Role of Galectin-3 Immunohistochemical Expression in Cirrhosis and Hepatocellular Carcinoma Complicating Chronic Hepatitis

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Abstract

Background and objectives: Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related mortality. The primary risk factor for HCC seems to be liver cirrhosis. A large majority of these patients have a history of viral hepatitis. Some experiments suggested possible role of Galectin-3 in the promoting of inflammation and the development of chronic liver disease, cirrhosis and HCC. Our aim was to evaluate the role of immunohistochemical expression of Galectin 3 in cirrhotic liver and HCC complicating chronic hepatitis as well as to study its subcellular distribution to examine the possible involvement of galectin-3 in neoplastic progression and to clarify if expression of galectin-3 is related to the clinicopathological characteristics and prognosis of hepatocellular carcinoma (HCC) patients or not.

Materials and methods: The material of this work included 20 cases with cirrhosis and 20 cases with hepatocellular carcinoma, both have serological evidence of chronic hepatitis. 10 cases of normal liver tissue also included as control group. Hematoxylin and eosin-stained sections from all cases were re-evaluated and then further stained immunohistochemically using antibodies against Galectin 3.

Results: The high expression of galectin-3 were observed in 35 % of cirrhosis and it was found to be 28.6%, 42.9%, 28.6% in cirrhosis secondary to hepatitis B, C and in hepatitis negative serological evidence of both infection; but this difference was not statistically significant ($P = 0.459$). The primary HCC tumor tissues showed significantly upregulated galectin-3 expression relative to normal tissues and cirrhosis ($P = 0.006$) and it is independent of whether the patient had prior specific type of viral infection. High positive expression of Galectin-3 was significantly associated with tumor grade, vascular invasion and patients with underlying cirrhosis ($P = 0.034, 0.037, 0.001$) respectively. As regard the pathological stage, multiplicity, tumors with large size (>5 cm) tended to be associated with the high positive expression but not statistically significant ($P = 0.144, 0.389, 0.292$) respectively. Galectin 3 expression displayed no association with age, gender and with underline cause of hepatitis ($P > 0.05$). Based on the dominant type of localization, cytoplasmic expression tended to be related to large size, solitary tumors, poorly differentiated, absent of vascular invasion compared with negative expression, but not significant.

Conclusion: These results suggested that high expression of galectin-3 in cirrhosis may be guiding us about its progression to HCC and it is related to the prognosis of it. its expression independent of whether the patient had prior specific type of viral infection and galectin-3 could serve as a novel biomarker and therapeutic target in HCC.

Keywords: Galectin-3, Cirrhosis, Hepatocellular carcinoma, Chronic hepatitis

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related mortality. Various factors have been identified as causes for cirrhosis and HCC, but Chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV) or both is the most common risk factor for cirrhosis and HCC worldwide (*Mehmet et al., 2015*). Some experiments suggested possible role of Galectin-3 in promoting inflammation and the development of chronic liver disease, cirrhosis and HCC (*Unal et al., 2015*). Oncogenic and viral stimulation can change galectin-3 expression and galectin-3 increases in several human tumors (*Mehmet et al., 2015*).

Galectin-3 (GAL-3) is a protein that binds to -galactosidase residues on cell surface glycoproteins. This marker has been implicated in cell adhesion, cell-matrix interaction, angiogenesis, regulation of normal cellular proliferation and apoptosis, as well as malignant transformation and the metastasis of cancer cells (*Connie et al., 2010*).

The family of lectins are predominantly localized in the cytoplasm, may translocate to the perinuclear membrane, nucleus and/or get secreted from the cytoplasm (*Aiad et al., 2008*) and (*Nangia-Makker et al., 2007*). The biological role of galectin-3 is defined by its cellular localization, which depends on the cell type, proliferation status and neoplastic progression, while the regulation of localization remains unknown (*Dumic et al., 2006*). In the cytoplasm, galectin-3 acts as an anti-apoptotic factor and is involved in the modulation of cell proliferation, differentiation, survival and apoptosis through binding with activated K-Ras and interacting with Bcl-2 (*Zhixiong et al., 2014*), (*Amalina et al., 2014*). In the nucleus, galectin-3 is involved in gene transcription through the enhancement or stabilization of transcriptional factors and modulates the cell resulting in cell cycle arrest without cell death (*Hea et al., 2013*). Extracellular galectin-3 mediates cell adhesion through its ability to bind cell surface glycoproteins and glycosylated components of the extracellular matrix (*Tian et al., 2014*), as well as

through a cross-linking of glycosylated membrane receptors to trigger signal transduction cascades (*Jesús et al., 2015*).

The normal hepatocytes do not express galectin-3, but this protein can be present in HCC and it was also found that focal regenerating nodules of cirrhotic tissue also express galectin-3 (*Hsu et al., 1999*).

In this study, we aimed to evaluate Galectin-3 expression in normal liver, cirrhotic liver and HCC both secondary to chronic hepatitis as well as its subcellular distribution to examine the possible involvement of galectin-3 in neoplastic progression as well as its relation to the related to the clinicopathological characteristics and prognosis of hepatocellular carcinoma.

Materials and Methods

Tissue sample

The blocks were collected from the surgical files of the Histopathology Department of Al-Azhar University Girl's Hospital and from the archives of some private laboratories, covering the period from January 2013 to April 2016, after obtaining an informed consent and approval of local ethical committee. The clinicopathological data such as age, gender, histological grade, stage, tumor multiplicity, size of the tumor, vascular invasion, associated cirrhosis, as well as viral serology were obtained from the available histopathological charts.

This study was carried out on 20 cases with cirrhosis and 20 cases with hepatocellular carcinoma both have serological evidence of chronic hepatitis and 10 cases of normal liver tissue as control group. (These normal liver biopsies were obtained from normal regions in cases of intrahepatic cholangiocarcinoma).

Twenty cases of cirrhosis were secondary to HBV (7 cases), HCV (10 cases) and negative to B and C viral hepatitis (3 cases).

Twenty cases of Hepatocellular carcinoma were secondary to HBV (7 cases), HCV (9 cases) and negative to both viral hepatitis (4 cases).

Positive HBV infection is defined by presence of serum HBV surface antigen or antibodies to HBV surface antigen. Positive HCV infection is defined by presence of serum HCV surface antigen or antibodies to HCV surface antigen. Negative Cases were negative serologically of both infections.

Representative samples were stained with H&E to re-evaluate the diagnosis. The histologic grade of hepatocellular carcinoma was assigned according to the Edmondson Steiner grading system (*Yu et al., 2012*), and the tumors were grouped as well (I), moderately (II) and poorly (III/IV) degrees of differentiation. Pathological staging (PT stage) was determined according to the American Joint Committee on Cancer grading system (7th edition) (*Kee et al., 2013*).

Immunohistochemical study:

Immunostaining for Galectin 3 was performed on 3-5 µm sections of formalin-fixed, paraffin-embedded endometrial specimens using standard streptavidin –biotin–peroxidase complex (ABC) methods. Tissue sections were incubated at 50°C for 2 hour, deparaffinized in xylene, hydrated in a graded alcohol dilutions, a rinse in distal water and washed in PBS. Endogenous peroxidase activity was block by incubating the sections for 15 min with 0.3% hydrogen peroxide in absolute methanol. Then, after a 10-min rehydration in PBS, the sections were heated in a microwave oven for 10 min at 60 C° in citrate buffer for antigen retrieval. After cooling at room temperature, the sections were incubated in primary antibodies overnight .The antibodies used were, mouse monoclonal anti- galectin-3 MAb A3A12 (*Liu et al., 1996*) antibody at 10 µg/ml.

After washing with PBS, the slides were incubated with multilink secondary antibody, after which goat anti-mouse streptavidin –biotin–peroxidase reagent was applied for 30 minutes (Dako, Japan). Lastly, sections were incubated with diaminobenzidine and counter-stained with hematoxylin then cleared and mounted. The primary antibodies was omitted and served as negative control. The positive control for galectin-3 is conventional papillary thyroid carcinoma.

Positive and negative controls were run simultaneously.

Interpretation of the staining

Assessment of Galectin 3 labeling index

The expression of galectin-3 was analyzed by the degree of expression and localization. For the degree, the overall expression rate was defined by the total galectin-3 immunostaining, score was calculated as the sum of the percent of positive stained tumor cells and staining intensity. Briefly, the percentage of positive staining was scored as 0 (<5%, negative), 1 (5–25%, sporadic), 2 (25–50%, focal), or 3 (>50%, diffuse). Staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The total immunostaining score was calculated as the value of percent positivity score × staining intensity score; that_ranged from 0 to 9. We defined galectin-3 expression levels as: – (score 0–1), + (score 2–3), ++ (score 4–6) and +++ (score > 6). Based on their levels of galectin-3 expression, patients were divided into two groups: low galectin-3 (– and +) and high galectin-3 (++ and +++) (*Shan et al., 2014*). Regarding the cellular localization, the galectin-3 expression was detected in the cytoplasm only, in the cytoplasm and the nucleus or in the cytoplasm with nucleus and the membrane or nucleus and membrane. Several types of localization were observed even in the same specimen of HCC tissue so the localization was considered by the dominant type in the specimen (*Matsuda et al., 2008*).

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. Qualitative data were presented as number and percentages. The comparison between two groups with qualitative data were done by using **Chi-square test** and/or **Fisher exact test** was used instead of Chi-square test when the expected count in any cell was found less than 5. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

Probability (P-value)

- P-value ≤ 0.05 was considered significant.
- P-value ≤ 0.001 was considered as highly significant.
- P-value > 0.05 was considered insignificant.

Results

A total of 40 cases (20 cases with Cirrhosis, and 20 cases with HCC) were included in this study. Male/female ratios were 11/9 and 8/12, respectively. The median age was 52 years with range 32 -63 in cirrhotic group, and 54 with range 45- 70 in HCC group.

As regard to 20 cases of cirrhosis complicated chronic hepatitis there were secondary to HBV in (7 cases), HCV (10 cases) and negative serological evidence of both infections in (3 cases).

As regard Hepatocellular carcinoma associated with hepatitis there were 7 cases secondary to HBV , 9 cases secondary to HCV and non- B, non C viral hepatitis was found in 4 cases. fifteen cases (75%) showed underlying cirrhosis.

According to size of the tumor in hepatocellular carcinoma, 60% (12 cases) were smaller than 5 cm, and 40 % (8 cases) were larger than 5cm. As regard to tumor multiplicity, tumor was solitary in 18 cases and multiple in only 2 cases.

As regard tumor differentiation, 4 of them were well differentiated (20 %), 6 cases (30 %) were moderately differentiated and 10 cases (50%) were poorly differentiated. 5 cases (25 %) had vascular invasion.

As regard stage of the tumor, it was classified into 9 cases (45%) were stage I, 5 cases (25%) were stage II and 6 cases (30%) were stage III. Clinico-pathological characteristics of HCC Patients, all are included in (**Table 1**).

Table (1): Clinico- pathologic characters of 20 cases of hepatocellular carcinoma	
Variable	Number of cases
Age	
Medium (range)	54y (45-70)
Gender	
Male / female	8/12
Tumor Size	
5 cm	12 (60.0%)
5 cm	8 (40.0%)
Tumor Multiplicity	
• Solitary	18 (90%)
• Multiple	2 (10%)
Grade	
• Well diff. (%)	4 (20 %)
• Moderate diff. (%)	6 (30 %)
• Poorly diff. (%)	10 (50 %)
Pathological Staging	
• I	9 (45%)
• II	5 (25%)
• III	6 (30%)
Vascular Invasion	
• Absent	15 (75%)
• Present	5 (25%)
Associated Hepatitis	
HBV	7 (35%) cases
HCV	9 (45%) cases
Non B non C	4 (20%) cases
Associated Cirrhosis	
• Absent	5 (25%)
• Present	15 (75%)

Immunohistochemical results of galectin-3 expression :

Normal liver tissues

In the normal liver tissues, galectin-3 expression was found in cholangiocytes and in Kupffer cells

in all cases and it was found only in (2/10) 20% of cases in normal hepatocytes, where the positivity represented less than 5% of total cells and was weak and cytoplasmic (**Fig. 1**).

Figure (1): Galectin 3 expression in normal liver; (a) positive expression of Galectin 3 Kupffer cells (X 100). (b) high power of previous picture (X 300)

Expression of Galectin-3 in cirrhotic human liver

In cirrhotic liver specimens that not associated with HCC, galectin-3 expression were observed in the cholangiocytes and Kupffer cells in 16/20 (80%) of cases and strong cytoplasmic staining of hepatocytes were observed in 7/20 (35 %) of cases , while the remaining 13 cases (65%)

showed low expression of galectin-3. All positive cases had more than 20 % galectin-3-positive hepatocytes in 4 cases , the expression were at the periphery of the cirrhotic nodules adjacent to the inflamed and bridging fibrosis, in 2 cases all of the hepatocytes in the nodule were positive for galectin-3 and in one case , the expression was seen at the center of cirrhotic nodules (**Fig. 2**).

Figure (2): Galectin 3 expression in cirrhosis; (a) the expression was present at the periphery of cirrhotic nodules adjacent to the inflamed and bridging fibrosis and in cholangiocytes (X 100). (b) High power of previous picture (X 300). (c) Expression was seen at the center of cirrhotic nodules (X 100). (d) All of the hepatocytes in the nodules (X 100).

The high expression of galectin-3 in cirrhotic patients was found to be; 28.6% in cirrhosis secondary to hepatitis B, 42.9% in cirrhosis secondary to hepatitis C, and 28.6% in hepatitis

negative serologically for both infections ,but this difference was not statistically significant ($P = 0.459$) (Table 2).

Table (2): Galectin-3 expression in 20 cases of cirrhosis according to viral etiology							
Galectin 3 expression	No =20	High expression (7 cases 35%)		Low expression (13 cases 65%)		Chi-square test	
		No.	%	No.	%	X ²	P-value
Cirrhosis secondary to HBV	7	2	28.6%	5	38.5%	1.559	0.459
Cirrhosis secondary to HCV	10	3	42.9%	7	53.8%		
Cirrhosis with negative serological evidence of both infection	3	2	28.6%	1	7.7%		

Expression of Galectin-3 in cases of hepatocellular carcinoma

to normal tissues (20%) and cirrhosis (35%) ($P = 0.006$) (Table 3).

High expression of Galectin-3 was increased significantly in primary HCC cases (75%) relative

Table (3): Correlation between Galectin 3 expression in HCC relative to normal and cirrhotic liver tissue								
Gala 3	Normal (10 cases)		Cirrhosis (20 cases)		HCC (20 cases)		Chi-square test	
	No.	%	No.	%	No.	%	X ²	P-value
High	2	20.0%	7	35.0%	15	75.0%	10.337	0.006*
Low	8	80.0%	13	65.0%	5	25.0%		

In cases of HCC there were high expression was observed in 75% of cases (15/20). Stained cells were distributed diffusely in the tissues and have different locations. Based on the dominant type of

localization, the expression was cytoplasmic in 10 cases, cytoplasmic and nuclear in 6 cases, cytoplasmic, nuclear and membranous in 4 cases (Fig .3).

Figure (3): The subcellular localization of galectin-3 expression in hepatocellular carcinoma. Expression of galectin-3 in tumor tissues was detected in (a) cytoplasm only, (b) cytoplasm and nucleus, (c & d) cytoplasm, nucleus and on the membrane (X100).

The significance of the localization on the prognosis was estimated by comparison of the groups. Only cytoplasmic expression tended to be related to small size, solitary tumors, poorly differentiated, cases of absence of vascular

invasion compared with negative expression, but this relation is not statistically significant. Correlation between galectin-3 localization and prognosis are included in (Table 4).

Table (4): Correlation between Galectin-3 localization and prognosis					
	Cytoplasmic	Cytoplasmic and nuclear	Cytoplasmic, nuclear and membranous	X ²	P value
Tumor size					
5 (12 cases)	6	4	2	0.278	0.870
>5 (8 cases)	4	2	2		
Tumor multiplicity					
Solitary (18)	8	6	4	2.222	0.329
Multiple (2)	2	0	0		
Grade					
Well (4)	1	3	0	3.153	0.532
Moderate (6)	2	1	3		
Poor (10)	7	2	1		
Vascular invasion					
Absent (15)	9	5	1	0.915	0.632
Present (5)	1	1	3		
TNM stage					
I (9)	5	3	1	1.6	0.808
II (5)	3	1	1		
III (6)	2	2	2		

Correlation of Galectin 3 expression of HCC with clinicopathological variables

To verify the biological function of Galectin 3 in HCC, we correlated Galectin 3 expression in 20 HCC samples with widely recognized clinicopathological features (**Table 5**).

High Positive expression of galectin-3 was significantly associated with tumor grade ($P = 0.034$), vascular invasion ($P = 0.037$) and patients with underlying cirrhosis ($P = 0.001$).

As regard the pathological stage, multiplicity, tumors with large size (>5) tended to be associated with the high positive expression ($P =$

0.144, 0.389, 0.292) respectively, but not statistically significant. Galectin 3 expression displayed no association with age, gender and cases with underlying hepatitis ($P > 0.05$).

From 15 cases of HCC complicating cirrhosis, the cirrhotic nodules showed high Positive expression of Galectin-3 in 14 cases. The staining patterns were similar to those seen in cirrhotic livers without HCC but 50% of cases had more than 20% Galectin-3 positive hepatocytes, from which, 5 cases showed diffuse cytoplasmic expression and only one case showed positive expression at the periphery of cirrhotic nodules with nuclear localization (**Fig. 4**).

Table (5): Correlation of Galectin 3 expression in HCC with clinicopathological parameters

Clinical feature	No =20	High		Low		Chi-square test	
		No .	%	No.	%	X ²	P-value
Gender							
Males	8	5	33.3%	3	60.0%	1.111	0.292
Females	12	10	66.6%	2	40.0%		
Age							
45	17	13	86.7%	4	80.0%	0.131	0.718
> 45	3	2	13.3%	1	20.0%		
Size							
5 cm	12	8	53.3%	4	80.0%	1.111	0.292
>5 cm	8	7	46.7%	1	20.0%		
Tumor Multiplicity							
Solitary	18	14	93.3%	4	80.0%	0.741	0.389
Multiple	2	1	6.7%	1	20.0%		
Tumor Differentiation							
Well	4	1	6.7%	3	60.0%	6.756	0.034*
Moderate	6	5	33.3%	1	20.0%		
Poor	10	9	60.0%	1	20.0%		
Pathologic Stage							
I	9	5	33.3%	4	80.0%	3.881	0.144
II	5	4	26.7%	1	20.0%		
III	6	6	40.0%	0	0.0%		

Vascular Invasion							
Absent	15	13	86.7%	2	40.0%	4.365	0.037*
Present	5	2	13.3%	3	60.0%		
Associated Hepatitis							
HBV	7	5	33.3%	2	40.0%		
HCV	9	7	46.7%	2	40.0%	0.085	0.959
Non B nor C	4	3	20.0%	1	20.0%		
Associated Cirrhosis							
Absent	5	1	6.7%	4	80.0%	10.756	0.0018
Present	15	14	93.3%	1	20%		*

Figure (4): Galectin 3 expression in cirrhotic nodules associated HCC; (a) Expression of Galectin 3 were present at the periphery of cirrhotic nodules with nuclear localization (X100). **(b)** Diffuse cytoplasmic expression (X100).

Discussion

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy of adults. It is the sixth most common cancer worldwide and the third most common cause of cancer death (Dai *et al.*, 2014). In Egypt, liver cancer forms 11.75% of the malignancies of all digestive organs and 1.68% of the total malignancies (Mokhtar *et al.*, 2007). HCC represents the main complication of cirrhosis, and shows a growing incidence in Egypt, which may be the result of a shift in the relative importance of hepatitis B virus (HBV) and HCV as primary risk factors (Gomaa *et al.*, 2014).

The liver is unique in its capacity for regeneration following injury, also gives rise to this malignancy commonly associated with the inflammatory state of advanced fibrosis, or

cirrhosis (Newell *et al.*, 2008). Some experiments suggested possible role of Galectin-3 in the promoting inflammation and the development of chronic liver diseases, cirrhosis and HCC (Unal *et al.*, 2015).

In this study galectin-3 was minimally or unexpressed in normal hepatocytes and it was detected in the cytoplasm only and it was found in cholangiocytes and Kupffer cells in all cases. Results were in line with those of Hsu *et al.*, 1999 and Matsuda *et al.*, 2008 who did not find any expression in normal liver except epithelial cells lining the bile duct and Kupffer cells. However stained hepatocytes represented less than 5% of total cells in most of the sections. Galectin-3 was weak and cytoplasmic only. Another study by Jiang *et al.*, 2014 found weak to negative expression of galectin-3 in normal adjacent liver tissue in comparison to tumorous one.

In cirrhotic liver specimens that not associated with HCC, galectin-3 expression was observed in 35 % of cases with cytoplasmic localization except in one case showed nuclear expression. These might be partly explained by the impaired hepatic removal and/or by higher hepatic synthesis of galectin-3 that appears to contribute to tissue protection. Previous studies by *Hsu et al., 1999* and *Jiang et al., 2014* reported that galectin-3 expression was distributed peripherally in cirrhotic nodules with nuclear localization in all evaluated patients.

Further investigation showed that positive galectin-3 expression in cirrhosis is independent of whether the patient had specific type of infection or not (HBV, HCV as well as in cases without serological evidence of both infection) *Hsu et al., (1999)*. Analysis of galectin-3 expression in HCC from patients with or without serological evidence of HBV infection revealed that galectin-3 expression was not strictly correlated with HBV infection (positive galectin-3 expression in 78% of HBV⁺ patients and 71% of HBV⁻ patients). Another study by *Mehmet et al., 2015* found that the serum level of Galectin 3 levels more pronounced in case of HCV, suggest that hepatitis is a basic response to hepatic injury, regardless of the initiating agent or disease process.

In our study there were high expression was observed in (75%) of cases of HCC. Stained cells were distributed diffusely in the tissues. This observation is similar to another demonstrated positive rate of Galectin-3 in the tissue of HCC was 78.2%, dramatically higher than that in para cancerous tissues (*Fang et al., 2011*).

From 15 cases of HCC that associated with cirrhosis, cirrhotic nodules were positive in 14 cases. HCC was significantly associated with cirrhosis ($P = 0.001$). These finding revealed that regenerating nodules in cirrhotic liver expressed galectin 3 is linked to proliferative cell status and the hepatocytes are undergoing rapid proliferation. This is in concordance with previous studies by *Hsu et al., 1999* who reported the regenerating nodules in cirrhotic liver express galectin-3 is worthy of comment. The staining

patterns were similar to those seen in cirrhotic livers without HCC but 60% of cases had more than 20% galectin-3 positive hepatocytes. This is in concordance with previous studies by *Chung et al., 2002* who reported the up-regulation of Galectin-3 in HCC human tissues with respect to their normal counterparts. Moreover, by gene expression analysis, *Luo et al., 2006* also reported the overexpression of galectin-3 gene in HCC tissues respect to cirrhosis and adjacent non-tumoral tissues. Another immunohistochemistry analysis by *Jiang et al., 2014* revealed that galectin-3 was abundantly accumulated in the cytoplasm of Hepatocellular cancer cells.

We analyzed the correlation between expression of galectin-3 and clinicopathological parameters and found statistically significant high Positive expression of galectin-3 associated with vascular invasion ($P = 0.037$). This may be explained by *Jiang et al., 2014* study who demonstrates a positive correlation between galectin-3 protein and Micro Vessel Density (MVD). Proliferation of blood vessels may provide nutrients and pathways for tumor cells and improve its ability of invasion and metastasis, thus affecting the biological behavior of Hepatocellular Carcinoma.

High Positive expression of galectin-3 also associated with the pathological differentiation ($P = 0.034$). These results were in agreement with *Matsuda et al., 2008* and *Bacigalupo et al., 2013* who identified significant correlation between expression of galectin-3 and histological differentiation and vascular invasion in HCC patients in spite of the varying localizations, which have different functions. However, *Bacigalupo et al., 2013* found no apparent relationship between galectin-3 expression and other clinicopathological parameters; including differentiation status (both $P > 0.05$). The reasons for these differences may include different genetic backgrounds and a larger study cohort.

The pathological tumor stage, large size tumor tended to be associated with the positive expression, but not statistically significant ($P = 0.144, 0.292$) respectively. Another multivariate analysis by *Jiang et al., 2014*, observed that galectin-3 expression, together with some

traditional prognostic factors (tumor size, histologic grade) were independent risk factors in the prognosis of HCC patients. Similar findings have been reported in other malignancies (*Prieto et al., 2006 & Cheng et al., 2013*).

The expression of Galectin 3 in HCC displayed no association gender, age and underlying cases of hepatitis ($P > 0.05$). The positive galectin-3 expression in HCC is not strictly correlated with certain type of infection, where in HBV patients (33.3%), HCV (46.7%) patients as well as in cases without serological evidence of both infection (20%). This data suggests that factors other than viral hepatitis infection can result in galectin-3 expression. This was similar to *Mehmet et al., (2015)*, who found that galectin-3 expression in HCC is independent of whether the patient had prior HBV infection or not.

In the current study regarding the dominant type of localization, cytoplasmic expression tended to be related to large size, solitary tumors, poorly differentiated, absent of vascular invasion compared with negative expression, but not significant. This closely agreed with *Matsuda et al., 2008* and *Jiang et al., 2014* studies. *Matsuda et al., 2008* found that all types of localized expression tended to be related to a worse prognosis compared with negative expression in particular, nuclear localization of galectin-3 expression was related to a poor prognosis but *Califice et al., 2004* report that cytoplasmic over-expression of galectin-3 in the of prostatic cancer can promote its anti-apoptotic activity as well as increase cell proliferation, tumor growth, invasion, and angiogenesis while Galectin-3 expression in the nucleus decreases cell proliferation. This means that the effect of the localization of galectin-3 on tumor progression is dependent on the cell type.

Collectively these results suggest that high expression of Galectin-3 in cirrhosis may be guiding us about tumor progression and related to the prognosis of HCC. Galectin-3 expression independent of the presence of specific type of viral infection. Different subcellular localization of Galectin-3 in HCC suggests different biological roles for galectin-3, that depend on its

localization and our observations suggested that Galectin-3 could be a novel tumor marker and therapeutic strategies to treat a broad range of inflammatory diseases.

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