CD34 Expression as a marker of progression of chronic hepatitis C

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Abstract

Background

In Egypt, the rate of death/transplantation, decompensation and HCC in patients with compensated HCV cirrhosis is 4.58%, 6.37% and 3.36%, respectively. It is estimated that Egyptian patients with HCV cirrhosis had an annual incidence of HCC with 5.3%. CD34 is a 110-kDa transmembrane glycoprotein present on leukemic cells, endothelial cells and stem cells). CD34 is preferentially expressed on the surface of regenerating or migrating endothelial cells and is a marker of proliferating endothelial cells in the growing sprouts during angiogenesis.

Aim of the work: The aim of the present study was to analyses the correlation between CD34 and the clinical severityof CHC.

Methods: A total of 70 adult patients with chronic hepatitis C infection in various stages with no evidence of cirrhosis, were recruited for the study. We studied the expression of CD34 in liver specimens from chronic HCV infected patients using a computer-based analysis of immunohistochemical staining and confirmed it by Western Blot.

Results: CD34 significantly related with AST and ALT levels. Also, it is significantly related with alpha-fetoprotein and prothrombin concentration. Also, There was a significant relation between CD34 and fibrosis stage.

Conclusion: A direct correlation existed between endothelial cell markers CD34 expression and the progression of fibrosis in chronic hepatitis C. The immunohistochemical methods of this molecule can be important clue in future prognostic strategies.

Introduction

Worldwide, more than 170 million people are infected with hepatitis C. InEgypt the prevalence of hepatitis C infection is considered the highest in the world [1] .In Egypt, the rate of death/transplantation, decompensation and HCC in patients with compensated HCV cirrhosis are 4.58%, 6.37% and 3.36%, respectively [2]. It is estimated that Egyptian patients with HCV cirrhosis had an annual incidence of HCC with 5.3% [3].

CD 34 is a cell surface, sialomucin-like glycoprotein expressed on hematopoietic progenitor cells, normal vascular endothelium and fibroblasts [4]. CD34 is an endothelial antigen used to highlight the density of vessels as a direct marker of the degree of neo-angiogenesis in tumors [5] and to distinguish well-differentiated HCC from non-neoplastic liver [6]. Understanding the process of angiogenesis might suggest an effective therapeutic target that would prevent disease progression. Data pertaining to

the assessment of angiogenesis in the liver tissue of CHC patients are scarce [6].

Angiogenesis is an integral part of tumor progression, it has also been observed in different inflammatory, fibrotic, and ischemic diseases [9]. Chronic liver diseases (CLDs) do not represent an exception to this rule. The suggestion thatangiogenesis may significantly contribute to fibrogenesis and disease progression relies first on the fact that vascularremodeling is a common finding in human liver withadvanced fibrosis, irrespective of etiology [6,8].Capillary structures formed by ECs in inflamed portal tracts have been observed both in chronic hepatitis C(CHC) and chronic hepatitis B (CHB) [9,10]. Evidence of angiogenesis was significantly more frequent in HCV positive patients compared with HBV-positive patients or controls [9-11].Theaim of the present study was to analyses the correlation between CD34 and the clinical severityof CHC

Material and methods

70 patients with chronic hepatitis C had included in our study,all patients had undergone liver biopsy under ultra-sonographic guideat the Department of Tropical Medicine And Gastroenterology of Sohaguniversity hospitals, Sohag, Egypt.

The diagnosis of chronic HCV will be based on positive HCV antibody by ELISA test and HCV RNA by PCR for more than 6 months.The study was approved by the Ethical Committee of the Medical University of Sohag;Informed consent was obtained from all patients.

Histopathological assessment:

Seventy liver biopsies were included in the study and submitted tohistopathological examination to assess both the grade and the stage of chronic viral hepatitis, using the METAVIR classification system [12], which evaluates fibrosis stage in a scale from F0 to F4. Necro-inflammatory activity will be scored with a scale from A0 to A3.

Immunohistochemistry

CD34: For micro vessel density (MVD) assessment, we first identified „hot spots"(areas with the highest micro vessel concentration) by scanning the section at low magnification (40x) using Olympus microscope CX21FS1, then we counted the number of positive vessels in three hot spots at a magnification of 200x, single immunoreactive endothelial cell, or endothelial cell clusters separate from other microvessels, were counted as a vessel (Weidner, 1995). The presence of blood cells or fibrin without any detectable endothelial cells was not sufficient to define a microvessel. Vessels with muscular walls were not counted (Svagzdys et al., 2009). The mean microvessel density for CD34 was calculated as the mean value of the vessel count in the three fields (Trojan et al., 2004).

Statistical analysis:

Data was analyzed using SPSS computer program version 22.0.Quantitative data was expressed as means ± standard deviation, median and range. One-Way ANOVA test was used for normally distributed data. Spearman's correlation was used for testing of correlation between different quantitative variables. Chi-Square test was used for comparison between qualitative variables. P-values 0.05 were considered statistically significant.

Results

From September 2014 to august 2016, 70 adult patients with chronic hepatitis C infection had fulfilled our inclusion criteria and were included in this study. Their ages ranged from 20-59 years (40.5 ± 11.4 SD). 42 males, 28 females.Males and female were comparable according to age [p value 0.457].Fibrosis stage of the patients using METAVIR classification system was done. The result were represented in table [1]

Fibrosis stage	Number	percentage
F1	28	28 (40%)
F2	22	22 (31.4%)
F3	20	20 (28.6%)

CD34 significantly related with AST and ALT levels. Also, it is significantly related with alpha-fetoprotein and prothrombin concentration.

Table [2] Correlation between CD34 and laboratory parameters of the studied patients (n= 70).

Group	R	P-value
HB (g/dl)	-0.056	0.647
RBCS (x1,000,000/mm3)	0.032	0.792
WBCS (x1000/mm3)	-0.181	0.133
Platelets (x1,000/mm3)	-0.164	0.175
ALT (IU/l)	0.264	0.027*
AST (IU/l)	0.295	0.013*
Bilirubin (mg/dl)	0.034	0.783
Albumin (mg/dl)	-0.189	0.118
Prothrombin time (sec)	0.214	0.075
Prothrombin concentration	-0.279	0.02*
Alpha fetoprotein	0.332	0.005*
HCV PCR	-0.022	0.860

There was asignificant relation between CD34 and fibrosis stage [p value=0.05].

Table [3]: Relation between fibrosis stage and laboratory data of the studied patients

Parameter	Fibrosis stage			P-value
	F1 (N= 28)	F2 (N= 22)	F3 (N= 20)	
HB (g/dl) Mean± S.D.	14.4 ± 1.5	14.1 ± 2.1	13.9 ± 1.8	0.640**
RBCS (x1,000,000/mm^3) Mean± S.D.	5.5 ± 0.9	5.1 ± 0.7	5.2 ± 0.8	0.305*
WBCS (x1000/mm^3) Mean± S.D.	6.9 ± 2.2	7.4 ± 3.5	5.7 ± 1.5	0.142*
Platelets (x1,000/mm^3) Mean± S.D.	244.3 ± 62.1	257.9 ± 65.6	201.6 ± 55.9	0.011**
ALT (IU/l) Mean± S.D.	46.2 ± 81.3	41.8 ± 26.4	73.2 ± 45.9	0.001*
AST (IU/l) Mean± S.D.	33.6 ± 28.8	41.7 ± 19.9	61.9 ± 32.9	< 0.001*
Bilirubin (mg) Mean± S.D.	0.7 ± 0.3	1.6 ± 3.7	0.7 ± 0.3	0.582*
Serum albumin (mg/dl) Mean± S.D.	4.2 ± 0.7	4.1 ± 0.4	4.01 ± 0.3	0.605*
Prothrombin time (sec) Mean± S.D.	11.9 ± 2.04	11.6 ± 0.9	12.6 ± 0.8	0.001*
Prothrombin concentration Mean± S.D.	93.3 ± 12.6	96.6 ± 9.3	86.4 ± 7.6	< 0.001*
Alpha fetoprotein Mean± S.D.	3.05 ± 1.3	5.01 ± 5.1	25.6 ± 38.4	0.001*
HCV PCR Low viremia (%) Moderate viremia(%) High viremia (%)	0 (0.0%) 8 (28.6%) 20(71.4%)	1 (4.5%) 4 (18.2%) 17 (77.3%)	3 (15%) 3 (15%) 14 (70%)	0.205***
CD34 levelMean± S.D.	15.7 ± 6.6	16.6 ± 7.3	26.1 ± 16.3	0.05*

Discussion

In the present study, there was a significant relation between CD34 and fibrosis stage. Theseobservations indicate that angiogenesis is particularlylinked to HCV infection, suggesting a possible contributionto HCV-related liver oncogenesis.Many studies reported that CD34 expression,which is a marker of neovascularization, was found in theperiportal area of lobules and increased in parallel with thefibrosis stage. [13, 14].

Other studies reported that In CHC, newCD34 vessels are found in areas of lymphoid neogenesiswhich occurs in response to local cytokines in the portaltract to form expanded portal-associated lymphoid tissue[9, 16, and 17].

This observation additionally supports an interactionbetween the inflammatory activity and neoangiogenesis. It is possible that activated cytotoxic T cells may up-regulatethe expression of vascular adhesion molecules, whichenhance angiogenesis [15, 16]. The new vessels are an integralpart of tissue remodeling that accompanies chronicinflammation and provide a portal of entry for the continuingrecruitment of inflammatory cells. They also delivernutrients and oxygen to areas of chronic inflammation thatare relatively hypoxic [16].CHC patients present a proangiogenic profile of angiogenesissoluble markers [17,18]. CD34 is increased in viral hepatitis and their concentrationscould be valuable markers of the evolution of liverinflammation, disease progression, and response to therapy[18, 19].

Conclusion

A direct correlation existed between endothelial cell markers CD34 expression and the progression of fibrosis in chronic hepatitis C. Theimmunohistochemical methods of this molecule can be important clue in futureprognostic strategies.

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الملخص العربى

كدلاله لتطور الالتهاب الكبدي الفيروسي سي.34 CD ظهور عامل

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في مصر معدل الوفيات / زرع الكبد و سرطان الكبد في المرضى الذين يعانون من تليف الكبد هو 4.58٪ و 3.36٪، على التوالي. وتشير التقديرات إلى أن المرضى المصريين الذين يعانون من تليف الكبد كان لديهم حدوث سنوي من سرطان الكبد بنسبة 5.3٪.
CD34 هو بروتين سكري غشاء بقدرة 110 كيلو دالتون موجود على خلايا اللوكيميا والخلايا البطانية والخلايا الجذعية). يتم التعبير عن CD34 بشكل تفضيلي على سطح تجديد أو الخلايا البطانية المهاجرة وهو علامة من الخلايا البطانية المتكاثر في البراعم المتنامية أثناء الأوعية الدموية.

الهدف من البحث:
والشدة السريرية للالتهاب الكبدي الفيروسي سي. CD34 كان الهدف من هذه الدراسة هو تحليل العلاقة بين

طرق البحث:
تم توظيف مجموعه 70 مريضا بالغين ن هؤلاء المترددين علي قسم طب المناطق الحاره والجهاز الهضمي بطب CD34 في مراحل مختلفة مع عدم وجود تليف بالكبد. درسنا التعبير عن Cسوهاج يعانون من التهاب الكبد المزمن .Cفي عينات الكبد من المرضى المصابين التهاب الكبد المزمن

النتائج:
يرتبط بشكل كبير مع مستوي الانزيمات الكبديه. أيضا، يرتبط ارتباطا كبيرا مع ألفا-فيتوبروتين وتركيز CD34 .ومرحلة التليف CD34 البروثرومبين. أيضا، كانت هناك علاقة كبيرة بين