Serum Galectin-3 Levels in Children with Chronic Hepatitis B Infection and Inactive Hepatitis B Carriers

Authors' Contribution:
Study Design **A**
Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
Manuscript Preparation **E**
Literature Search **F**
Funds Collection **G**

ACD **Ünal Uluca**
BDF **Velat Şen**
CE **Aydın Ece**
BD **İlhan Tan**
EF **Duran Karabel**
AE **Fesih Aktar**
DE **Müsemma Karabel**
EF **Hasan Balık**
AF **Ali Güneş**

Department of Pediatrics, Dicle University, Medical School, Diyarbakır, Turkey

Corresponding Author: Ünal Uluca, e-mail: ulucaunal@hotmail.com
Source of support: Departmental sources

Background: Chronic hepatitis B virus (HBV) infection is common worldwide. Follow-up of patients by the use of non-invasive techniques may be valuable in clinical practice. The aim of this study was to investigate serum galectin-3 (GAL-3) levels for monitoring disease status in children with chronic HBV infection.

Material/Methods: Thirty-two patients with chronic hepatitis B (CHB), 30 inactive HBV carrier patients, and 30 matched healthy controls were enrolled in the study. We performed basic laboratory tests: serum glucose, albumin, alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyl transferase (GGT), total bilirubin, prothrombin time, and activated partial thromboplastin time. In addition, serum GAL-3 levels were measured by ELISA technique.

Results: Significantly higher serum GAL-3 levels (16.5 ± 3.6, 1.1 ± 0.3, 0.7 ± 0.5 ng/ml, respectively, $p < 0.001$) and ALT levels (80.2 ± 30.6, 26.8 ± 12.6, 28.1 ± 4.4 IU/L, respectively, $p < 0.001$) were found in the CHB group compared with the inactive carriers and the control groups. There were no significant differences in ALT levels and GAL-3 levels or between inactive HBV carriers and the control groups ($p > 0.05$, for each). Significantly higher GGT levels were found in the CHB group (51.3 ± 27.5 IU/L) compared with the inactive HBV carriers (35.7 ± 10.1 IU/L) and the control group (31.3 ± 9.5 IU/L) ($p < 0.001$, and $p = 0.004$, respectively). A significant correlation was found between GAL-3 and ALT levels in the CHB group ($r = 0.82$, $p < 0.001$).

Conclusions: Our results suggest that serum GAL-3 level may be a beneficial indicator of chronicity in hepatitis B infection in children.

MeSH Keywords: **Child • Galectin 3 • Hepatitis B, Chronic**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/894035

📄 1934 🗂 1 📊 1 📚 19

Background

Chronic hepatitis B virus (HBV) infection is one of the most common and serious infections worldwide. According to the World Health Organization, approximately two billion people have been infected with HBV and more than 240 million of them suffer from chronic HBV infection. Chronic HBV infection may be complicated by cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease [1–3]. All children with HBV infection should be monitored by physicians due to the risk of progression to chronicity, cirrhosis, and HCC. Progression to chronicity and development of cirrhosis or hepatocellular carcinoma can be detected by invasive methods such as liver biopsy. Therefore, monitoring these patients with non-invasive techniques has considerable clinical importance [4].

Galectins belong to a family of proteins that bind terminal galactose residues on macromolecules. Galectin-3 (GAL-3) is a 30 kDa protein that is important in many activities of malignant behaviors, including cancer cells and development of fibrosis [5,6]. GAL-3 may be important in the pathogenesis of liver fibrosis, as the previous literature suggest that the lack of GAL-3 may be associated with resistance to liver fibrosis development [6]. Recently, some experimental disease models suggested that GAL-3 was associated with the development of chronic liver disease secondary to inflammatory or toxic insults. Moreover, GAL-3 may be a potential target for treatment modalities [6].

GAL-3 can stimulate the production of some cytokines and chemokines via CD98 through interactions with macrophages. GAL-3 may play an important role in sustaining of HBV replication and may contribute to pathologic processes that resulted in chronic status in HBV infection. GAL-3 may stimulate fibrogenesis by decreasing IL-10 production. [7,8]. Based on these data GAL-3 was found to be crucial for promoting inflammation that may contribute in the progression of chronic infection and development of liver fibrosis [9]. Thus, GAL-3 may be useful for monitoring the chronicity in different phases of HBV infection. To our knowledge, there is no study regarding GAL-3 levels in children with CHB.

In this study, we investigated serum GAL-3 levels in children with CHB compared to inactive HBV carrier patients to help us understand whether GAL-3 levels may be beneficial for discrimination and monitoring of HBV chronicity.

Material and Methods

Study population

Thirty-two patients with chronic hepatitis B (CHB) with no antiviral treatment and 30 inactive HBV carrier children with hepatitis B were followed up between July 2013 and December 2013. Thirty age and gender matched healthy subjects were recruited as the control group. Patients with chronic hepatitis B were defined by the following criteria: 1) positive serum hepatitis B surface antigen (HBsAg) lasting at least 6 months prior to obtaining of blood samples; and 2) for hepatitis B e antigen (HBeAg) positive patients [serum HBV DNA level ≥20 000 IU/ml and serum ALT level ≥1.5×upper limit of normal values (ULN); for HBeAg negative patients, serum HBV DNA ≥2000 IU/ml and serum ALT ≥1.5×ULN]. Inactive HBV carrier patients were defined by the following criteria: 1) positive serum HBsAg from at least 6 months prior to obtaining of blood samples; 2) serum HBV DNA level <2000 IU/ml and serum ALT levels <ULN; and 3) negative serum HBeAg [10]. Control subjects were recruited from children who applied for routine check-up or elective surgery such as hernia repair or circumcision. Control subjects had no abnormal liver function or hepatitis B tests, and no history or findings of any systemic disease. The basic laboratory tests of all subjects including serum glucose, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin (TBIL), prothrombin time (PT) and activated partial thromboplastin time (aPTT) were recorded.

Patients that were under 2 years or older than 18 years of age were excluded from the study. Patients that received hepatotoxic drugs or had other causes of acute or chronic hepatitis (Wilson disease, autoimmune hepatitis, α1-antitrypsin deficiency, metabolic disease and co-infection or superinfection of hepatitis D virus infection and hepatitis C infection) were also excluded.

Informed consent was obtained from parents or legal guardians of the subjects and the study protocol was approved by the local ethics committee.

Biochemical analyzes

Serum glucose, albumin, ALT, AST, GGT, TBIL, PT, and aPTT were measured by enzymatic colorimetric method using an Abbot ARCHITECT C16000 (Illinois, United States). Basic laboratory tests were performed in the central biochemical laboratory of our hospital.

Measurement of serum GAL-3

Serum GAL-3 levels were measured by using a commercial quantitative enzyme-linked immune sorbent assay (ELISA)

Table 1. Demographic features and serum biochemical results of the study groups (mean ± standard deviation).

	CHB group (n=32)	Inactive Carriers (n=30)	Control group (n=30)	p
Age (years)	11.7±3.0	12.3±2.5	11.7±2.7	NS[†]
Gender (M/F)	19/13	16/14	14/16	NS[†]
BMI	18.5±3.1	19.4±4.3	18.6±3.1	NS[†]
Serum galectin-3 (ng/ml)	16.5±3.6	1.1±0.3	0.7±0.5	<0.0001[†], <0.0001[‡], <0.0001[§], <0.0001[*]
ALT (IU/L)	80.2±30.6	26.8±12.6	28.1±4.4	<0.0001[†], 0.001[‡], <0.0001[§], NS[*]
Albumin (g/d L)	3.9±2.7	4.0±0.3	4.0±0.3	NS[†]
Glucose (mg/dL)	93.5±12.0	93.5±10.6	96.2±12.8	NS[†]
GGT (IU/L)	51.3±27.5	35.7±10.1	31.3±9.5	<0.0001[†], 0.005[‡], <0.0001[§], NS[*]
PT INR	1.1±0.06	1.1±0.09	1.07±0.07	NS[†]
aPTT (IU/L)	28.3±3.0	31.2±7.6	30.1±8.1	NS[†]

CHB – chronic hepatitis B; ALT – alanine aminotransferase; GGT – gama glutamyl transferase; aPTT – activated partial thromboplastin time; PT – prothrombin time; INR – International normalized ratio; NS – not significant. Differences, [†] between three groups; [‡] between CHB group and inactive carriers; [§] between CHB group and the control group; [*] between inactive carriers and the control group.

(Eastbiopharm Company, Hangzhou, China) according to the manufacturer's instructions. Serum GAL-3 levels were shown as ng/ml. The lowest mean detection limit for GAL-3 level was 0.015 ng/ml.

Statistical analysis

All data were evaluated by using SPSS (Statistical Package for Social Sciences) 16.0 program for Windows. Data were shown as the mean ± standard deviation. Categorical variables were given as counts and percentages. Kolmogorov-Smirnov test was used to examine distribution pattern of the data. Analyses of variance test followed by post hoc Scheffe test was used to compare three independent groups. Pearson's correlation analysis was used to check correlations between numerical data. A p-value of less than 0.05 was accepted as statistically significant.

Results

The study group included 32 CHB patients (19 Male /13 Female), 30 inactive HBV carrier patients (16 Male / 14 Female) and 30 healthy children (16 Male /14 Female). The age was 11.7±3.0 years in the CHB group, 12.3±2.5 years in the inactive HBV carrier group, and 11.7±2.7 years in the control group. Demographic characteristics including age, gender, body mass index, and laboratory test results of CHB, inactive HBV carrier, and the control groups are summarized in Table 1. Significantly
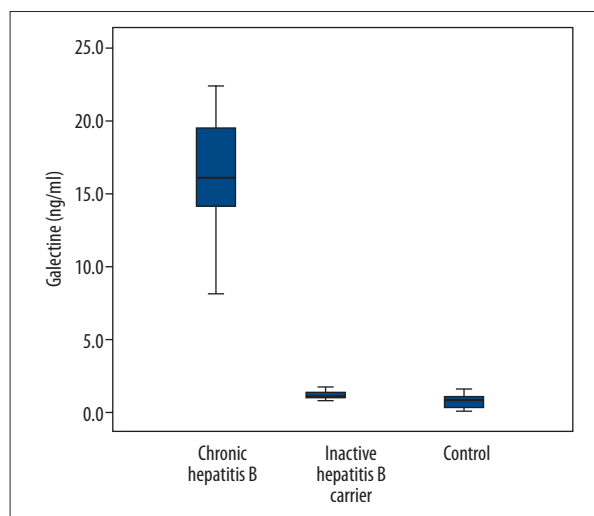

Figure 1. Comparison of serum galectin-3 levels among chronic hepatitis B, inactive hepatitis B carriers and the control groups

higher serum GAL-3 levels were found in the CHB group compared with HBV carrier and the control groups (16.5±3.6 ng/ml, 1.1±0.3 ng/ml, 0.7±0.5 ng/ml, respectively, p<0.001) (Figure 1) and no significant difference was found in the GAL-3 levels of inactive HBV carrier and the control groups (p>0.05). Similarly, serum ALT levels (80.2±30.6, 26.8±12.6, 28.1±4.4 IU/L, respectively, p<0.001) were found to be significantly higher in the CHB group than other groups (p<0.001) and no significant difference was found in the ALT levels of inactive HBV carrier

and the control groups (p>0.05). CHB group had significantly higher GGT levels (51.3±27.5 IU/L) compared with the inactive HBV carriers (35.7±10.1 IU/L) and the control group (31.3±9.5 IU/L) (p<0.001, and p=0.004, respectively). No significant differences were found in aPTT, INR, and serum albumin and glucose levels between the three groups (p>0.05) (Table 1). There was a positive correlation between GAL-3 and serum ALT levels in the CHB group (r=0.82, p<0.001). However, no correlations of GAL-3 were found with serum albumin, glucose, GGT, aPTT, and INR values in the CHB, inactive HBV carrier, or the control groups (p>0.05 for each).

Discussion

In this study, we analyzed GAL-3 levels in different phases of HBV infections and healthy subjects by ELISA to investigate the possible benefit of GAL-3 as a diagnostic marker for chronicity. The results of the present study indicated that, in patients with serologically-proven CHB, serum levels of GAL-3 levels were significantly higher than those of inactive HBV carrier and healthy controls. In addition, GAL-3 levels were associated with ALT levels.

CHB infection is a common infection worldwide and has some important clinical complications such as cirrhosis and HCC. Treatment decision is crucial for preventing cirrhosis and HCC. Treatment decision is based on ALT levels, HBeAg positivity, HBV DNA level, and liver histology. Family history of HCC, co-existing liver diseases, and patient's treatment history were also important points for treatment decision [10,11].

Inteleukin-10 (IL-10) is a cytokine which is produced by T helper type 2 cells. IL-10 has effect on inhibition of pro-inflammation cytokines, regulation of humoral immunity, and termination of inflammation [7]. An experimental autoimmune encephalomyelitis model demonstrated that Gal-3 plays an important role in decreasing IL-10 production [8]. Decreasing IL-10 may contribute to the sustaining of HBV replication and may initiate chronic HBV infection, which may direct fibrogenesis via inhibition of IFN-γ secretion [7]. Although we did not measure IL-10 in our patients, GAL-3 may contribute to chronicity of HBV infection by decreasing IL-10. Measurement of IL-10 together with GAL-3 in children with hepatitis B may be a subject of further studies.

Histologic changes of liver can be shown by liver biopsy, which is an invasive technique. In addition to being an invasive method, liver biopsy is a painful process and accuracy of biopsy depends on suitability of biopsy specimen and experience of pathologist. Although, some non-invasive techniques such as FibroScan have been suggested in order to predict to chronicity and fibrosis status of chronic CHB infection, these

methods are still not validated [4,10,12]. In a multicenter study, FibroScan was shown to be useful for determining diagnostic accuracy for cirrhosis, but not significant for fibrosis [13]. Therefore, further non-invasive methods are needed to predict entering chronic phase or development of complications with avoidance of liver biopsy.

Because of possible role of GAL-3 in the promoting of inflammation and leading to chronic hepatitis, it can be accepted as a useful biomarker indicating liver inflammation and chronic phase of CHB as a non-invasive technique. To the best of our knowledge, the presented study is the first that investigates association between CHB infection and serum GAL-3 levels in childhood.

GAL-3 has a carbohydrate recognition domain which is activated by the ligand interaction and has powerful affinity for β-galactosides. GAL-3 may contribute some cellular processing which may result in fibrosis development, cancer progression, and tissue remodeling [14]. Moreover, galectins are expressed in immune cells such as activated macrophages, mast cells, and eosinophils. That's why they may have acute and chronic immunologic effects such as production of some cytokines and mediators as well as apoptosis and chemoattraction [15]. In a previous experimental study, lower collagen deposition was shown in the Galectin-3$^{-/-}$ mice. Thus, inhibition of galectin may be used as therapeutic target in inflammatory diseases [15,16].

The severity of the liver damage during the CHB infections was assessed by some biochemical markers including ALT, AST, GGT, alkaline phosphatase, bilirubin, serum albumin, and globulin levels [7]. In present study, ALT levels, which reflect ongoing liver damage, were found to associate with GAL-3 levels in CHB group. Thus, GAL-3 levels may reflect disease activity in CHB patients. Similarly we found that higher GGT levels in CHB group. Higher GGT activities have also been reported in cholestatic liver disease, and other hepatic disorders related to the biliary tract. In addition, previous studies have suggested that increased levels of serum GGT indicated advanced liver fibrosis [17,18]. The results of our study showed no significant positive correlation between serum GGT and GAL-3 level. Probably, our finding related to lack of correlation between GGT and GAL-3 levels may be a reflection of hepatocyte injury due to CHB infection rather than cholestatic hepatitis in our patient group. This point also needed to be explained by further studies.

The pathogenesis of chronic HBV infection and how HBV infection maintains its chronic persistent infection is not fully understood [19]. GAL-3 may contribute to the understanding of the pathogenesis of chronic HBV infection. We believe that our results may be evidence for the role of GAL-3 in both

the inflammatory process and the development of chronicity in CHB infections.

The main limitation of this study was the absence of a liver biopsy. Serum IL-10 levels of our study population were unavailable which might increase the value of the results.

Conclusions

The present study demonstrated that GAL-3 may be beneficial for predicting chronicity in childhood chronic HBV infection. Understanding the role of GAL-3 in HBV infections may contribute to the explanation of the pathogenesis of CHB infections. Further investigates are needed to explain the precise mechanism(s) of GAL-3 in CHB infection.

References:

1. Della Corte C, Nobili V, Comparcola D et al: Management of chronic hepatitis B in children: an unresolved issue. J Gastroenterol Hepatol, 2014; 29: 912–19

2. Doo EC, Hoofnagle JH, Rodgers GP: NIH consensus development conference: management of Hepatitis B. Introduction. Hepatology, 2009; 49: S1–3

3. Kibar F, Goruroglu Ozturk O et al: Role of KIR genes and genotypes in susceptibility to or protection against hepatitis B virus infection in a Turkish cohort. Med Sci Monit, 2014; 20: 28–34

4. En V, Uluca U, Ece AN et al: Serum prolidase activity and oxidant inverted question markantioxidant status in children with chronic hepatitis B virus infection. Ital J Pediatr, 2014; 40: 95

5. Song L, Mao J, Zhang J et al: Annexin A7 and its binding protein galectin-3 influence mouse hepatocellular carcinoma cell line *in vitro*. Biomed Pharmacother, 2014; 68: 377–84

6. Traber PG, Chou H, Zomer E et al: Regression of fibrosis and reversal of cirrhosis in rats by galectin inhibitors in thioacetamide-induced liver disease. PLoS One, 2013; 8: e75361

7. Li X, Liu X, Tian L, Chen Y: Cytokine-mediated immunopathogenesis of hepatitis B virus infections. Clin Rev Allergy Immunol, 2014 [Epub ahead of print]

8. Jiang HR, Al Rasebi Z, Mensah-Brown E et al: Galectin-3 deficiency reduces the severity of experimental autoimmune encephalomyelitis. J Immunol, 2009; 182: 1167–73

9. Dragomir AC, Sun R, Mishin V et al: Role of galectin-3 in acetaminophen-induced hepatotoxicity and inflammatory mediator production. Toxicol Sci, 2012; 127: 609–19

10. Sokal EM, Paganelli M, Wirth S et al: Management of chronic hepatitis B in childhood: ESPGHAN clinical practice guidelines: consensus of an expert panel on behalf of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. J Hepatol, 2013; 59: 814–29

11. Turhan O, Coban E, Inan D, Yalcin AN: Increased mean platelet volume in chronic hepatitis B patients with inactive disease. Med Sci Monit, 2010; 16: CR202–5

12. European Association For The Study Of The L. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol, 2012; 57: 167–85

13. Bessone F: Re-appraisal of old and new diagnostic tools in the current management of chronic hepatitis B. Liver Int, 2014; 34: 991–1000

14. de Boer RA, van Veldhuisen DJ, Gansevoort RT et al: The fibrosis marker galectin-3 and outcome in the general population. J Intern Med, 2012; 272: 55–64

15. Liu FT, Rabinovich GA: Galectins: regulators of acute and chronic inflammation. Ann NY Acad Sci, 2010; 1183: 158–82

16. Henderson NC, Mackinnon AC, Farnworth SL et al: Galectin-3 regulates myofibroblast activation and hepatic fibrosis. Proc Natl Acad Sci USA, 2006; 103: 5060–65

17. Huang CF, Yeh ML, Tsai PC et al: Baseline gamma-glutamyl transferase levels strongly correlate with hepatocellular carcinoma development in non-cirrhotic patients with successful hepatitis C virus eradication. J Hepatol, 2014; 61: 67–74

18. Everhart JE, Wright EC: Association of gamma-glutamyl transferase (GGT) activity with treatment and clinical outcomes in chronic hepatitis C (HCV). Hepatology, 2013; 57: 1725–33

19. Shi YY, Dai MJ, Wu GP et al: Levels of interleukin-35 and its relationship with regulatory T-cells in chronic hepatitis B patients. Viral Immunol, 2015; 28: 93–100