Evaluation of Tumor Necrosis Factor-α, Lactate Dehydrogenase and Gamma -Glutamyl Transferase as Independent Predictors for Monitoring Progressive HCV-Chronic Liver Disease

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ABSTRACT

Objective: Hepatocellular carcinoma (HC) ranks as the 5th most common malignant cancer and the 3rd most frequent cause of death worldwide. The majority of HCC patients are not amenable to curative therapy as they are detected at late stages. Therefore, our aim was to evaluate the clinical implications of TNF-α level and its correlation with the activities of GGT and LDH in monitoring the progression of HCV-chronic liver disease.

Materials and methods: This study comprised forty eight patients suffering from HCV-chronic liver disease; 24(50%) cases with HCC, 14(29.2%) cases with LC and 10(20.8%) cases with CH. Twenty five healthy individuals (HI) served as control group. Sera of all individuals were examined for the activities of TNF-α, LDH, GGT, AFP and its correlation with other laboratory investigations.

Results: Serum levels of TNF-α, LDH, AFP were elevated significantly in HCC patients compared to LC and CH but the difference between LC and CH was elevated significantly only ($p<0.0001$) in TNF-α. Significant association was recorded between LDH and TNF-α, GGT, AFP, ALT and AST levels. Linear regression for TNF-α, LDH and AFP showed significant prediction for progression of HCV-chronic liver disease.

Conclusion: Serum level of LDH and TNF-α could be used simultaneously with AFP for the evaluation of chronic inflammation leading to cancer development.

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as the 5th most common malignant cancer and the 3rd most frequent cause of cancer leading death worldwide [1,2,3]. HCC is an environmental related cancer, with both viral and chemical carcinogens involved in multi stage process [4]. However, the reasons for viral persistence and transformation from acute to chronic infection are not clear, but it is known that both viral and host characteristics can influence the outcome of the infection [5]

The host response to hepatitis viruses involves various components of the immune system, including T-lymphocyte immune-regulatory cytokines [6,7]. TNF-α, a monocyte/macrophage-derived cytokine, is known to possess anti-neoplastic, anti-viral, and potent immunomodulatory activities [8]. It has been reported that TNF-α is involved in the pathogenesis of a diversity of liver diseases including viral hepatitis and HCC [9].

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The majority of HCC patients are not amenable to curative therapy as they are detected at late stages [4]. Therefore, several tumor markers are used currently for the evaluation of tumor progression and prognosis of patients with HCC including AFP level, Lens Culinaris agglutinin A-reactive fraction of AFP (AFP-L3) [10]. However, AFP is a fairly specific but insensitive marker for HCC. Therefore, to improve the sensitivity of HCC detection by serum markers, various markers are used in combination with AFP [11].

GGT is a microsomal enzyme present in hepatocytes and biliary epithelial cells, renal tubules, pancrease and intestine [12]. Serum GGT activity is a sensitive marker of hepatobiliary disorder. It is generally accepted as the most sensitive marker of cholestasis and pancreatic disease or enzymatic induction by alcohol and drugs [13]. However, GGT activity is not necessarily considered a routine test in the evaluation of liver disease because it is believed to contribute little diagnostic information [14,15,16].

Lactate dehydrogenase (LDH), which is a key enzyme in the conversion of pyruvate to lactate under anaerobic environment [17] has been recognized as an indirect marker of the extent of tumor hypoxia a key biological mechanism for the development of treatment resistance in cancer cells [18,19]. It was known that LDH had 5 isoenzymes, and each of them might function differently in the tumor progression [20]. Therefore, this study was conducted to evaluate the clinical significance of TNF-α level and its correlation with the activities of LDH and GGT in HCV-progressive liver disease.

SUBJECTS AND METHODS

This study comprised forty eight (44 men and 4 women) patients suffering from HCV-chronic liver diseases admitted at Gastroenterology surgical Center, Mansoura university, Egypt. HCV-chronic liver disease patients had positive reactivity for HCV Abs with detectable HCV RNA and with no serologic evidence of co-infection with other hepatotropic viruses or human immunodeficiency virus. Twenty five (15 men and 10 women; mean age 25.3±4.63 yrs, range 19-34 yrs) healthy individuals served as control group were selected without a clinical history of hepatitis and without symptoms or signs of liver diseases.

HCV-infected patients were histopathologically diagnosed and accordingly divided into: 24(50%) patients with HCC (23 men and 1 women; mean age 56.4±6.4yrs; range 50-66 yrs); 14(29.2%) patients with liver cirrhosis (13 men and 1 women; mean age 52.6±9.14yrs, range 30-61yrs) and 10(20.8%) patients with chronic hepatitis (8 men and women; mean age 48.9±12.4 yrs; range 26-65yrs).

Serum samples of all individuals were collected and stored at -70°C until used. All sera were investigated for ALT, AST, Albumin, T.bilirubin, LDH and GGT levels using Hitachi 750XRC Analyzer. Serum AFP level was detected using AFP kit (Abbott Laboratories, USA) and the results were automatically calculated using 1Mx Abbott equipment. In vitro human TNF-alpha ELISA kit (RayBiotech, Inc., www.raybiotech.com) was used for the quantitative measurement of TNF-alpha in serum.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 (version 17, Sydney, NSW, Australia). Continuous variables were expressed as mean±SD and categorical variables were expressed as frequencies and percentages. To

calculate the significance between categorical variables; Chi-square test or Fisher's exact test was used and the difference in continuous variables, Kruskal Wallis and Mann-Whitney U test was used. Linear regression analysis was used for correlation's statistical analysis. Differences between variables were considered significant at p<0.05 [21].

RESULTS

Demographic and base line characteristics of patients with HCV-chronic liver disease and healthy individuals were listed in table (**1**). The difference was considered significant between HCC and LC as regard to age (p=0.038), ALT (p=0.003), AST (p=0.017), Alb. (p=0.004) and HCV viral load (p=0.005) but the difference between LC and CH was detected significant only in ALT (p=0.004) and T.bilirubin (p=0.02). All study groups (HCC, LC, CH) showed highly significant difference (p<0.0001) compared to HI as regard to all variables.

Serum concentrations of AFP, LDH, GGT and TNF-α in all individuals were listed in table (**2**). Significant difference was recorded between HCC and LC in AFP (p<0.0001), LDH (p=0.001), and TNF-α (p<0.0001) and the difference between HCC and CH was detected significant as regard to AFP (p=0.003), LDH (p=0.008), and TNF-α (p<0.0001). However, the difference between LC and CH patients was also highly significant (p<0.0001) in TNF-α only. Compared to HI, significant difference was recorded with HCC (p<0.0001) as regard to AFP, GGT, LDH, TNF-α; with LC as regard to LDH and TNF-α (p<0.0001) and GGT (p=0.005); with CH patients, the difference was considered significant in GGT and LDH (p<0.0001) and in TNF-α (p=0.019). However, there is no significant difference as regard to AFP neither between HI with either LC or CH nor between LC and CH.

Laboratory investigation and biochemical markers in HCV-infected patients was recorded as regard to viral load (data not shown) but there is no significant difference with all variables within all groups except in LDH group (388.5±130.56 vs 271.25±15.47, p=0.01).

Correlation between TNF-α, LDH, GGT and AFP in HCV-infected patients was listed in table (**3**). AFP was associated with LDH (r=0.35, p=0.015), TNF-α was associated with LDH (r=0.37, p=0.009), GGT was associated with LDH (r=0.549, p<0.0001), ALT (r=0.457, p=0.001) and AST (r=0.34, p=0.018). Therefore, LDH showed significant correlation with AFP, TNF-α, GGT (**Fig 1**) in addition to ALT (r=0.336, p=0.02) and AST (r=0.52, p<0.0001). LDH (p=0.008), TNF-α (p<0.0001) and AFP (p=0.028) showed significant prediction for disease progression in HCV chronic liver disease (**Fig 2**) by linear regression analysis.

DISCUSSION

Several serum tumor markers are used currently for the evaluation of tumor progression and prognosis of patients with HCC, including AFP level [10]. The diagnostic capacity of AFP depends on its elevation in the serum; concentration of AFP greater than the upper reference limit indicate the presence of HCC but values below this level are less useful because they may also occur in chronic liver disease [11]. In the present study, serum concentration of AFP in HCC group with average value 170.98±284.0 ng/ml was considered significant (p<0.0001) compared to LC (6.15±3.39ng/ml) and CH (24.9±61.6ng/ml, p=0.003). Positive serum AFP (>15ng/ml) was detected in 79.2% of HCC patients and in only one case of CH (10%) but all cases with liver

cirrhosis were detected negative for AFP. Therefore, the sensitivity of AFP in HCC patients in current study is comparable to that recorded by **Raedle et al.**[22] and **Gadelhak et al.**[11] (69.3%, 58.46% respectively). However, this low sensitivity of AFP makes its value limited in the diagnosis and the prognosis of HCC, but it is independent of other predictors [23]. Therefore, to improve the sensitivity of HCC detection by serum markers, various biomarkers are used in combination with AFP.

TNF-α plays a central role in the host's immunomodulatory response to infective agents [24] and hepatitis infection is associated with increased transcriptional expression of the TNF-α gene in the liver with high serum levels of TNF-α [25]. Currently, the level of TNF-α was elevated significantly (p<0.0001) in the sera of HCC patients compared to LC and CH and also, elevated in LC than CH (p<0.0001). Elevated serum TNF-α level have been observed by other researchers even in patients with mild liver inflammation, indicating that this cytokine could be used as a predictor of liver inflammation [26]. TNF-α level was not correlated with ALT, AST, or viral load in current study and these results are in consistence with other reports, that, serum TNF-α level was not correlated with serum ALT or AST activities neither in HBV nor in HCV infected patients [27]. Such results have also been reported by other authors and it is concluded that, measurement of TNF-α levels reflects liver injury despite normal levels of liver enzymes[28,29].

Increased GGT activity is associated with liver injury and with mortality in the general population but less is known about its association with chronic hepatitis (HCV) outcomes [30]. Among patients with chronic HCV, higher GGT activity has been associated with more severe liver disease in a number of cross sectional studies [31-35].

On contrary, other cross sectional studies provide in conclusive evidence that GGT is associated with liver disease progression [36]. GGT elevation in current study was increased in HCC patients than in LC and CH but significant association with liver disease progression was not detected suggesting that GGT is not a marker for disease severity.

Patients with elevated GGT activity had significantly higher serum level of AST but no association between ALT, viral load and GGT [37]. Also, **Silva et al.** [33], demonstrated that there is no significant association between increased GGT levels and ALT in logistic regression analysis. In current study, GGT showed significant association with ALT (r=0.457, p=0.001) and AST (r=0.34, p=0.018) but there is no significant association with viral load. These results are in agreement with **Hwang et al.** [38] who demonstrated that, a highly significant correlation was recorded between serum level of ALT and GGT but elevated serum GGT was not correlated with serum HCV RNA titer or HCV genotype. Such results suggest that the histological damage could represent a common origin of the alteration of both enzymes ALT and GGT activities [39].

LDH activity is a commonly used serum biomarker, which is easily and cheap to detect and, thus, appropriate for the use in routine clinical practice [19]. In current study, serum activity of LDH was highly elevated in patients with HCC than in LC (p=0.001) and CH (p=0.008) but there is no significant difference between LC and CH, therefore, elevated LDH is a possible indicator of disease progression[40]. LDH was also correlated positively with liver disease markers ALT (r=0336, p=0.02) and AST (r=0.52, p<0.0001) suggesting that, LDH is an enzyme that is expressed at higher levels when cells are distressed

and damaged. LDH, TNF-α, and AFP showed significant prediction for disease progression in HCV infected patients of our study. Therefore, our results concluded that, LDH and TNF-α could be used simultaneously with AFP for the evaluation of chronic inflammation associated with HCV infection leading to HCC development.

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Table (1): Demographic and base line characteristics of patients with HCV-chronic liver disease and healthy individuals

	HCC	LC	CH	HI[#]
Age (yrs)	56.38±4.6•	53.43±9.5•	48.9±12.4	25.3±4.6
Gender (M/F)	23/1	13/1	8/2	15/10
ALT (u/ml)	67.3±42.7•	36.1±7.5•*	69.4±41.8*	22.6±3.9
AST (u/ml)	78.5±31.02•	53.35±14.99•	70.5±40.14	23.8±5.3
Albumin (g/dl)	3.37±0.55•	2.8±0.36•	3.5±1.2	4.49±0.3
T.Bilirubin (mg/dl)	2.06±1.2	2.7±0.82*	12.68±0.97*	0.612±0.03
Pt count (x10^9)	81.46±42.1	85.9±68.5	114.5±61.5	218.5±35.4
Creatinin (mg/dl)	0.96±0.17	0.94±0.12	0.8±0.13	0.65±0.13
HCV RNA (x10^6)	2.45±2.97•	0.73±0.92•	0.07±2.4	-
Values are expressed as mean ±or number (%) •The difference was considered significant between HCC and LC as regard to age (p=0.038), ALT (p=0.003), AST (p=0.017), Albumin (p=0.004), HGCV RNA (p=0.005) *The difference was considered significant between LC and CH patients as regard to ALT (p=0.004) and T.bilirubin (p=0.02). [#]Significant difference was recorded (p<0.0001) between HI and HCC, LC, CH with all variables.				

**Table (2):** Serum level of AFP, LDH, GGT, TNF-α in all cases of the study group

	HCC N=24	LC N=14	CH N=10	HI N=25
AFP (ng/ml) mean±SD % of positivity	170.98±284.0•* 19/24(79.2%)	6.15±3.39• 0/14(0%)	24.9±61.6* 1/10(10%)	5.03±1.8 0/25(0%)
LDH (u/l) mean±SD % of positivity	413.2±84.6•* 24/24(100%)	320.0±41.51• 12/14(85.7%)	341.6±114.95* 7/10(70%)	217.3±51.1 6/25(24%)
GGT (u/l) mean±SD % of positivity	76.3±75.5 9/24(37.5%)	37.6±12.3 0/14(0%)	71.6±81.3 3/10(30%)	23.7±9.5 1/25(4%)
TNF-α (pg/ml) mean±SD % of positivity	520.6±48.4•* 24/24(100%)	414.3±43.1•+ 13/14(92.9%)	315.5±34.8*+ 2/10(20%)	288.8±25.5 1/25(4%)
•The difference was considered significant between HCC and LC as regard to AFP (p<0.0001), LDH (p=0.001), TNF-α (p<0.0001) *The difference was considered significant between HCC and CH as regard to AFP (p=0.003), LDH (p=0.008), TNF-α (p<0.0001) +The difference was considered significant between LC and CH as regard to TNF-α (p<0.0001)				

Table (3): Correlation between TNF-α, LDH, GGT with other biochemical markers in HCV-chronic liver disease

	GGT	LDH	AFP	TNF-α	HCV RNA	ALT	AST
GGT	1						
LDH	0.549** 0.000	1					
AFP	0.175 0.235	0.35* 0.015	1				
TNF-α	0.17 0.228	0.37** 0.009	0.279 0.055	1			
HCV RNA	0.03 0.823	0.22 0.13	0.045 0.76	0.061 0.68	1		
ALT	0.457** 0.001	0.336* 0.02	0.06 0.66	0.13 0.38	0.163 0.268	1	
AST	0.34* 0.018	0.52** 0.000	0.213 0.146	022 0.13	0.39** 0.006	0.686** 0.000	1
	GGT	LDH	AFP	TNF-α	HCV RNA	ALT	AST

(a)   **(b)**   **(c)**
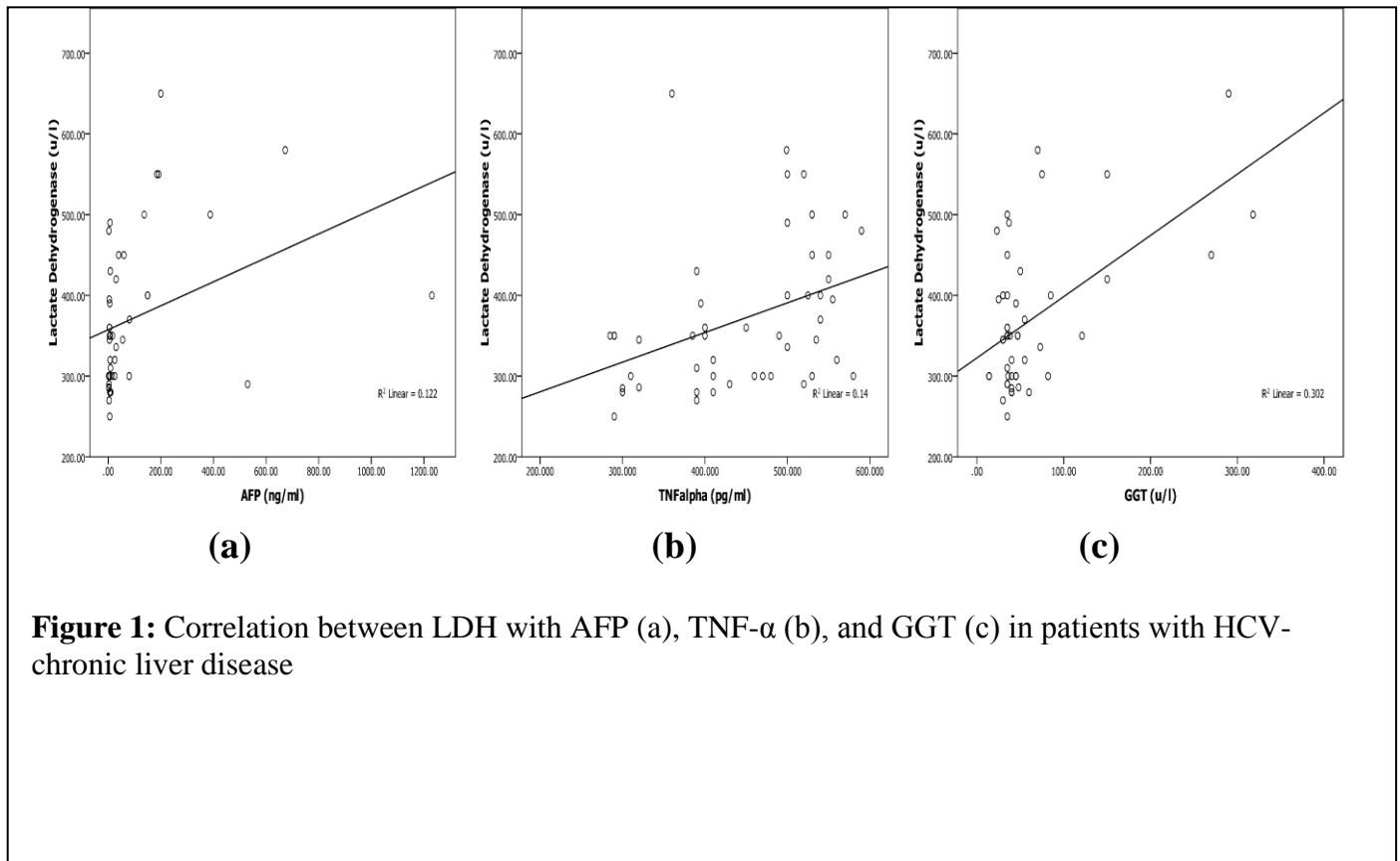
Figure 1: Correlation between LDH with AFP (a), TNF-α (b), and GGT (c) in patients with HCV-chronic liver disease
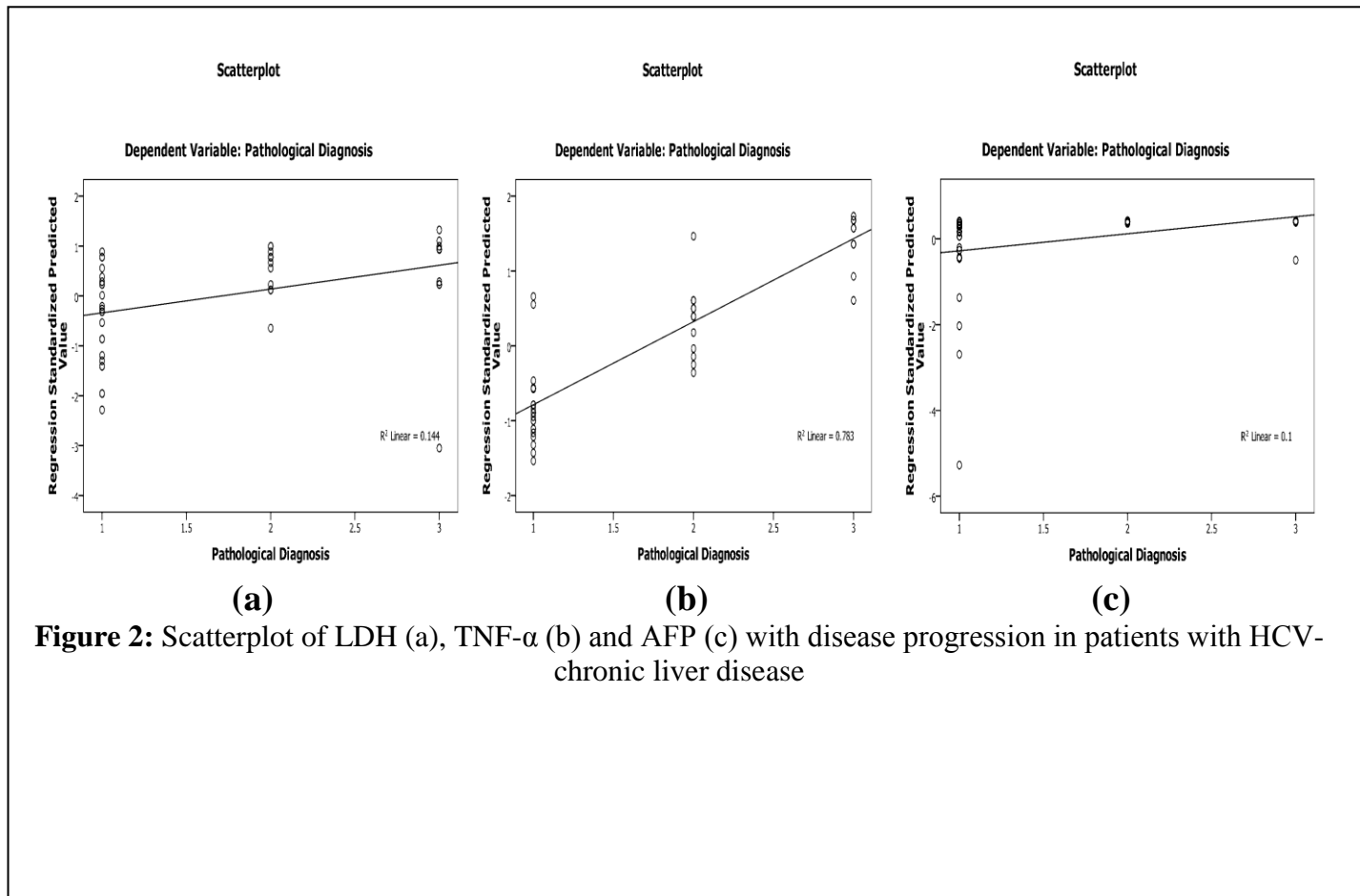
Figure 2: Scatterplot of LDH (a), TNF-α (b) and AFP (c) with disease progression in patients with HCV-chronic liver disease