Role of Cyclin D1 in Patients with Chronic Hepatitis C

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Abstract

Background: Hepatitis C infection is often asymptomatic but chronic infection with hepatitis C virus is associated with an increased risk for the development of fibrosis, cirrhosis, and hepatocellular carcinoma.

Objective: To assess the expression of cyclin D1 in liver tissues of patients with chronic hepatitis C virus and related with different parameters such as gender, age, stage of fibrosis and histological active index.

Patients and Methods: Twenty one formalin fixed, paraffin-embedded liver tissue blocks were included (16 males and 5 females), age range (15-60) years. They were collected from the archives of histopathology laboratories of Hepatology and Gastroenterology Teaching Hospital in Baghdad, Iraq, the present study done in department of Microbiology – College of Medicine – University of Diyala during the period from 1st August 2014 till 1st March 2015. All the samples are related to the period between 2009 to 2012. Histopathological sections were made for these liver biopsies and stained by hematoxylin and eosin stain for definitive diagnosis. The molecular detection of cyclin D1 in those tissue blocks were performed by using immunohistochemistry.

Results: Distribution of 21 patients with chronic active hepatitis diagnosed by 3rd generation enzyme linked immunosorbant assay revealed that 16 were males are higher (76.19%) than females 5(23.8%). The mean age was 38 years, most infection occurs within age group 31-45 years. The majority of the liver cases were diagnosed to have histological active index was 5/18 total number was 10 cases. Among liver tissues, 5 out of 21 (23.8%) showed 1/6 and 2/6 stage of fibrosis. Expression of cycline D1 demonstrated that 16 cases was positive while 5 was negative. Statistical analysis revealed significant differences between expression of cycline D1 and age, grade, HAI, but not significant with stage of fibrosis.

Conclusion: Cyclin D1 play important role in development and progression of hepatitis C virus, these findings support the concept that the cell cycle regulation may play a role in initiation and development of hepatocellular carcinoma; need to be confirmed by further large scale studies.

Key words: Chronic hepatitis C virus, immunohistochemistry, cyclin D1.

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Introduction

Hepatitis simply means inflammation of the liver. Hepatitis may be caused by a virus or a toxin substance such as alcohol. Viruses that can cause injury to liver cells include the hepatitis A, hepatitis B and hepatitis C viruses. These viruses are not related to each other or to hepatitis B virus

and differ in their structure they are spread among individuals [1].

It is estimated that approximately 130-170 million people worldwide are infected with hepatitis C virus. According to data from world health organization (WHO) community and blood donor surveys, the African and Eastern Mediterranean countries report the highest prevalence rates (>10%). The rates of infection in the general population and the incidence of newly-acquired cases indicate an appreciable change in the epidemiology of the infection [2].

Hepatitis C virus (HCV) is an enveloped RNA virus belonging to the flaviviridae family. Hepatitis C virus contains a single-stranded plus polarity RNA genome of approximately 9500 nucleotides [3]. Blood tests use to measure albumin level, liver function tests, prothrombin time and liver biopsy as well as genetic testing is done to check for the type of hepatitis C also can use enzyme immune assay (EIA) assay to detect hepatitis C antibody and hepatitis C RNA assays to measure virus levels (viral load) [5].

Cyclin D is a member of the cyclin protein family that is involved in regulating cell cycle progression. The synthesis of cyclin D is initiated during G1 and drives the G1/S phase transition. Cyclin D protein is anywhere consists of 155 to 477 amino acids in length [6]. The effects of HCV proteins on hepatocarcinogenesis have undergone intense investigation during the recent years. These studies have implicated three viral proteins (Core, NS5A, and NS3) in hepatocarconogenesis. The involvement of all three proteins have been described as being in control of cell cycle through alteration of or interaction with key cellular regulator proteins such as p53, p21, cyclins as well as transcription factors, proto-oncogenes, and growth factors/ cytokines [7].

In Iraq many studies done about HCV, study of [8]. Who determine the anti-HCV seroprevalence in pregnant women and shows that 3.21% rate of infection. Another study done by [9].Shows that the prevalence of anti-HCV antibodies was 7.1% in the general population. while other study determines genotype of virus among thalassemic, chronic liver disease and blood donors [10].

To the best of our knowledge, there is no previous study in Iraq about cylin D and hepatitis so this study design to assess the expression of cyclin D1 in patients with chronic hepatitis C virus and related with different parameters such as gender, age, stage of fibrosis and histological active index.

Patients and Methods

Ninety the subjects included in this study were represented by their archival formalin-fixed, paraffin embedded tissue blocks with liver tissue. Twenty one formalin-fixed, paraffin embedded blocks tissues were obtained, from patient's liver tissue had positive test for anti-HCV antibodies (3rd generation ELISA, plasmatic laboratory products Ltd, United Kingdom) from the archives of Hepatology and Gastroenterology Teaching Hospital in Baghdad. Their age ranged from 15 to 60 years. This study was carried out in department of Microbiology - College of Medicine - University of Diyala during the period beginning from 1st August 2014 till 1st March 2015. While the pathological samples were collected during the period from 2011 to 2012.

In Liver tissue sections were cut at 4 µm and placed on positively-charged slides; one section was stained with hematoxylin and eosin [11]. And second used for the detection of cyclin D1 by immunohistochemistry technique (IHC). Anti-cyclin D1 antibody-

(ab16663), and Rabbit specific HRP/DAB detection IHC Kit (ab64261) from Cambridge Science Park. England was used.

The slides were deparaffinized and rehydrated by xylene and serially graded alcohol for 5 minutes each , then distilled water. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 minutes. Slides were washed in phosphate-buffered saline, then treated with protein block, and incubated at 37°C for 5 minutes, slides washed with PBS thereafter. Primary antibody was applied to cover slides and incubated for 1 hours in humidity chamber at 37°C (primary antibody was prepared at dilution 1:100). Slides were rinsed gently in PBS. The secondary antibody was added for 10 minutes at room temperature, followed by the addition of Streptavidin-HRP antibodies for 10 minutes at 37˚C. After washing, samples were stained with diluted liquid DAB for 15-45 minutes at room temperature. Slides were counterstained with hematoxylin for 30 second and washed well in running tap water, then dehydrated and mounting with permanent-mounting medium (DPX), examined under light microscope first at 10 then at 400 magnifications was finally done. Interpreted as positive when >10% of the infected cell expressed the marker with a moderate to strong intensity of staining. Also we study the relationship between cyclin D1 overexpression and different variables Also we study the relationship between cyclin D1 overexpression and different variables.

Statistical analysis

Statistical analysis were conducted to describe different variables and parameters in this research and to describe relationships with each other as well. Chi-square and Fischer exact test was used to find out the effect of different patients criteria on the reading of each marker of immunohistochemistry.

Results

A twenty one causes where obtain from patient with chronic hepatitis C infection 16 (76.1%) were males and 5 (23.8%) were females. Statistical analysis show the significant differences between males and females.

Table (1): Distribution of patient with chronic hepatitis C infection according to their gender.

Gender	Number	Percentage	Comparison of Significance Chi^2-value	Sig
Male	16	76.19%	5.762	0.016 *
Female	5	23.8%		
Total	21	100%		

***Significant**

In the present study mean age was 38.90 years, distribution of age which demonstrated that most causes 13(61.9%) of chronic HCV patient were in age group (3145) while age group (15-30) and (46-60) low percent. Respectively (14.28%) and (23.8%). The statistical analysis demonstrated significant differences table(2).

Table (2): Distribution of patients with chronic hepatitis C infection according to their age.

Age stratum	Number	Percentage	Comparison of Significance Chi^2-value	Sig.
15-30	3	14.28%	8.000	0.018*
31-45	13	61.9%		
46-60	5	23.1%		
Total	21	100%		

***Significant**

According to stage of fibrosis, the results show in table (3) which revealed that 10 cases (47.61%) have 5/18, while 5 causes (23.8%) have 4/18 and 2 causes (9.52%) for 3/18 and 8/18 and 1 causes (4.76%) for 6/18 and 9/18. On other hand statistical analysis was demonstrated highly significant difference in different group.

Table (3): Distribution of patients with chronic hepatitis C infection cases according to Histological Active Index.

HAI	Number	Percentage	Comparison of Significance Chi^2-value	Sig.
3/18	2	9.52%	17.571	0.004*
4/18	5	23.8%		
5/18	10	47.61%		
6/18	1	4.76%		
8/18	2	9.52%		
9/18	1	4.76%		
Total	21	100%		

According to the fibrosis, histological diagnosis revealed that 5 causes (23.8%) had 1/6 and 2/6 fibrosis and 3 causes (14.28%) had 5/6 fibrosis while 3/6, 4/6 and 6/6 fibrosis had same percent 2 causes (9.52%) as shown in table (4). Statistical analysis not find significant difference.

Table (4): Distribution of patients with chronic hepatitis C infection according to stage of fibrosis

Fibrosis	Number	Percentage	Comparison of Significance Chi^2-value	Sig.
0	2	9.52%	4.000	0.677
1/6	5	23.8%		
2/6	5	23.8%		
3/6	2	9.52%		
4/6	2	9.52%		
5/6	3	14.28%		
6/6	2	9.52%		
Total	21	100%		

The expression of cyclin D1 in tissues from patient with chronic hepatitis C infection was76.19 % (16 out of 21) show positive expression, while 23.80% (5 out of 21) show negative expression, Significant differences noticed according to expression as shown in figure (1).

Table (5): IHC positive and negative cyclin D1 expression in patient with chronic hepatitis C infection

IHC result	Number	Percentage	Comparison of Significance Chi^2-value	Sig.
Cyclin D-Positive	16	76.19 %	5.762	0.016*
Cyclin D-Neggative	5	23.80%		
Total	21	99.99%		

*Significant

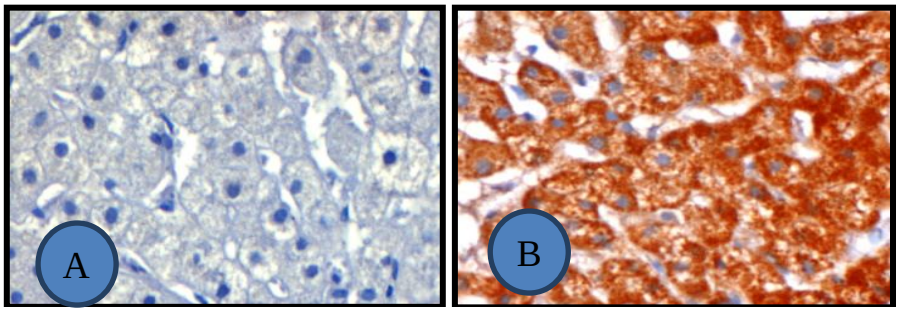

Figure (1): Immunohistochemistry- cyclin D1 expression in tissues of patient with chronic hepatitis C infection**,** stained by DAB chromogen and counter stained with heamatoxylin is shown as radish brown in positive cases (magnification power, 400). **A**-Negative expression **B**-Positive expression.

Table (6) demonstrated that expression of cyclin D 1 according to score distribution, out of 16 positive expressions revealed that 12 cases had high expression, intermediate expression and 3 had low expression, 75%, 6.25% and 18.75% respectively.

Table (6): Score for positive expression of cyclin D1- IHC in patient with HCV.

Variable	Positive	Comparison of Significance Chi^2-value	Sig
High	12(75%)	2.875	0.002*
Intermediate	1(6.25%)		
Low	3 (18.75%)		
Total	١٦		

*Significant

Table (7) showed that positive expression of cyclin D1related to different parameters. Statistical analysis showed significant differences between cyclin D1 expression with age, gender and HAI while non-significant with stage of fibrosis.

Table (7): Immunohistochemistry expression of positive and negative cyclin D1 relation to different parameter.

Variable		Positive	Negative	Comparison of Significance Chi^2-value	Sig
Age	15-30 31-45 46-60	2(12.5%) 11(68.75%) 3 (18.75%)	1(20%) 2(405%) 2(40%)	9.125	0.010*
Gender	Male Female	12(75%) 4(80%)	4(25%) 1(20%)	4.000	0.046*
	3/18 4/18 5/18 6/18 8/18 9/18	1(6.25%) 3(18.75%) 8(50%) 1(6.25%) 2(12.5%) 1(6.25)	1(20%) 2(40%) 2(40%) 0 0 0	14.000	0.016*
Fibrosis	0 1/6 2/6 3/6 4/6 5/6 6/6	1(6.25%) 3(18.75%) 5(31.25%) 1(6.25%) 2(12.50%) 2(12.50%) 2(12.50%)	1(20%) 2(40%) 0 1(20%) 0 1(20%) 1(20%)	5.000	0.544

Discussion

Current study which demonstrated that the prevalence of HCV infection was found in males 16(76.19%) more than females 5(23.8%), this results was in agreement with the findings of Li (2014), who reported that males high percent compare with females in HCV infection [12], Also agreement with studies done by other researchers [13, 14]. This may be related to the fact that males in Iraq are more socially active than female. Furthermore, they are more exposed to male-related risk factors than females due to their work (e.g. hair dressing and circumcision). This result of present study disagree with large cross-sectional studies have not demonstrated gender differences in the rate of chronicity in hepatitis C infection which revealed similar rates of HCV chronicity among both men and women [15, 16].

According to age distribution the preset study revealed that 61.9% occur in patient in age group (31-45 years), followed by age group (46-60), this results in agreement with finding of Azza *et al*., (2010), who show that age (>45 year), male gender, alcohol drinking, hypertension, creatinine and HCV infection were the significant factors associated with the presence of HCV [13]. This age related increase of incidence of hepatic neoplasia in HCV infected subjects appears to be an attributed to the inherent decline of the immune system and macrophage surveillance in old patients in addition to the increasing incidence of mutations of HCV infected hepatocytes [17].

Prevous study of [20] have reported that infection with chronic hepatitis C virus is typically characterised by slowly progressive hepatic fibrosis, with progression from stage 0 (no fibrosis) to stage 4 (cirrhosis) taking place at approximately 0.10-0.15 fibrosis units (median) per decade [20]. However, it is recognised that some patients do not progress while others rapidly develop significant fibrosis. Many studies have assessed the factors that could influence this rate of fibrotic progression [20, 21].

The present study showed high percent within 1/6 and 2/6 fibrosis, these finding in an agreement with McCaughan and George (2004) who demonstrated same results [22].These results agreement with findingof [14].

The scores in the present cases show that majority of the cases 10(47.61%) had 5/18 moderate to severe degree of necroinflammatory activity and this agree with classification of knodell and Scheuer [23, 24]. The staging of chronic liver disease is related to its time course and has an important. According to Knodell, HAl 12% cases showed scores ranging between 1-3, in 32% score ranged between 4-8 and in 48% score ranged between 9-12. According to Scheuer HAl 8% scored 1-3, 52% scored 4-8 and 40% scored 9-12 [24].

The role of cyclin D1 in disease development and to a lesser extent the other D-type cyclins, is frequently deregulated in cancer and is a biomarker of cancer phenotype and disease progression. The ability of these cyclins to activate the cyclin-dependent kinases (CDKs) CDK4 and CDK6 is the most extensively documented mechanism for their oncogenic actions and provides an attractive therapeutic target. Is this an effective means of targeting the cyclin D oncogenes, and how might the patient subgroups that are most likely to benefit be identified [25].

In the current study, expression of cyclin D1 was found in the hepatocytes of HCV-infected. However, the expression was significantly elevated and this result an agreement with study done by [25]. Who was highlighting the increased hepatocyte turnover in chronic HCV infection. The upregulation of cyclin D1 in cirrhotic livers cases suggests that its expression may play an important role in the process of tumorigenesis. This goes in hand with the findings of other investigators [26, 27]. Who reported that cyclin D1 overexpression accelerates and shortens the G1-phase of the cell cycle, leading to a more rapid entry into the S-phase and also increases the number of cell cycle divisions. Study done by Raychaudhuri *et al.*, who revealed that HCV non-structural protein 5A was responsible for stimulation of rRNA transcription. The activation of rRNA transcription appears to be due to stimulation of phosphorylation of upstream binding factor (UBF1) possibly as a result of up-regulation of cyclin D1/cdk4 by the NS5A polypeptide. These results could, at least in part, explain a mechanism by which HCV contributes to transformation of liver cells [28].

In this study statistical analysis revealed significant differences between expression of cycline D1 and age, grade, HAI, but not significant with stage of fibrosis, strong relationship between cycline D1 expression and stage of fibrosis but in this study non-significant may be related with limited sample size.

In conclusion, expression of cyclin D1 is very high among HCV positive cases in present study Cyclin D1 play important role in development and progression of hepatitis C virus, these findings support the concept that the cell cycle regulation may play a role in initiation and development of hepatocellular carcinoma; need to be confirmed by further large scale studies.

References

[1]Sorrell MF, Belongia EA, Costa J, Gareen IF, Grem JL, Inadomi JM, *et al*. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis B. Ann Intern Med. 2009;150:104-10.

[2]Baldo V, Baldovin T, Trivello R, Floreani A. Epidemiology of HCV infection. Curr Pharm Des. 2008; 14(17):1646-54.

[3]Raychaudhuri S, Vanessa F, Bhaswati B and Asim D. Activation of Ribosomal RNA Transcription by Hepatitis C Virus Involves Upstream Binding Factor Phosphorylation Via Induction of Cyclin D1.Cancer Res. 2009; 69(5): 2057–2064.

[4] O'Leary JG, Davis GL. Hepatitis C. In: Feldman M, Friedman LS, Brandt LJ, eds. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9th ed. Philadelphia, Pa: Elsevier Saunders; 2010:chap 79.

[5] Ming-Jen Hsu, Chi-Hong Chao, Shao-Hung Wang, Li-Ru You and Yan-Hwa Wu Lee: Mechanisms for Inhibition of Hepatitis B Virus Gene Expression and Replication by Hepatitis C Virus Core Protein. Journals of biological chemistry 2003; 278(1): 591-607.

[6]Hardwick JM. Cyclin' on the viral path to destruction. 2009; 2 (11): E203-204.

[7]Kasparzk, k; Adamek, A. Role of hepatitis C virus proteins in hepatic carcinogenesis. Hepatology Research 2008;38:1–26.

[8].Al-Kubaisy WA, Niazi AD, Kubba K. History of miscarriage as a risk factor for hepatitis C virus infection in pregnant Iraqi women. East Mediterr Health J. 2002;8(2-3):239-244.

[9]Khattab OS. Prevalence and risk factors for hepatitis C virus infection in hemodialysis patients in an Iraqi renal transplant center. Saudi J Kidney Dis Transpl. 2008;19(1):110-5.

[10]Khalid MD ، BassimaAA. Hepatitis C virus genotypes in Iraq. Iraqi J Biotech. 2012;11 (2):475-480.

[11]Luna GL: Manual of histological staining of Armed Forces Institute of pathology.3ed Ed., 1968. McGraw-Hill. USA: p18.
[12]Li WC1, Lee YY, Chen IC, Wang SH, Hsiao CT, Loke SS. Age and gender differences in the relationship between
hepatitis C infection and. J Viral Hepat. 2014; 21(10):706-15.
[13] Azza EE, Mona MN, Mona KZ, Noha AA, Ahmed M A, Ibrahim MI, Suher Z, Abdel-Hakeem SE, Nora EE. Differential expression of cell cycle regulators in HCV infection and related hepatocellular carcinoma World J Hepatol 2010; 2(1): 32-41.
[14] Mushtaq S, Muhammad M, Muhammad TK, Waheed-UT, Nadim M. A Morphological Study of Histological Activity Index (HAl) and Scoring System in Hepatitis-C: JPMA.1997; 47:162- 165.
[15] Bellentani S, Tiribelli C. The spectrum of liver disease in the general population: lesson from the Dionysos study. J Hepatol. 2001; 35(4):531-7.
[16]Stephen LC and Timothy RM.The Natural History of Hepatitis C Virus (HCV) Infection Int J Med Sci 2006; 3(2):47-52.
[17]Burns EA, Leventhal EA. A immunity and cancer. Cancer Control.2000; 7: 513-22.
[18]Vogt M. *et al*. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med. 1999; 341(12):866-70.
[19]Bellentani S, Tiribelli C. The spectrum of liver disease in the general population: lesson from the Dionysos study. J Hepatol. 2001; 35(4):531-7.
[20]Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C.Hepatology2002; 36:S47-56.
[21]Poynard T, Bedossa P, Opolon P, *et al.* Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet1997;349:825-32.
[22]McCaughan GW، J George. Fibrosis progression in chronic hepatitis C virus infection. Gut 2004; 53:318-321.
[23]Knodell RC, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N *et al*., formulation and application of a numerical scoring system for assessment histological activity in asymptomatic chronic active hepatitis. Hepatology. 1981; 1(5):431-5.
[24]Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991; 13: 372-374.
[25]Elizabeth A. Musgrove, C. Elizabeth Caldon, Jane Barraclough, Andrew Stone & Robert L. Sutherland.Cyclin D as a therapeutic target in cancer Nature Reviews Cancer. 2011. 11, 558-572.
[26]Fu M, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview:Cyclin D1: normal and abnormal functions. Endocrinology 2004; 145: 5439-5447.
[27]Bahnassya, AA, Abdel-Rahman NZ, Samah AL, Waleed SM, Amrallah AM, Salem ES, *et al*. The role of cyclins and cyclin dependent kinases in development and progression of hepatitis C virus-genotype 4-associated hepatitis andhepatocellular
carcinomaExperimental and Molecular Pathology. 2011; 91(2): 643–652.
[28] Raychaudhuri S, Vanessa F, Bhaswati B and Asim D. Activation of Ribosomal RNA Transcription by Hepatitis C Virus Involves Upstream Binding Factor Phosphorylation Via Induction of Cyclin D1. Cancer Res. 2009; 69(5): 2057–2064.