholic hepatitis, hepatocellular carcinoma, and viral hepatitis [4]. Chronic hepatitis C (CHC) is thought to affect more than 170 million people worldwide, and it has been shown that steatosis occurs in approximately 50% of patients with CHC [5]. Steatosis also occurs more than twice as frequently in patients with CHC than in the general population [6]. Most steatosis is mild,

with the more severe cases usually occurring in genotype 3 virus infections [7]. Genotype 3 is a steatogenic virus and the severity of hepatic steatosis is related to high viral load in the serum as well as high intrahepatic viral load. In this case, steatosis usually resolves with successful antiviral therapy [8]. In Egypt, more than 90% of patients with HCV were infected with HCV genotype 4 [9]. In cases of chronic hepatitis B (CHB) infection, the clinical significance of steatosis and its relation to HBV geno types are unknown [10]. The frequency of hepatic steatosis in CHB patients is higher than that reported for the general population, but lower than that in CHC patients [11]. It has been shown that the incidence of hepatic steatosis in CHB patients was approximately 32% [12]. In this study, we evaluated serum AFP and its clinical significance in Egyptian patients with chronic hepatitis B and C with and without steatosis.

## 2. Subjects and Methods

This prospective study included 50 patients with chronic viral hepatitis which were divided into 25 patients with chronic hepatitis B and 25 patients with chronic hepatitis C. Both groups further subdivided based on liver biopsy into chronic hepatitis B with, without steatosis and chronic hepatitis C with, without steatosis. Both groups were adjusting as regarding age, sex, and for risk factors for steatosis (BMI, DM, and hyperlipidemia). They were referred to Zagazig university Hospitals, from January 2012 to December 2012, for liver biopsy and searching for chronic hepatitis B and C

management. The study protocol conformed to the ethical guidelines of 1975 Declaration of Helsinki. Informed consent was obtained from all patients. CHB was defined according to positive serum HBsAg for at least 6 months and elevated levels of serum aminotransferases (AST, ALT). CHC was defined according to positive serum HCV-Ab and HCV-RNA for at least 6 months and elevated levels of serum aminotransferases (AST, ALT). The patient's medical histories were taken including, age, gender, weight, height, and body mass index (BMI).

AFP was studied with ELISA method using Abbott laboratory reagents, USA (normal level of AFP was defined as  $<8.1$  ng/mL); serum fasting triglyceride, fasting blood sugar, albumin, AST, ALT and, total bilirubin were determined.

Biochemical tests, HBsAg and HCV-RNA were performed by autoanalyzer (Selecta, Germany) and Cobas Amplicore monitor version 2 (Roche Molecular Systems, Branchburgh, NJ, USA), respectively.

BMI was calculated by the following formula: weight (kg)/height<sup>2</sup> (m<sup>2</sup>). Exclusion criteria were patients with diabetes mellitus, hypertension, and patients who had any serological evidence of infection with other viruses (HDV and HIV), all other known causes of liver diseases were excluded on the basis of analytical, clinical, and epidemiological data: autoimmunity, metabolic and genetic disorders, alcohol intake, drug toxicity and patients with decompensated cirrhosis. Hepatocellular carcinoma and other causes of high levels of AFP like cancer of the testes or ovaries and metastatic liver cancer were excluded using ultrasound as the predominant screening method.

Percutaneous liver biopsy ( $\geq 15$ mm in length) was performed for all the patients. Liver biopsy specimens were reviewed by a single pathologist. For each liver biopsy specimen, hematoxylin and eosin and Masson's trichrome stains were available. The extent of hepatic steatosis was assessed and graded as none labeled as 0 (steatosis  $< 5\%$ ), mild steatosis labeled as 1 (steatosis 5–33% of hepatocytes), moderate steatosis labeled as 2 (steatosis 34–66% of hepatocytes), and severe labeled as 3 (steatosis  $> 66\%$  of hepatocytes) according to histological scoring system of Kleiner et al., [13]. Liver fibrosis stages were evaluated semi-quantitatively according to the Metavir scoring-system (Bedossa and Poynard, 1996) [14]. Fibrosis was staged on a scale of 0 to 4: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis.

### 3. Statistical Analysis

The statistical analysis of data was done by using *Excel* program and *SPSS* program (statistical package for socialscience) version 10. Data are expressed as the mean  $\pm$ SD. Mean values were compared with the Student's *t*-test (variables with normal distribution) or Mann-Whitney U test (variables with non normal distribution). Categorical variables were compared using the chi-square test. Correlations were done using Pearson's correlation.

All the tests performed were two sided and a *P* value  $<0.05$  was considered to be statistically significant.

### 4. Results

This prospective study included 50 patients with chronic viral hepatitis which were divided into 25 patients with chronic hepatitis B and 25 patients with chronic hepatitis C. According to results of liver biopsy out of 25 patients with chronic hepatitis B; 9 (36%) patients had an evidence of hepatic steatosis and the rest 16 (64%) showed no steatosis. While in chronic hepatitis C patients; 14 (56%) patients had steatosis and the others 11 (44%) had no evidence. By comparing these results, there were statistically significant increment ( $p < 0.05$ ) of the number and percentage of patients with steatosis in CHC patients in comparison to CHB patients (figure 1-5).

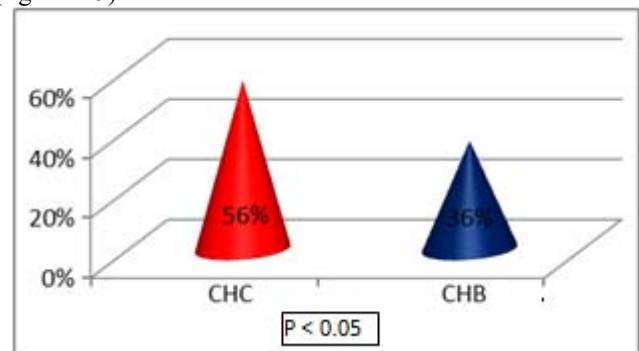


Figure 1: Comparison between the percentage of steatosis in patients with CHB and CHC

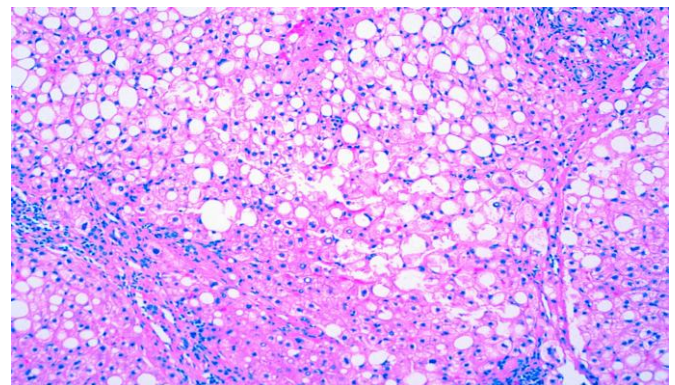


Figure 2

This liver biopsy specimen from patient with chronic hepatitis C Shows active steatohepatitis with moderate steatosis. The distribution of steatosis is accentuated around the fibrous septa, foci of inflammatory cell aggregates in the lobules and ballooned hepatocytes containing Mallory hyaline are evident (H&E,  $\times 100$ ).

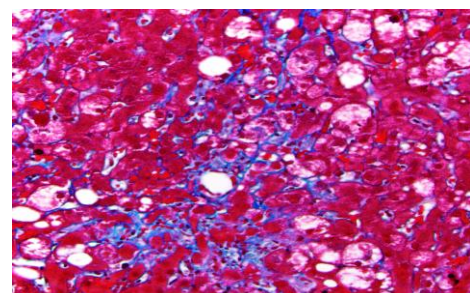
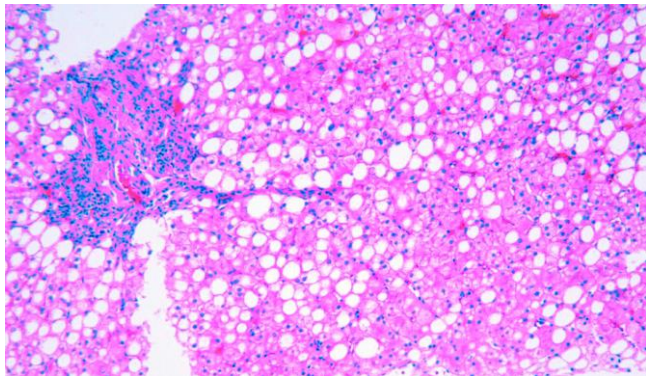


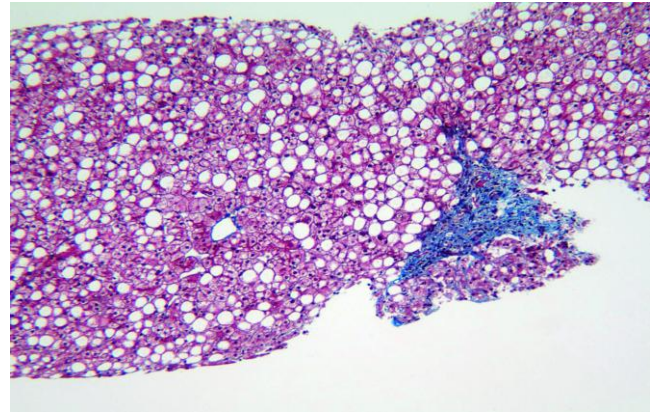
Figure 3

This liver biopsy specimen from patient with chronic hepatitis C Shows active steatohepatitis with Perivenular/pericellular fibrosis. (Masson trichrome, ×200).



**Figure 4**

This liver biopsy specimen from patient with chronic hepatitis B Shows active steatohepatitis with moderate steatosis with inflammation and fibrosis; inflammatory cell aggregates in the lobules and ballooned hepatocytes (H&E, ×100).



**Figure 5**

This liver biopsy specimen from patient with chronic hepatitis B Shows active steatohepatitis with moderate steatosis with inflammation and fibrosis (Masson trichrome, ×100).

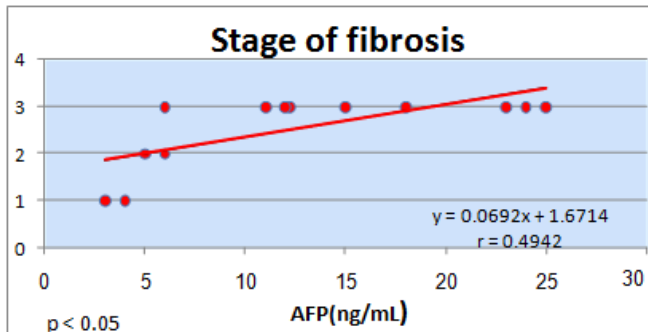
**Table 1:** Clinical, biochemical and histopathological characteristics of chronic hepatitis B and C patients with and without steatosis

	Chronic hepatitis B (n: 25)		Chronic hepatitis C (n:25)		p
	With steatosis (n:9)	Without steatosis (n:16)	With steatosis (n:14)	Without steatosis (n:11)	
Age (years)	44.2±11.3	41.9±14.3	42.6±9.4	39.9±11.8	> 0.05
S.Triglyceride (mg/dL)	112.3±18.3	106±11.8	118.8±21.5	109.4±12.4	> 0.05
FB glucose (mg/dL)	105.2±14.1	101.8±11.2	107.9±12.8	103.8±10.8	> 0.05
BMI	29.9 ± 5.6	29.1 ± 5.1	30.4 ± 5.5	29.9 ± 6.1	> 0.05
Albumin (gm/dl)	3.6 ± 0.6	3.9 ± 0.5	3.5 ± 0.6	3.6 ± 0.7	> 0.05
S.Total Bilirubin (mg/dL)	<u>0.81 ± 0.36</u>		<u>0.86 ± 0.41</u>		> 0.05
	0.89 ± 0.33	0.74 ± 0.29	0.92 ± 0.36	0.81 ± 0.32	< 0.05
	0.89 ± 0.33		0.92 ± 0.36		> 0.05
Prothrombin time concentration %	<u>86.9 ± 9.7</u>		<u>89.7 ± 11.4</u>		> 0.05
	89.9 ± 9.1	83.6 ± 8.7	92.3 ± 11.1	86.1 ± 10.2	< 0.05
	89.9 ± 9.1		92.3 ± 11.1		> 0.05
AST (u/L)	<u>51.1 ± 21.3</u>		<u>56.3 ± 22.4</u>		> 0.05
	56.2 ± 19.4	36.7 ± 11.9	62.6 ± 21.5	44.8 ± 17.3	< 0.05
	56.2 ± 19.4		62.6 ± 21.5		> 0.05
ALT (u/L)	<u>54.3±20.4</u>		<u>61.2±22.8</u>		> 0.05
	59.1 ± 20.7	47.1 ± 15.5	70.7 ± 22.3	50.1 ± 20.7	< 0.05
	59.1 ± 20.7		70.7 ± 22.3		> 0.05
Percentage of patients with steatosis	<u>36%</u>		<u>56%</u>		< 0.05
Grade of steatosis	2 ± 08		2.07 ± 0.8		> 0.05
Stage of fibrosis	<u>1.6 ± 1.3</u>		<u>1.92 ± 1.3</u>		> 0.05
	2.55 ± 0.91	1.06± 1.3	2.57 ± 1.1	1.09±1.3	< 0.01
	2.55 ± 0.91		2.57 ± 1.1		> 0.05
AFP (ng/mL)	<u>8.64 ± 5.1</u>		<u>9.9 ± 7.3</u>		> 0.05
	12.82±5.3	6.29± 4.9	13.01± 7.2	5.94±5.9	< 0.01
	12.82±5.3		13.01± 7.2		> 0.05

There were no significant differences of the mean values of the age, fasting blood glucose, triglyceride, BMI, albumin, total billirubin, prothrombin time concentration %, AST,

ALT, stage of fibrosis and AFP between patients with CHB and CHC. However, the values were slightly higher in patients with CHC which were not statistically significant. In

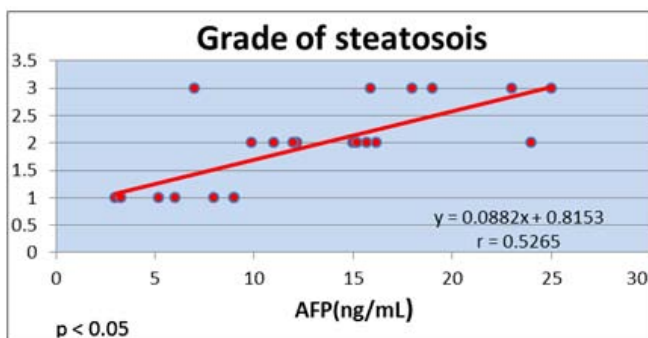
patients with CHC, there were statistically significant differences between patients with steatosis and the patients without as regard mean values of total billirubin, prothrombin time concentration %, AST, ALT ( $p < 0.05$ ) and highly significant difference as regard stage of fibrosis and AFP ( $P < 0.01$ ). In CHC patients with steatosis, there was significant positive correlation between AFP and stage of fibrosis ( $p < 0.05$ ,  $r = 0.49$ ) Figure 6



**Figure 6:** Correlation of AFP and stage of fibrosis in CHC patients with steatosis

In patients with CHB, there were statistically significant differences between patients with steatosis and the patients without as regard mean values of total billirubin, prothrombin time concentration %, AST, ALT ( $p < 0.05$ ) and highly significant difference as regard stage of fibrosis and AFP ( $P < 0.01$ ). In CHB patients with steatosis, there was no significant correlation between AFP and stage of fibrosis ( $p > 0.05$ ,  $r = 0.16$ )

There were no significant differences of the mean values of the age, fasting blood glucose, triglyceride, BMI, albumin, total billirubin, prothrombin time concentration %, AST, ALT, stage of fibrosis ,AFP and grade of steatosis between patients of CHB with steatosis and CHC with steatosis. However, the values were slightly higher in patients with CHC which were not statistically significant. In all patient with steatosis whatever CHC or CHB (n:23), there was significant positive correlation between AFP and grade of steatosis ( $p < 0.05$ ,  $r = 0.52$ ) Figure 7



**Figure 7**

## 5. Discussion

Hepatic steatosis is characterized by the accumulation of lipids in hepatocytes and is associated with diverse systemic conditions and various primary liver diseases [15]. In addition, steatosis induces chronic hepatic inflammation, reactive oxygen species, and DNA damage

in animal models [3]. So steatosis is associated with more degree of inflammation and fibrosis. Hepatic progenitor cells (HPCs) arise in the periportal region of the liver and may be responsible for liver regeneration. Alpha fetoprotein is a substance produced by a fetus' liver that can be found in the amniotic fluid and in the mother's blood [2]. But may also be found at an elevated level in the sera of adults having, ataxia-telangiectasia syndrome, hereditary tyrosinemia, cirrhosis, alcoholic hepatitis, hepatocellular carcinoma, and viral hepatitis [4] and its level in amniotic fluid can be used to detect certain fetal abnormalities, including Down syndrome and spina bifida [4,16]. In this study, 36% of hepatitis B patients and 56% of hepatitis C patients showed evidence of steatosis. Similar results were obtained by Asselah et al., (2006) in HCV patients [17]. And Bondini et al., (2007) in HBV patients [18]. The reported prevalence of steatosis depending on the features of the population studied in terms of alcohol consumption, prevalence of obesity, diabetes, and other risk factors [17]. In our study, these risk factors were justified in all groups but still the prevalence of steatosis in CHC patients was significantly higher than CHB patients. These results were almost similar to Thomopoulos et al., [19], suggesting that HCV may directly cause steatosis, at least in some patients. Viral effects include a decrease of adiponectin levels, and changes to hepatic lipid metabolism that lead to triglyceride accumulation [20]. All HCV genotypes are steatogenic, but numerous reports showed that steatosis was more frequent and more severe in patients infected with genotype 3 [15]. In Egypt, more than 90% of patients with HCV were infected with HCV genotype 4 [9]. Similar to previous study [21, 22] we found that AFP was significantly increased in patients with chronic hepatitis B and C associated with steatosis than patients without steatosis. Also there was significant positive correlation with the severity of steatosis. This significant elevation and correlation with steatosis could be explained by the fact that steatosis is associated with more degree of inflammation, reactive oxygen species, and DNA damage and a lot of Hepatic progenitor cells (HPCs) arise in the periportal region of the liver that express high levels of AFP, certain keratin markers, and GGT [23]. So HPCs and the extent of the ductular reaction as provided by Clouston et al., provide a potential mechanism whereby steatosis contributes to the increase in AFP [24]. Another interesting point, we found significant positive correlation of AFP and severity of fibrosis in patients with CHC associated with steatosis. These data supported by that done by Nasser et al., (2012) and Chu et al., (2001) [25, 26]. These results could be easily understood if we knew that activation of HPCs (developed and activated due steatosis and responsible for AFP elevation) has been documented in parallel with cells associated with the development of fibrosis (stellate cells) [27]. So in a large portion of cases, steatosis is associated with worsening fibrosis. However despite this attractive explanation, it cannot explain why in our study, AFP had no significant correlation with the severity of fibrosis in patients with CHB associated with steatosis. In fact, this failure might be related to small number of the patient (only 9 patients). The amino transferases are also important biological markers that are widely used for liver diseases. Elevation of the activity of these enzymes in serum is believed to result from their leakage from damaged cells, and so this reflects hepatocyte injury. [25]. In this study the levels of amino transferases AST and ALT are significantly

increased in patients with steatosis whatever CHC or CHB in comparison to patients without steatosis, indicating more hepatocytes damage and necrosis in patients with steatosis. Our result was in agreement with Hepburn et al., (2005) who found significant increase in ALT and AFP level among patients with steatosis versus patients without steatosis [28].

## 6. Conclusion

Alpha fetoprotein levels significantly increased in patients with chronic hepatitis B and C with steatosis than patients without steatosis. The percentage of patients with steatosis was significantly higher in CHC than CHB. AFP levels had significant positive correlation with the severity of steatosis in patients with chronic hepatitis C and B and severity of fibrosis in chronic hepatitis C only. So in the absence of traditional causes of elevated serum AFP, steatosis should be among the differential diagnoses of elevated serum AFP levels and elevated AFP level could be considered as a marker of steatosis in chronic hepatitis B patients and marker of steatosis and fibrosis in chronic hepatitis C patients.

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