Liver Cells Apoptosis Biomarkers among Egyptian Patients with Chronic Hepatitis-C Virus and Hepatocellular Carcinoma

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Abstract

Chronic Liver Damage (CLD) is the main cause of Hepatocellular carcinoma (HCC). Hepatitis C-Virus (HCV) infection resulting in inflammation of liver which may developed from acute to chronic that may develop into HCC. The aim of this work was to evaluate Apoptotic markers including (Transforming Growth Factor β1) TGF-β1 and CD90 and Annexin–V in patients with chronic liver disease (CLD) Fibrosis, HCC and healthy individuals. The present study included 51 patients with Fibrosis (F2-F4), 30 Hepatocellular Carcinoma (HCC) patients, in addition 40 normal healthy individuals were enrolled in this study as control group. AFP and CEA were estimated in all groups. TGF-β1, CD90 and Annexin-V were estimated using Flow Cytometry technique. Results from this study revealed that there was high significance difference in APRI ratio, FIB-4 ratio between HCC, Fibrosis comparing with Control group (p<0.05), also there was high prevalence TGF-β1 and CD90 in HCC patients comparing with both Fibrosis and healthy control group (P<0.005). Based on our observation in this study TGF-β1 has diagnostic value importance in assessment of hepatocellular carcinoma. CD90 could be used as Cancer Stem Cell (CSC) biomarker in hepatocellular carcinoma patients. Chronic HCV resulting in increasing apoptosis..

Keywords: Hepatocellular carcinoma (HCC); Transforming growth Factor beta1 TGF-β1; Alpha feto protein (AFP); Carcinoembryonic antigen (CEA).

Introduction

Chronic liver injury causes morbidity and mortality worldwide. Patients with chronic liver injury may progress from initial liver fibrosis to cirrhosis, which may developed into HCC [1-13]. The main aetiologies of chronic liver injury are chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infections [14]. In this study our subjects were divided into three different groups. Group I which including 51 Fibrosis classified according to METAVIR classification (F2-F4) patients, 42 males (82.4%) and 9 females (17.6 %), their age ranged from 43-87 years with a mean of 59.9±10.1 years. Group II which including 30 HCC patients, 23 males (76.7%) and 7 females (23.3%) and their age ranged from 43-74 years with a mean of 57±8.81 years. Group III which including 40 unrelated healthy adults with no liver diseases as a control group 28 males (70%) and 12 females (30%), and their age ranged from 20-51 years with a mean of 30.8±7.57 years (Table 1). Our data was in agree with Hussein et al. [15], Hernandez-Castillo et al. [16] and Massoud et al. [17], who reported that the mean ages among HCC cases were 53.7±10.1, 57.4±8.7, and 55.2±8 years, respectively (Figure 1). In the present study ALT activity was increased in Fibrosis (F2-F4) and HCC patients significantly compared with Healthy control group Mean±SD. were 59.9±36.7, 56.3±35.7 and 12.15±2.6; respectively (P<0.005). Also, AST activity was increased in both Fibrosis(F2-F4) and HCC patients comparing with Healthy control group Mean SD. were 73.2±35.6, 70.1±28.3 and 12.4±2.7; respectively (p<0.005). Serum bilirubin was increased significantly in both HCC and Fibrosis patients comparing with Health control group with values 7.4±11.0, 4.1±6.3 and 0.3±0.18; respectively (p< 0.005). In our study albumin was decreased significantly in both HCC and Fibrosis patients comparing with Healthy control group with values 2.91±0.25, 3.2±0.15 and 4.3±0.43; respectively (p<0.005) (Table 2).

Table 1: Individual characters in all studied groups.

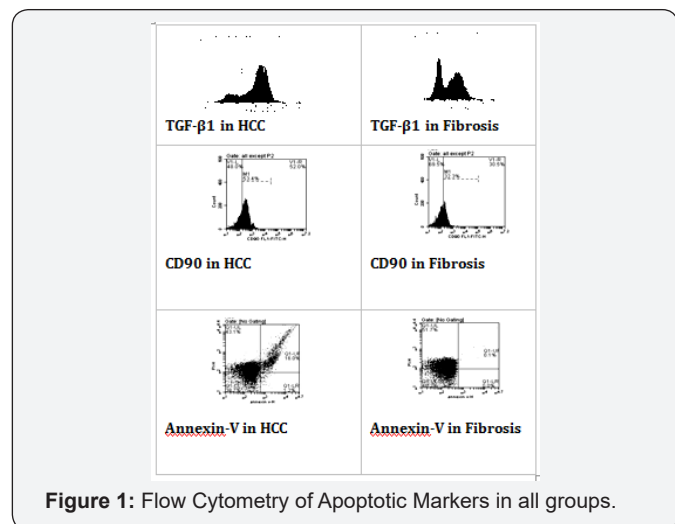| Groups / Characters	Group I N= 51	Group II N= 30	Group III N= 40
Age	59.9 ±10.1	57±8.81	30.8 ±7.57
Males	42 (82.4 %)	23 (76.7 %)	28 (70 %)
Females	9 (17.6 %)	7 (23.3 %)	12 (30 %)

Figure 1: Flow Cytometry of Apoptotic Markers in all groups.

Table 2: Biochemical Parameters in all groups.

Biochemical Parameters	Group I N= 51	Group II N= 30	Group III N= 40	P – value
ALT (U/ml)	59.9±36.7	56.3±35.7	12.15±2.6	P<0.005
AST (U/ml)	73.2±35.6	70.1±28.3	12.4±2.7	P<0.001
Platelets (×109)	200.8±47.9	153.8±54.5	289.3±16.6	P=0.000
Bilirubin(mg/dl)	4.1±6.3	7.4±11.0	0.3±0.18	P<0.005
Albumin(g/dl)	3.2±0.15	2.91±0.25	4.3±0.43	P<0.001
AST/ALT ratio	1.4±0.7	1.5±0.58	1.07±0.37	P<0.005
APRI ratio	1.3±1.3	0.98±0.61	0.1±0.2	P<0.001
FIB-4 ratio	4.2±3.6	3.7±2.3	0.38±0.13	P<0.005

Our data agree with Durazo et al. [18] who found that the mean value of AST activity in HCC was 3.5 times the upper limit of normal, and also with Okonkwo et al. [19] who found that the AST activity in HCC was 1.39 times the upper limit of normal. Serum ALT activity showed significant difference between the HCC group and the non-HCC group, which was in agreement with Durazo et al. [18].

Alpha-Fetoprotein (AFP) is a glycoprotein with molecular weight about 72kDa. AFP normally synthesized during fetal life, first in the yolk sac and then in fetal liver [20]. In the present study tumor markers including both AFP and CEA were estimated using ELISA technique in all studied groups the mean values of AFP concentration in Fibrosis, HCC and healthy control were 25.08±22.2, 423.3±4.7 and 1.68±0.74; respectively (p<0.001). The mean value of CEA concentration in Fibrosis, HCC

and healthy control were 2.64±0.66 and 3.61±0.6 and 1.26±0.13; respectively, this was in agreement with Hussein et al. [15] who showed a significant elevation of serum AFP in HCC patients.

Transforming growth factor β1 (TGF-β1) is a multi-functional cytokine over expressed in HCC compared to that of chronic hepatitis C. It is suggested that TGF-β1 may be associated with the malignant transformation of hepatocyte or the progression of HCV- associated HCC [21]. TGF-β1 is suggested to play a role in development, growth or progression of hepatocellular carcinoma (HCC). In our study blood peripheral mononuclear cells TGF-β1 was increased in HCC and Fibrosis patients comparing with Healthy control group with values Mean±SD. 78.6±9.0, 55.5±18.0 and 12.07±1.62 respectively (p<0.005). Table 3 this agree with number of studies suggest that activation of TGF-β1 promote HCC development Hoshida et al. [22].

Table 3:TGF-β1 in all groups.

Groups TGF-β1 (M1%)	Group I N = 51	Group II N= 30	Group III N = 40	P-Valu
Mean ± SD	55.5 ± 18.0	78.6 ± 9.0	12.07± 1.62	P<0.005
Range	24.3 – 87.0	59.9 – 88.6	10.1–15.3	

CD90 is glycophosphatidyl inositol -anchored protein expressed in many cells such as T-cells, thymocytes, neurons, endothelial cells and fibroblast. CD90 contributes an important regulator of cell to cell and cell to matrix interaction, apoptosis, adhesion, migration, cancer and fibrosis [23]. In our study blood peripheral mononuclear cells CD90 (M1%) was increased significantly in HCC, Fibrosis patients comparing with Healthy control group and Mean±SD. were 46.29±4.0, 39.66±5.49 and 6.13±2.84 respectively (P<0.005). This is in accordance with some previous studies on HCC cell lines and human samples, where CD90 was highly expressed in malignant hepatocytes and the presence of CD90+/CD44+ cells contributed to an aggressive phenotype with more frequent metastatic lesions in the lung Zhu et al. [24]. Our data was in agree with Bahnassy et al. who found that CD90 was significantly higher in the blood of HCC patients compared to those in the CH and control groups (P < 0.001).

«Annexin-V» is Ca²⁺ dependent phospholipid-binding protein with high affinity for the membrane phospholipid phosphatidylserine (PS). Annexin-V can be conjugated to fluorochromes thus serves as a sensitive probe for flow cytometric analysis of cells undergoing apoptosis [25]. In the present study peripheral blood mononuclear cells(PBMC) Annexin V+/PI– which representing early apoptotic cells were increased significantly in both HCC and Fibrosis patients comparing with healthy individuals with Mean± SD. values 12.7±3.05 and 14.1±4.0 and 0.88±0.78; respectively. In our study (PBMC) Annexin V–/PI+ which representing necrosis cells were increased significantly in both HCC and Fibrosis patients comparing with healthy control with values 9.7±3.91 and

22.7±2.6 and 0.7±1.36; respectively (p<0.005). Also, (PBMC) Annexin V+/PI+ representing late apoptosis cells were increased significantly in both HCC and Fibrosis patients comparing with healthy control with values 25.3±4.54 and 15.0±2.9 and 0.07±0.06; respectively (p<0.005).

Conclusion

Based on our observation in this study TGF-β1 has diagnostic value in assessment of patients with chronic hepatitis-c virus and hepatocellular carcinoma. CD90 could be used as Cancer Stem Cell biomarker in HCC patients. In our study, liver function tests including (AST), (ALT), albumin and total bilirubin, were measured using standard methodologies Routine blood pictures including platelets counting were determined. The AST/ALT ratio, FIB-4 and APRI (AST/platelets count ratio index) Table 2. Tumor Markers including AFP and CEA were estimated using ELISA technique Table 4. Transforming Growth Factor-β1, CD90 and Annexin-V were estimated using flow cytometry technique in all groups.

Table 4: AFP and CEA in all groups.

Tumor Markers	Group I N =51 Mean ± SD.	Group II N =30 Mean ± SD.	Group III N =40 Mean ± SD.	P Value
AFP (ng/ml)	25.08 ± 22.2	423.3 ± 4.7	1.68 ±0.74	P<0.005
CEA (μg/l)	2.64 ± 0.66	3.61 ± 0.6	1.26 ±0.13	P<0.005

Table 5: CD90 Flow Cytometry in all groups.

Annexin -V	Group I	Group II	Group III	P value
Viable Cells	47.4±3.07	51.0±6.23	98.0±2.04	P< 0.001
Early Apoptosis	14.1±4.0	12.7±3.05	0.88±0.78	P< 0.005
Late Apoptosis	15.0±2.9	25.3±4.54	0.07±0.06	P<0.001
Necrosis	22.7±2.6	9.7±3.91	0.7±1.36	P<0.001

Discussion

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