Research Article

Frequency of CD8$^+$T lymphocyte expression in HCV induced Hepatocellular carcinoma

Amel M. Kamal Eldin*, Lamiaa H. Ali*, Mohammed A. Abd El Hakeem*, Wael M. Abd El Ghany and Rana R. Ahmed***

* Department of Clinical pathology, faculty of medicine, Minia university

** Department Tropical medicine, faculty of medicine, Minia university

Abstract

Hepatitis C in humans is a global public health problem that causes chronic viral hepatitis which progresses to liver cirrhosis and hepatocellular carcinoma(HCC). HCC is the most frequent primary liver malignancy and the third cause of cancer-related deaths. Alpha-fetoprotein (AFP) is the most practical and widely used serum biomarker for HCC diagnosis. This prospective study was carried out in the Clinical Pathology Department and department of Tropical medicine, Faculty of Medicine, El-Minia University Hospital. Subjects included 4 groups, divided as follows: group I included 25 patients of HCV induced HCC, group II included 25 patients of HCV induced liver cirrhosis, group III included 25 patients of chronic HCV infection without HCC or liver cirrhosis, and group IV included 25 of apparent healthy volunteers as control. Methodology: AFP was performed by ELISA, and CD8$^+$T lymphocyte expression was performed by flowcytometry. Results: AFP level was significantly increased in group I when compared with groups III and IV, while there was a non-significant difference comparing group I and II. Also, AFP was significantly increased in group II when compared to groups III and IV. CD8 % was significant up regulation in group I compared to group III and IV. While there was no significant difference between group I and II. In group II CD8% was significantly increased when compared to group IV, while this increase was non significant when compared to group III. Group III showed a significant increase in CD8 % when compared to group IV. Conclusion: frequency of CD8$^+$T lymphocyte expression was upregulated in HCC, liver cirrhosis, and chronic HCV patients, as well as AFP level was increased in HCC and liver cirrhosis pateints.

Key words: hepatocellular carcinoma, liver cirrhosis, Alpha fetoprotein, CD8$^+$T lymphocyte

Introduction

Hepatitis C in humans, which is caused by an HCV infection, is a global public health problem. The global prevalence of HCV infection has been estimated to be around 2–3%, which equates to 130–170 million people living with the infection (Hajarizadeh et al., 2013). HCV infection frequently causes chronic hepatitis, which progresses to liver cirrhosis and hepatocellular carcinoma (HCC) (Fénéant et al., 2014).

Hepatocellular carcinoma (HCC) is the most frequent primary liver malignancy and the third cause of cancer-related death. The causes of HCC are chronic liver infections, nonalcoholic fatty liver disease, consumption of aflatoxins and tobacco smoking. Clinical presentation varies; patients can be asymptomatic while symptomatology extends from abdominal pain and weight loss to jaundice and lethargy (Dimitroulis et al., 2017).

Although several trials in diagnosis of HCC, it remains a challenge to early diagnose. AFP is the most practical and widely used serum biomarker for HCC diagnosis.

AFP is a specific glycoprotein produced primarily by fetal yolk sac and fetal liver. Its level decline to <10 ng/mL within 300 days of birth. Its value is often elevated at a milder level in patients with chronic HCV infection in the absence of HCC. HCC can produce a range of AFP values from normal to >100000 ng/mL. AFP >400-500 ng/mL is considered diagnostic for HCC. Prognosis has been shown to be reduced when AFP levels are >1000 ng/mL (Sterling et al., 2012). Monitoring AFP levels can be helpful in the diagnosis of recurrent

disease, although this is largely restricted to patients with AFP producing tumors (Zhou et al., 2006).

$CD8^+$ T cells are known to be key effector cells in controlling viral infections via cytotoxicity activity and cytokine secretion. In chronic hepatitis C (CHC), the $CD8^+$ T cells fail to control HCV. The failure is partially the result of altered function of the virus-specific $CD8^+$ T cells during chronic HCV infection, including proliferation, differentiation, and interferon-γ (IFN-γ) production (Chen et al., 2015). $CD8^+$ TILs are expressed in various tumor types such as HCC. It is conspicuous that CD8+ T cells can express multiple co-inhibitory immune checkpoints, which are manifested to mark the dysfunction of CD8+ TILs (Woo et al., 2012).

Aim of the work: this work aims to determine the frequency of $CD8^+$T lymphocyte expression in HCC, liver cirrhosis, and chronic HCV patients, as well as compare it with conventional AFP.

Subjects and methods

The present study was carried out in the Clinical Pathology Department and department of Tropical medicine, Faculty of Medicine, El-Minia University Hospital. It was conducted through the period from December, 2018 to March, 2019. This study included 4 groups, divided as follows: group I included 25 patients of HCV induced HCC, group II included 25 patients of HCV induced liver cirrhosis, group III included 25 patients of chronic HCV infection without HCC or liver cirrhosis, and group IV included 25 of apparent healthy volunteers as control. All individuals in the study were subjected to the following: Comprehensive medical history taken, Clinical examination, radiological imaging(Abdominal ultrasound, triphasic C.T. scan, and chest X-ray), and Laboratory Investigations which included: Routine investigations (CBC, liver function tests, INR, and hepatitis C virus antibodies "HCV Ab" and hepatitis B virus surface antigen "HBsAg" by ELISA), and Special investigations (serum levels of AFP by ELISA, and CD8 expression by flowcyto-metry).

Results

Alpha-fetoprotein (AFP) level in group I ranged from 3.1 to 360 ng/mL, with mean±SD (85.06±116.4), and median 11.6 (5.1-165.7). In group II, it ranged from 0.9 to 325 ng/mL, with mean±SD (57.4±103.1), and median 11.0 (2.7-55.9). In group III, it ranged from 1.0 to 61 ng/mL, with mean±SD (12.2±13.6), and median 8.7 (3.416.8). In group IV, AFP ranged from 0.9 to 8.7 ng/mL, with mean±SD (3.3±2.2), and median 3.0 (1.1-4.7). As shown in figure(1).

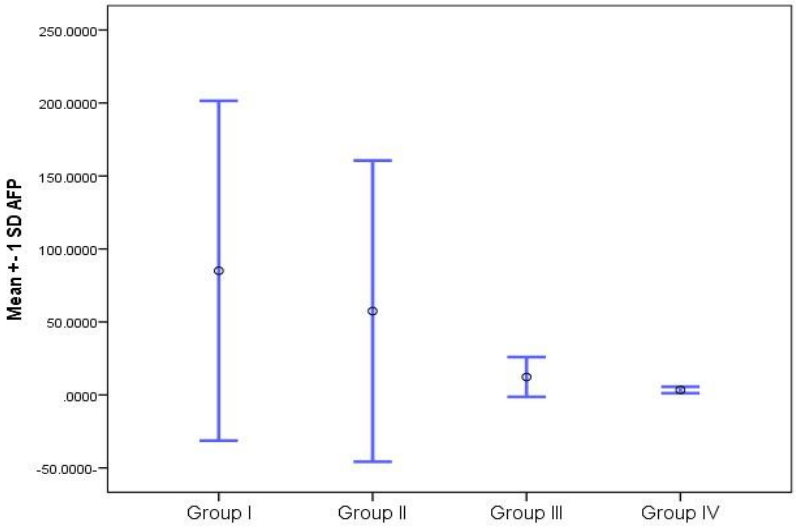
There was no significant difference between group I and II with P value (0.216), and group III and IV with P value (0.690). While AFP level in group I was significantly higher than its level in groups III and IV with P value (0.001 and < 0.001 respectively). In group II AFP level was significantly higher when compared to its level in groups III and IV with P value (0.045 and 0.018 respectively). As shown in table (I)

Regarding CD 8%, in group I it ranged from 6 to 44 %, with mean±SD (22.4±7.6). In group II, it ranged from 9 to 49.5%, with mean±SD (27.7±10.7). In group III, CD 8% ranged from 13.5 to 49.5%, with mean±SD (31.1±8.6). In group IV, it ranged from 11.9 to 30%, with mean±SD (18.2±4.9). as shown in figure (2)

There was significant upregulation of CD8% in group III and IV when compared to group I with P value (0.005) and (0.010) respectively. While there was non-significant upregulation of CD8% in group II when compared to group I with P value (0.166). there was non-significant increase in CD8% in group III compared to group II with P value (0.149), while there was a significant upregulation of CD8% in group II and III compared to group IV with P value (<0.001). As shown in table (I)

Table (I): comparison between different studied groups regarding AFP and CD8%:

Variable	Group I N=25	Group II N=25	Group III N=25	Group IV N=25	P-value					
					I&II	I&III	I&IV	II&III	II&IV	III&IV
AFP(ng/ml) Range	3.1-360	0.9-325	1-61	0.9-8.7	0. 001					
Mean± SD	5.06±116.4	57.4±103.1	12.2±13.6	3.3±2.2						
Median	11.6 (5.1-165.7) 3.1-360	11(2.7-55.9) 0.9-325	8.7(3.416.8) 1-61	3(1.1-4.7) 0.9-8.7	0.216	0.001	<0.001	0.045	0.018	0.690
CD8 % Range	6-44	9-49.5	13.5-49.5	11.9-30	<0.001					
Mean± SD	22.4±7.6	27.7±10.7	31.1±8.6	18.2±4.9	0.166	0.005	0.010	0.149	<0.001	<0.001



Figure(1): AFP level in different studied groups

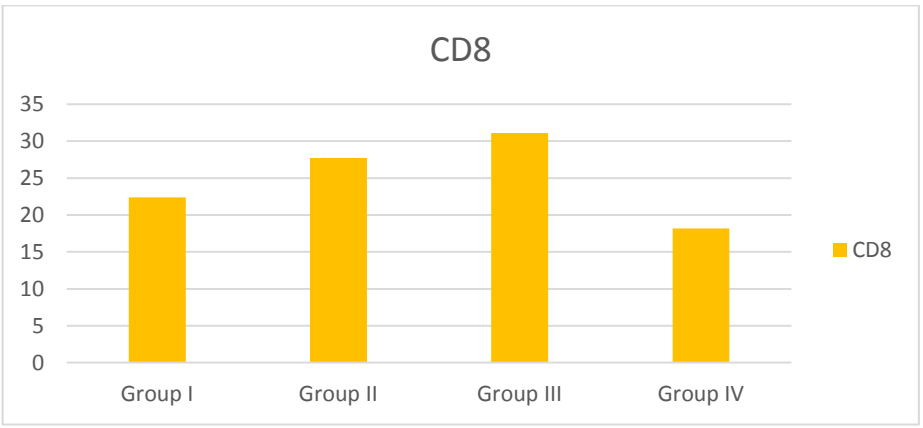


Figure (2): CD8 % in different studied groups

Discussion

Hepatocellular carcinoma (HCC) is an aggressive malignancy that typically develops in the setting of chronic liver disease or cirrhosis. HCC is the second leading cause of cancer-related death in the world. The leading risk factor for HCC is cirrhosis arising from viral hepatitis, alcoholic liver disease, and nonalcoholic fatty liver disease (Yarchoan et al., 2017). Early detection of HCC will increase the lifetime of HCC patients. Current strategies for the diagnosis of HCC fall into two main categories: biomarker tests and imaging (El emeery et al., 2017).

Alpha-feto protein has been the most practical and widely used serum biomarker for HCC diagnosis. However, it is non-specific, persistently elevated levels may indicate residual disease or incomplete resection. A gradual increase in AFP is frequently consistent with disease recurrence (Liu et al., 2019).

Results of the current study showed a significant increase in AFP in both HCC and cirrhosis patients groups compared to HCV and controls as a predictor of hepatitis C virus associated HCC in agreement with Saffroy et al., 2007 who reported that AFP is 75-91% specific for HCC. There was non significant relation when comparing AFP between HCC and cirrhotic patients group, this may be attributed to the sensitivity and specificity of AFP in HCC and chronic liver disease, that is agreed by Yang et al., 2017 and Caviglia et al., 2016 Who reported that AFP is significantly higher in patients with HCC compared with those without HCC, with high diagnostic performance when used as single biomarkers, and it also have been proposed in surveillance associated with ultrasound examination in patients at risk of HCC. Additionally it was recommended by the Japan society of hepatology (JSH) guidelines a combination of tumor biomarkers such AFP, AFP-L3 and DCP in addition to US for early HCC detection in patients with hepatitis B and C virus-related CLD. The strategy to adopt US and AFP has been shown to be helpful and cost-effective particularly in Eastern patients with cirrhosis (Chen et al., 2017). Also Kim et al., 2016 denoted that HCV-related HCC had a stronger association with raised AFP, however this was noted when compared to HBV-related HCC. Moreover, Alsalloom, 2016 denoted that Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP without the presence of tumor, so there is no significant correlation between AFP in both HCC and cirrhotic patients.

Results of the current study showed upregulation of CD8% in HCC, cirrhosis, and chronic HCV patients groups when compared to control group. This may be attributed to the fact that $CD8^{+}T$ cells have role in controlling viral infections via cytotoxicity activity and cytokine secretions, as approved by Chen et al., 2015.

Conclusion

Frequency of $CD8^{+}T$ lymphocyte expression was upregulated in HCC, liver cirrhosis, and chronic HCV patients, as well as AFP level was increased in HCC and liver cirrhosis pateints.

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