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Efficacy of Cd40l As an Early Marker in Diagnosis of Hcv-Related Hepatocelluler Carcinoma

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ABSTRACT

Background: Hepatocellular Carcinoma (HCC) is the commonest primary cancer of the liver. Incidence of HCC is increasing to be the fifth most common cancer and the second most frequent cause of cancer-related death globally. Kupffer cells and hepatocytes can express elevated levels of CD40 in hepatitis C virus-associated chronic liver disease. The CD40 CD40ligand co stimulatory pathway is associated with liver injury and hepatocyte apoptosis.

Objective: This study aimed to evaluate efficacy of soluble CD40 ligand as an early marker in diagnosis of HCV- related HCC.

Subjects and methods: This is a case control study. It was conducted in Internal Medicine and Microbiology Departments, Faculty of Medicine, Zagazig University Hospitals along six months. Ninety subjects were included in this study. They were classified into three groups as follows: Group 1 that included 30 (18 males and 12 females) apparently healthy subjects aged between 44 to 67 years old. Group 2 that included 30 patients (20 males and 10 females) aged between 41 to 65 years old with chronic HCV infection without HCC. Group 3 that composed of 30 patients (22 males and 8 females) aged between 44 to 65 years old with chronic HCV infection with HCC.

Results: There was statistical significance increase in CD40 level in group 3 compared to the other two groups (1 and 2). There was significant negative correlation between serum CD40L with both Hb and PLT, while there was significant positive correlation between CD40 level with T.bilirubin, D.bilirubin, ALP, INR and AFP.

Conclusion: Serum CD40L is significantly increased in patients with chronic HCV-related HCC. So, CD40L can be used as a non-invasive pro inflammatory marker for diagnosis of HCC.

Keywords: HCV infection, HCC, CD40l..

INTRODUCTION

Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily invades the liver [(1)]. The virus persists in the liver in about 75% to 85% of those initially infected. Over many years however, it often leads to chronic liver disease and cirrhosis [(2)]. In some cases, those with cirrhosis will develop complications such as liver failure, liver cancer, or dilated blood vessels in the esophagus and stomach [(1)].

There has been a remarkable increase of the proportion of HCC among Chronic liver disease (CLD) patients in Egypt from 4.0% to 7.2% over a decade. This rising proportion may be explained by the increasing risk factors such as the emergence of HCV over the same period of time, the contribution of HBV infection, improvement of the screening programs and diagnostic tools of HCC [(3)].

CD40 is a 40- to 45-kD type I membrane protein and a member of the TNFR superfamily that exists as a constitutional trimer complex on the cell surface. CD40 was initially characterized on B cells and is expressed on APCs, such as B cells, dendritic cells (DCs), macrophages, and monocytes, as well as on non-immune cells such as epithelial, endothelial, and mesenchymal (fibroblasts, myofibroblasts, synoviocytes, stellate cells, etc.) cells, platelets and tumors [(4)].

CD40L is produced as a type II transmembrane protein, CD40L may be expressed on the cell surface as a heteromultimeric complex. Apart from its 33-kDa form, the molecule is associated with two shorter versions of the protein of 31 and/or 18 kDa. These shorter soluble forms of CD40L retain their ability to form trimers, to bind CD40, and to deliver biological signals, thus indicating that CD40L might also act as a bona fide cytokine [(5)].

In cancer patients, sCD40L is more likely derived from activated platelets than from T cells, a notion supported by evidence that cancer patients have significant platelet activation, as well as inadequate T-cell activation [(6)].

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The CD40 CD40ligand co stimulatory pathway is associated with liver injury and hepatocyte apoptosis. Kupffer cells and hepatocytes can express elevated levels of CD40 in hepatitis C virus-associated chronic liver disease. CD40 activated B cells and macrophages produce inflammatory cytokines and contribute to the pathogenesis of necroinflammatory liver disease [7].

CD40-CD40L interactions play an important role in the production of several cytokines, including interleukin-12 (IL-12). Furthermore, both molecules have been presented in a natural soluble form, so they can act at distant sites. Finally, both CD40 and CD40L belong to the tumor necrosis factor-R (TNF-R) and TNF family. Families are sharing biological activities in processes such as cell growth, differentiation and death [5].

This study aimed to evaluate efficacy of soluble CD40 ligand as an early marker in diagnosis of HCV- related HCC.

SUBJECTS AND METHODS

This was a case control study. It was conducted in Internal Medicine and Microbiology Departments, Faculty of Medicine, Zagazig University Hospitals along six months. Ninety subjects were included in this study who were classified into three groups as follows:

- Group 1: composed of 30 (18 males and 12 females) apparently healthy subjects aged from 44 to 67 years old.
- Group 2: composed of 30 patients (20 males and 10 females) aged from 41 to 65 years old with chronic HCV infection without HCC.
- Group 3: composed of 30 patients (22 males and 8 females) aged from 44 to 65 years old with chronic HCV infection with HCC.

Ethical and patient approval:

Formal consent was obtained from all individuals and the study protocol was approved by the Medical Research Ethical Committee of Zagazig University. Five ml of venous blood was withdrawn by sterile venipuncture and used for routine investigations including CBC, LFTs RFTs and AFP HCV antibodies by ELISA as well as HCV-RNA by RT-PCR and CD40L serum level by ELISA technique.

Inclusion criteria:

1- Age 40 – 65 years old.
2- Both sexes.
3- Patients consent to enter the study.
4- Patients with liver cirrhosis due to chronic HCV with or without.

Exclusion criteria

1- Age < 40, > 65 years.
2- Patients refuse to enter the study.
3- Patients with liver cirrhosis resulting from causes other than HCV.
4- Diseases associated with elevated CD40 in sera such as patient with systemic lupus erythrematosis, rheumatoid arthritis with vasculitis, systemic sclerosis, Sjogren syndrome, mixed connective tissue disease and Kawasaki disease.

Each group was subjected to the following:

1. Complete history taking
2. Clinical examination
3. Routine laboratory investigations: complete blood picture and liver and kidney function tests.
4. Viral markers including: HCV Ab and HBsAg.
5. PCR
6. Alpha fetoprotein (AFP).
7. Specific laboratory investigations: measurement of serum CD40l levels using SunRed ELISA kit (Shanghai Sunred Biological Technology Co).
8. Imaging study: abdominal ultrasonography and /or triphasic CT abdomen for diagnosis of HCC

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program version 18.0. Results were expressed as means ± SD. Comparison between groups was done by Chi-Square test. ANOVA-F test was used to compare between more than two groups. Pearson's correlation coefficient was used to test correlation between variables. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Table (1): Demographic data of the control group and patient groups

Variable		Groups						ANOVA or Chi-Square	
		Control group N = 30		HCV group N = 30		HCC group N = 30		F or X^2	P-value
Age (Years)	Mean ± SD	54.333±7.581		54.267±8.212		55.433±6.399		0.233	0.793
Sex	Male	18	60.00	20	66.67	22	73.33	1.200	0.549
	Female	12	40.00	10	33.33	8	26.67		
Smoking:								4.680	0.096
No		20	66.7	18	60	12	40		NS
Yes		10	33.3	12	40	18	60		
Diabetes mellitus:								0.341	0.843
Absent		23	76.7	21	70	22	73.3		NS
Present		7	23.3	9	30	8	26.7		
Hypertension:								1.735	0.420
Absent		26	86.7	22	73.3	23	76.7		NS
Present		4	13.3	8	26.7	7	23.3		
Alcoholism:									
No		30	100	30	100	30	100		
Yes		0	0	0	0	0	0		

This table showed no statistical significance differences between studied groups regarding age, sex, smoking, alcohol intake and comorbid diabetes or hypertension.

Table (2): Comparison between control group and patient groups regarding CBC parameters

		Groups			ANOVA		TUKEY'S Test		
		Control group N = 30	HCV group N = 30	HCC group N = 30	F	P-value	C& HCV	C& HCC	HCV& HCC
Hb (g/dl)	Mean ± SD	11.437± 1.194	10.563± 0.879	8.193± 0.825	88.017	<0.001*	0.002*	<0.001*	<0.001*
WBCs	Mean ± SD	8.800± 1.169	7.583± 0.750	7.693± 1.013	3.702	0.029*	0.042*	0.071	0.973
Plateletes	Mean ± SD	191.633± 24.681	101.967± 10.759	73.867± 13.485	375.501	<0.001*	<0.001*	<0.001*	<0.001*

This table showed that there was statistical significance decrease in Hb, WBCs and PLT level in patient groups compared to control group.

Table (3): Comparison of LFTs between the control group and the patient groups

		Groups			ANOVA		TUKEY'S Test		
		Control group N=30	HCV group N=30	HCC group N=30	F	P-value	C& HCV	C& HCC	HCV& HCC
Total Bilirubin (µmol/L)	Mean ±SD	1.063± 0.116	1.710± 0.329	2.190± 0.477	82.249	<0.001*	<0.001*	<0.001*	<0.001*
Direct Bilirubin (µmol/L)	Mean ±SD	0.213± 0.073	0.333± 0.121	0.557± 0.179	52.302	<0.001*	0.002*	<0.001*	<0.001*
ALT (U/L)	Mean ±SD	24.500± 5.894	76.733± 10.302	78.467± 11.434	311.676	<0.001*	<0.001*	<0.001*	0.761
AST (U/L)	Mean ±SD	25.967± 6.754	76.767± 10.464	81.533± 21.736	136.023	<0.001*	<0.001*	<0.001*	0.412
Albumin (g/dl)	Mean ±SD	4.553± 0.552	2.933± 0.179	2.312± 0.408	239.431	<0.001*	<0.001*	<0.001*	<0.001*
Prothrombin Time (sec.)	Mean ±SD	12.930± 0.850	15.573± 0.899	16.413± 0.945	122.688	<0.001*	<0.001*	<0.001*	0.001*
INR	Mean ±SD	0.863± 0.174	1.094± 0.057	1.927± 0.313	214.233	<0.001*	<0.001*	<0.001*	<0.001*

This table showed statistical significance increase in liver enzymes and serum bilirubin in patient groups compared to control group, while serum albumin was significantly low in patient groups specially HCC group. Also, this table showed statistical significance prolongation of prothrombin time and increased INR level in patient groups compared to the control group.

Table (4): Comparison of CD40L level between the control group and the patient groups

Groups	CD40L	ANOVA	
	Mean±SD	F	P-value
Control group	1.772±1.177	14.262	<0.001*
HCV group	2.148±0.046		
HCC group	5.412±0.440		
TUKEY'S Test			
C&HCV	C&HCC	HCV&HCC	
0.871	<0.001*	<0.001*	

This table showed statistical significance increase in CD40L level of patient groups compared to control group.

Table (5): Correlation between age, laboratory data US finding &serum CD40l level in all groups

Correlations		
All cases	**CD40L**	
	R	**P-value**
▪ **Age (Years)**	0.160	0.221
▪ **Hb (g/dl)**	-0.313	0.015*
▪ **WBCs**	-0.107	0.414
▪ **Platelets**	-0.258	0.047*
▪ **Total Bilirubin (mg/dl)**	0.376	0.003*
▪ **Direct Bilirubin (mg/dl)**	0.394	0.002*
▪ **ALT (U/L)**	-0.003	0.982
▪ **AST (U/L)**	-0.157	0.231
▪ **Albumin (g/dl)**	-0.168	0.199
▪ **Prothrombin Time (sec.)**	0.162	0.215
▪ **INR**	0.321	0.013*
▪ **Urea (mg/dl)**	0.112	0.393
▪ **Creatinine (mg/dl)**	0.201	0.123
▪ **AFP (ng/ml)**	0.395	0.002*
▪ **Portal vein diameter (mm)**	0.353	0.006*
▪ **Splenic diameter (cm)**	0.214	0.101
▪ **PCR**	0.151	0.249
▪ **ESR (mm/hr)**	0.408	0.001*
▪ **CRP (mg/l)**	0.027	0.839

This table showed that there was significant negative correlation between serum CD40L with both Hb and PLT, while there was significant positive correlation between CD40L level with T. bilirubin, D. bilirubin, ALP, INR, AFP, P.V diameter and ESR.

Table (6): Sensitivity & accuracy of CD40L in diagnosis of HCV-related HCC

ROC curve between Control and Cases						
	Cutoff	**Sens.**	**Spec.**	**PPV**	**NPV**	**Accuracy**
CD40L	>1.55	71.67	70.00	82.7	55.3	66.2%
AFP (ng/ml)	>8	56.67 %	93.33	94.4	51.9	69.7%

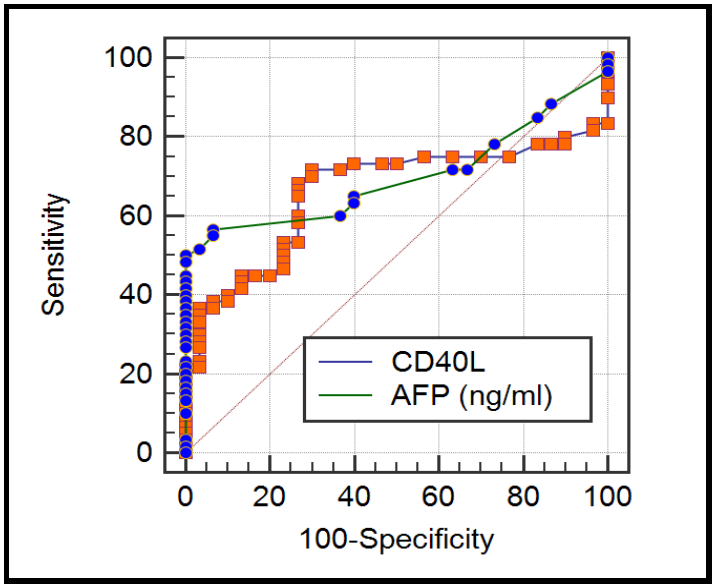

Figure (1): Receiver operating characteristic curve (ROC curve) showing sensitivity and specificity for AFP and CD40L in diagnosis of HCV-related HCC.

DISCUSSION

Widespread expression of CD40 in humans implies that its ligand has an important role in cancer pathogenesis: inhibiting apoptosis, facilitating metastases, increasing epithelial cell proliferation, motility and invasion and producing cytokines such as interleukin (IL)-10 that modulate the anti-tumour response of T lymphocytes [(8)].

The present study was carried out on 90 subjects classified into 3 groups: Group I that included 30 healthy subjects [18 were males (60.%) and 12 were females(40%)] with a mean age of 54.333 ± 7.581 years old, group II that included 30 patients with chronic hepatitis C without HCC [20 were males (66.67%) and 10 were females (33.33%)] with a mean age of 54.267 ± 8.212 years old and Group III that included 30 patients with chronic hepatitis C with HCC,22 [males (73.33%) and 8 were females (26.67%)] with mean age 55.433 ± 6.399 years old with no statistical significant differences between the three studied groups in age and sex distribution (p value > 0.05).

Hopf [(9)] reported that the incidence of HCC increases progressively with age, although this varies by country. **Abdel-Wahab *et al.*** [(10)] reported that males are more exposed to carcinogens and environmental factors as they participate more in out door activities than female**. Yu *et al.*** [(11)] explained that this variability may be due to the differences in exposure to risk factors as hepatitis B and C, which are more prevalent in male patients**.** The present study showed high statistical significant differences between the three studied groups as regards Hb (F = 88.017), WBCs (F= 3.702), PLT (F= 375.501), serum ALT (F= 311.676) and AST (F=136.023). The difference is between group 3 compared to both groups 1 and 2 (p value < 0.001*). **Hung *et al.*** [(12)] showed that the HCC subjects had lower platelet count compared to those with chronic hepatitis**. Hussein *et al.*** [(13)] reported that serum level of ALT, AST and bilirubin were higher among patients of HCC than those of chronic liver disease. Also, our study showed high statistical significant differences between the three studied groups as regards albumin level (F=239.431), prothrombin time (F=122.688) and INR (F=214.233). Also, there was statistical significant differences between the three studied groups as regards total and direct bilirubin (p value < 0.001*). The difference in Albumin level, prothrombin time and INR was between all groups (p value<0.001*). **Eltaher *et al.*** [(14)] found that serum albumin was significantly higher in control group than patient groups (P < 0.001).

The study showed high statistical significant level of CD40L in group 3 when compared to both groups 1 and 2 (p value < 0.001*) while there is no statistically significant difference between groups 1 and group 2 regarding CD40l level (p value = 0.871).

Eltaher *et al.* [(14)] presented that serum sCD40L level was significantly higher in HCC group than both HCV and control groups (P < 0.001). Serum sCD40L level was also significantly higher in HCC group than HCV group (P < 0.001).

There are statistically significant differences between the studied groups regarding CD40L and AFP levels with HCC group had the highest level of both markers. To evaluate the diagnostic accuracy of serum sCD40L and AFP for HCC, receiver operating characteristic curves were generated and area under the curve (AUC) was calculated. sCD40L level had AUC value with 71.67% sensitivity and 70% specificity at a cut-off of 1.55. In contrast, AFP level had 56.67% sensitivity and 93.33% specificity at a cut-off of 8 ng/ mL. Markedly high specificity and sensitivity, ~96.7%, were only possible when the two markers were combined.

CONCLUSION

It could be concluded the fundamental clinical evidence of the utility of sCD40L in everyday diagnosis and screening of HCC and routine follow-up of patients with HCV cirrhosis.

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