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Expression of Galactin-3 in Chronic Hepatitis C: Histopathological and Immunohistochemical Study

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Abstract: Hepatitis C virus (HCV) infection is characterized by a silent onset in most infected persons, increasing rate of viral persistence and the potential for development of severe chronic liver disease, ranging from chronic hepatitis to cirrhosis and hepatocellular carcinoma. Galactin-3 is a strongly pro-inflammatory signal as detected by both in vitro and in vivo cascade, participates in tumor genesis and tumor progression. The current study aimed to assess galectin-3 over-expression in 75 cases of HCV (15 of them complicated by HCC) by immune-histochemistry and in relation with their clinico-pathological features. Results showed that there was a statistically significant correlation between the galactin-3 and age, Metavir staging system as well as steatosis in HCV cases. Galactin-3 expression in HCC complicating HCV cases showed statistically significant correlations. Conclusion: Galactin-3 may act as a diagnostic marker for evaluation of fibrosis staging and inhibition of which may be a promising therapeutic strategy against tissue fibrosis. Also it may be potentially benefit for serving as a prognostic factor for HCC.

Key words: HCV · Metavir · HCC · Galactin-3

INTRODUCTION

Hepatitis C virus infection can progress to chronic infection in 75–80% of infected patients. The resolving or chronicity of the infection depends on a balance between the immunity and viremia as well as the critical role in the pathogenesis of chronic viral hepatitis [1, 2].

The Metavir scoring system is the most important scoring system for patients with hepatitis C. The scoring constitutes a grading and a staging system. The grade evaluates the activity or degree of inflammation and the stage evaluates the amount of fibrosis [3, 4]. Galectin-3 plays a very important role in regulation of inflammation. It has been involved in many biological events including cell proliferation, adhesion and survival. [5]. Its expression is negligible in normal liver and dramatically increased in cirrhotic nodule of hepatocytes. It is thought to play a critical role in fibrogenic processes in multiple tissues mainly through its immune-modulatory effect and seems to be included in neoplastic transformation and metastatic spread [6]. Moreover, it has been observed to be expressed in HCC tissues. Higher expression rate of nuclear galectin-3 has been reported in association with worse prognosis. These results highlighted a central role for galectin- 3 in HCC development and progression [7].

The aim of the work was to assess galactin-3 over-expression in cases with HCV and to correlate its expression with stage of fibrosis.

MATERIALS AND METHODS

Our study was performed on 75 retrospective, formalin fixed, paraffin embedded cases (52 cases of them percutaneous liver biopsies and 23cases resection hepatectomy specimens). All of them were serologically and histologically confirmed chronic hepatitis C (35 males and 25 females) and 15 of them complicated by HCC. All cases were collected from the pathology department laboratory at National Hepatology and Tropical Medicine Research Institute, Egypt.

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Histological Review: The paraffin blocks were serially sectioned at 3-5 *i*m thickness, stained with routine hematoxylin-eosin and Masson's trichrome stain for assessment of fibrosis and the other with reticulin stain to evaluate hepatic architecture. Each biopsy specimen was evaluated for the activity of chronic hepatitis C and the extent of liver fibrosis according to the criteria of Metavir scoring system [8, 9].

Immunohistochemical Staining: One section was mounted on positively charged slides for immunohistochemical staining with rabbit anti-galectin-3 polyclonal antibody (Dako) using En-Vision system (Dako).Galactin-3 staining, usually cytoplasmic and sometimes nuclear was scored subjectively by pathologists according to the percentage of the positively stained HSCS (staining of bile duct epithelium and endothelium of sinusoids were not considered in scoring) as follow**: Score 0** (negative): less than 5%,(score**1**) mild: 5-25%,(**Score 2**) moderate: 26-75% and (**Score 3**) severe: >75%. Both scores 0 and +1 were categorized as negative, tumors with scores of +2 or +3 were considered positive (galactin-3 over expression) [10].

Statistical Methods: Data were statistically described in terms of mean ± standard deviation and range, or frequencies (number of cases) and percentages. Comparison of numerical variables between the studied groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square (x^2) test was performed. Fisher's exact test was used instead when the expected frequency is less than 5. P values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science, version 15).

RESULTS

Our study was performed on 75 retrospective formalin fixed paraffin embedded HCV cases with male predominance, 15 of which were complicated by HCC. The age of the cases ranged between 24 and 59 years with median age of cases 49 ± 10 SD. We recorded that most of the studied cases showed insignificant (negative) and mild steatosis presented by 41.3 and 52% respectively. Of the 15 cases with complicated HCC 3 of which showed mild steatosis, the rest (12 cases) were considered negative for steatosis.

Table 1: Galactin-3 distribution in our studied cases.

Galactin-3	Frequency	Percent
Negative	5	6.6 %
Mild	23	30.7 %
Moderate	24	32 %
Marked	23	30.7 %
Total	75	100 %

Table 2: Correlation between galactin-3 expression and Metavir staging in the studied cases

		Fibrosis staging by Metavir					
Galactin-3		F0	F1	F2	F3	F4	Total
Negative	Count	2	0	0	3	0	5
	%	40%			60%		100%
Mild	Count	0	18	0	2	3	23
	%		78.1%		8.9%	13%	100%
Moderate	Count	0	1	10	9	4	24
	%		4.2%	41.7%	37.5%	16.6	100%
Marked	Count	0	0	0	10	13	23
	%				43.5%	56.5%	100%
Total	Count	2	19	10	24	20	75
	%	5%	31.7%	15%	31.7%	16.7%	100%

Table 3: Galactin-3 distribution in HCC complicating HCV cases.

Galactin-3	Frequency	Percent
Negative	3	20 %
Mild	5	33.33 %
Moderate	4	26.67 %
Marked	3	20 %
Total	15	100 %

On assessment of the activity score by Metavir scoring system, about 63% of the studied cases were categorized as A2 and A3. Evaluation of fibrosis by Metavir scoring system denoted that F0 was 2.7% of the studied cases, F1 was 25.3%, F2 was 13.3%, F3 was 32% and established cirrhosis (F4) was notable in 26.7% of the studied cases.

According to Galactin -3 immunostaining; we found that over-expression of galactin-3 in HCV cases was seen in 47(62.7%) cases [23 cases were moderate and 24 cases were marked]; while 28(37.3%) cases were negative [5 cases were negative and 23 cases were mild] (Table 1). There was a statistically significant correlation between the galactin-3 and age, Metavir staging system as well as steatosis (P value = 0.001) (Table 2 and Fig. 1). Whereas; no correlation was found between galactin-3 immunostaining and sex (P value = 0.304). Galactin-3 over expression in tumors area (HCC complicating HCV cases): seen in 7(46.7%) cases [4 cases were moderate and 3 cases were marked], but 8 (53.3%) cases were negative [3 cases were negative and 5 cases were mild] (Table 3 and Fig. 2)

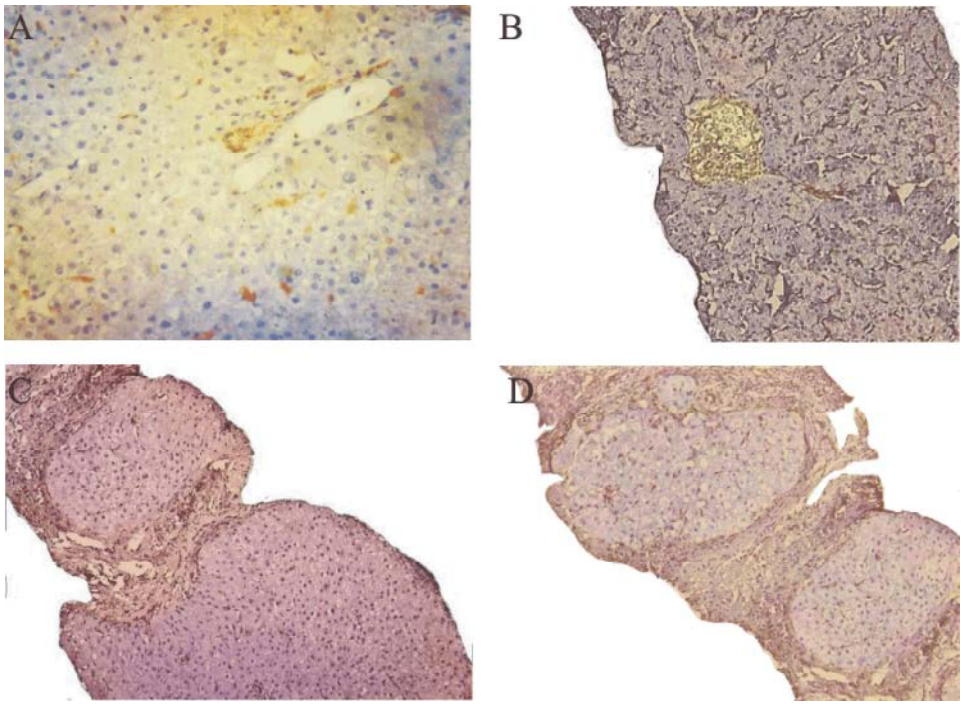

Fig. 1: A. HCV case (F0) showing negative galactin-3 expression. (G3 immunohistochemistry x40).
Fig. 1: B. HCV case (F2) showing mild galactin-3 expression (G3 immunohistochemistry x10).
Fig. 1: C. HCV case (F3) showing moderate galactin-3 expression (G3 immunohistochemistry x10).
Fig. 1: D. HCV case (F4) showing marked galactin-3 expression in studied cases with HCV (G3 immunohistochemistryx10).

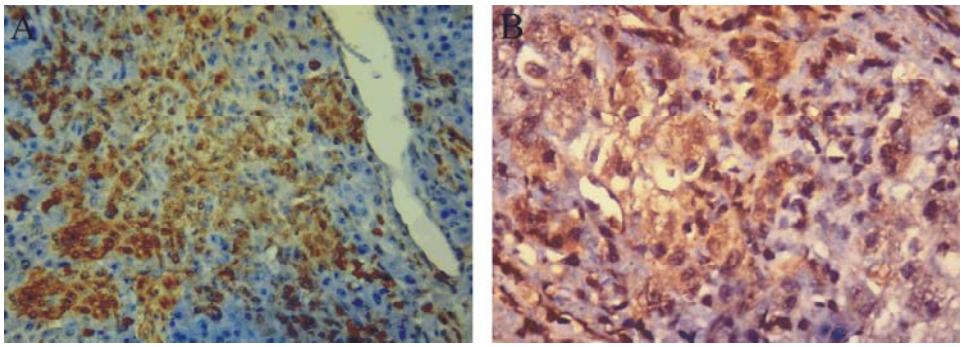

Fig. 2: A. Hepatocellular carcinoma complicating HCV case showing moderate galactin-3 expression (G3 immunohistochemistry x40).
Fig. 2: B. Hepatocellular carcinoma complicating HCV case showing marked galactin-3 expression (G3 immunohistochemistry x100).

DISCUSSION

Galectin-3 has been involved in many events of the inflammatory process including neutrophil and macrophage activation and function. The study of immunohistochemical expression of galectin-3 on our cases revealed that 30.7% of them had mild expression and the same percentage had marked expression. While, 32% of cases had moderate expression and 6.6% negative expression (62.7% of our study with a high expression of galectin-3). This result agrees with Hus *et al*. [11] who performed a study on 25 HCV cases and recorded that the expression of galactin-3 on their study was 35% mild, 30% moderate and 35% marked (65% of their study with a high expression of galectin-3).

In our work, we found statistically significant correlation between expression of galactin-3 and Metavir staging. Also; we noticed that galectin-3 was abundantly

expressed in cirrhotic liver in peripheral distribution within regenerating nodules. This goes with Hus *et al*. [11] who found that focal regenerating nodules of cirrhotic tissue express galectin-3. Moreover; this matches with other studies where galectin-3 expression was dramatically increased in the periportal areas, areas of bridging fibrosis and the cirrhotic nodules of hepatocytes suggesting that galectin-3 may promote fibrosis by stimulating myofibroblasts activation and matrix production by a (TGF-β)-independent mechanism [10, 12].

Galectin-3 is over-expressed in fibrotic liver disease. Also, affection of the galectin-3 gene markedly attenuates liver fibrosis with decrease collagen deposition and myofibroblasts activation. This finding suggests that galectin-3 may increase fibrosis by increasing myofibroblasts activation and matrix production by a (TGF-β)-independent mechanism [10].

Furthermore; Mehmet *et al*. [13] stated that galectin-3 expression in rapidly proliferating hepatocytes of cirrhotic liver may be associated with high mitotic index. This might explain the presence of HCC complicating our studied cases as we noted that 20% of all HCV cases were complicated by HCC (all complicated HCC cases were cirrhotic). These findings are compatible with Ziada D.H *et al.* [14] who performed a study on 514 HCV patients and recorded that 103 patients representing 22% of the total number of their cases developed HCC and this was mainly associated with decompensated cirrhosis.

The expression of galectin-3 in the tumor area of HCC complicating HCV cases in this study revealed that 20% of them showed negative expression, 33.3% showed mild expression, 26.7% were moderate and 20% were marked (46.7% of our study with a high expression of galectin-3). However, in a study previously done by Shan-Shan *et al.* [12] on 165 HCC cases on top of HCV and revealed that 81.8% of their cases showed high expression of galectin-3 (moderate and marked expression). This difference is probably due to small number of patients and random sample included in the current study.

In fact, galectin-3 contributes to tumor genesis and tumor progression through multiple different mechanisms, including progression of oncogenesis, angiogenesis, invasion and metastatic spread [12]. Galectin-3 is prominently up-regulated in HCC tissues. Higher expression rate of nuclear galectin-3 denoted worse prognosis [7].

In this current research, there was a highly statistically significant positive relationship between the degree of hepatic steatosis and expression of galectin-3 of the studied cases (P value 0.001). This is in line with what were reported by both Laderach *et al.* [15] and Eisa *et al.* [16] who found a parallel increasing of galectin-3 expression with steatosis and illustrated that in patients with varying degrees of hepatic impairment (steatosis, hepatitis, cholestasis and cirrhosis); liver functions were impaired and galectin-3 levels rise. This might be due to the strong pro-inflammatory signal produced by galatin-3 detected by both in vitro and in vivo cascade [10].

CONCLUSIONS

We concluded that galactin-3 might have a potential profibrotic role in fibrosis in cases of HCV. So it can be used as a diagnostic marker for evaluation of stage of fibrosis and inhibition of which may represent a promising therapeutic strategy against tissue fibrosis. We also noted that 46.7 % of HCC cases of the current study showed galactin-3 over-expressing, these findings may be potentially benefit for serving galectin-3as a prognostic factor for HCC. Further studies with a larger sample size on HCC can clarify the probable role of galectin-3 in the prognosis, progression and metastasis of HCC.

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