

Received: 2014.09.03
Accepted: 2014.11.19
Published: 2015.03.13

Serum Galectin-3 as a Potential Marker for Gastric Cancer

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

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Source of support: This work was supported by a Grant from the National Natural Science Foundation of China (No. 81301835)

Background: The identification of cancer biomarkers can advance the possibility for early detection and better monitoring of tumor progression. The aim of this study was to assess the diagnostic and prognostic value of serum galectin-3 (Gal-3) in patients with gastric cancer (GC).





Material/Methods: We measured serum Gal-3 levels using ELISA method in 87 patients with GC, 53 patients with benign gastric lesions, and 51 healthy controls.

Results: Serum levels of Gal-3 in patients with GC were significantly higher than those in benign disease patients and healthy controls ($P < 0.001$), but no difference was found between benign disease patients and healthy controls ($P = 0.635$). Additionally, serum Gal-3 level was associated with lymph node metastasis ($P = 0.001$) and distant metastasis ($P < 0.001$), whereas it was not related to gender ($P = 0.204$), age ($P = 0.269$), tumor size ($P = 0.399$), location ($P = 0.715$), TNM stage ($P = 0.385$), differentiation ($P = 0.135$), or invasion depth ($P = 0.273$). The Kaplan-Meier survival analysis revealed that overall survival rates in patients with high Gal-3 levels were not significantly different than those with low Gal-3 levels ($P = 0.099$).

Conclusions: Results of the current study suggests that serum Gal-3 represents a potential diagnostic marker for patients with GC, and may be an adjunct to determine the individual prognosis of these patients.

MeSH Keywords: **Diagnosis • Galectin 3 • Serum • Stomach Neoplasms**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/892386>

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Background

Gastric cancer (GC) is the fourth-most common cancer and the second-most common cause of cancer-related death worldwide, especially in many Asian countries, such as China, Japan, and Korea [1–3]. In 2008, there were approximately 980 000 new cases of GC and 738 000 deaths worldwide [4]. Because a more favorable outcome of GC is closely related to how early the disease is diagnosed and subjected to proper treatment, efficient diagnostic methods, and estimation of prognosis, and effective therapeutic strategy are urgently needed in GC medical process. However, on the basis of current evidence, there are few reliable biomarkers for the diagnosis of GC [5]. Thus, development of new biomarkers for early detection and evaluation of prognosis for GC is of considerable clinical importance.

The galectins are an ancient family of carbohydrate-binding proteins with an affinity for β -galactosides [6], sharing a conserved carbohydrate recognition domain (CRD) of about 130 amino acids [7,8]. Galectin-3, a member of the galectin family, is localized not only in intracellular space such as cytoplasm or the nucleus, but also in extracellular space such as the cell surface or the extracellular matrix [9,10]. Accumulating experimental and clinical evidence have demonstrated that Gal-3 participates in several biological processes: cell migration [11], cell adhesion [12], cell activation [13], cell growth and differentiation [14], angiogenesis [15], and apoptosis [16]. Moreover, Gal-3 is often overexpressed in various human tumors, including colon [17], prostate [18], thyroid [19], and breast cancer [20], and its altered expression correlates with the stage of tumor progression [21]. Therefore, several attempts to use serum levels of Gal-3 as a diagnostic indicator are under development. In many malignant tumors the significance of serum Gal-3 has been evaluated and some investigators have examined the efficacy of Gal-3 as a diagnostic marker, such as colorectal cancer [22], lung cancer [23], pancreatic carcinoma [24], bladder cancer [10], thyroid cancer [25], prostate cancer [26], and head and neck squamous cell carcinoma [27].

To date, numerous studies demonstrated that Gal-3 expression is altered in GC. Gal-3 expression was observed significantly to be stronger in metastatic lymph nodes than in the primary GC [21]. Gal-3 expression was also reported to show a slight increase in primary gastric adenocarcinomas compared to normal tissues [28]. Conversely, Gal-3 protein expression was at least 1.5-fold reduced in 50% of gastric tumors [29]. In addition, reduced Gal-3 expression was reported to be correlated with nodal status, lymphatic invasion, pathological stage, and histological parameters [30]. Therefore, neither the expression profile nor the clinical significance of Gal-3 has been elucidated in GC thus far.

In this study, we aimed to analyze the serum Gal-3 levels in GC patients, investigated the associations between serum

Gal-3 levels and clinicopathological parameters, and evaluated the diagnostic and prognostic value of serum Gal-3 for GC.

Material and Methods

Patients

Between September, 2005 and June, 2008, a total of 87 histologically confirmed primary GC patients were recruited from the No. 202 Hospital in Shenyang, China. The group was composed of 45 men and 42 women with a mean age of 55.2 ± 13.5 years (range 29–78 years). None of patients had received chemotherapy or radiotherapy prior to surgery. The clinical characteristics of GC patients are presented in Table 1. In addition, 53 patients with benign gastric lesions at the Gastroenterology Endoscopy Center and 51 healthy individuals at the Health Examination Center were enrolled. The 53 patients with benign gastric lesions included 32 males and 21 females with a mean age of 53.7 ± 11.8 years (range 27–69 years). Of 53 patients with benign gastric lesions, 26 had chronic gastritis, 16 had benign gastric ulcers, and 11 had hyperplastic polyps. The 50 healthy individuals included 29 males and 21 females with a mean age of 49.6 ± 15.3 years (range 26–67 years), and all healthy individuals had no personal/family history of GC. This study was reviewed and approved by the Ethics Committee of No. 202 Hospital. The written informed consent was obtained from every subject or a legally authorized representative before blood sampling.

Follow-up

After surgery, patients were followed up every 3 months for the first 3 years and thereafter every 6 months for the fourth and fifth years. Overall survival (OS) was defined as from surgery to death or last follow-up. Clinical follow-up was performed by telephone visit and questionnaire letters.

Assay for serum Gal-3

After overnight fasting, preoperative blood samples were obtained by venous puncture, centrifuged at 2000 g for 10 min, and stored at -80°C until use. Serum Gal-3 levels were measured by ELISA method using specific kits, according to the manufacturer's instructions (Bender MedSystems, Austria).

Statistical analysis

The statistical analysis was performed using SPSS software (version 15.0; Chicago, IL, USA). The data are presented as mean \pm standard deviation (SD). Comparisons between 2 groups were performed using the Mann-Whitney test. In case of multiple groups, Kruskal-Wallis tests were used, with post hoc comparisons according to Mann-Whitney method. Receiver operating

Table 1. Serum Gal-3 levels in gastric cancer patients according to clinicopathological characteristics.

	Numbers	%	Gal-3 (ng/ml)	P value
Gender				
Male	45	51.7	17.36±6.71	0.204
Female	42	48.3	19.35±7.74	
Age (years)				
≤60	51	58.6	19.048±.11	0.269
>60	36	41.4	17.29±5.76	
Tumor size				
≤5.0 cm	52	59.8	17.78±7.46	0.399
>5.0 cm	35	40.2	19.12±6.95	
Location				
Upper	44	50.6	18.04±7.45	0.715
Middle/lower	43	49.4	18.61±7.12	
TNM stage				
I+II	46	52.9	17.68±7.41	0.385
III+IV	41	47.1	19.04±7.08	
Differentiation				
Well	28	32.2	16.29±5.73	0.135
Moderate	35	40.2	18.60±7.00	
Poor	24	27.6	20.28±8.74	
Invasion depth				
T1+T2	38	43.7	17.35±7.06	0.273
T3+T4	49	56.3	19.07±7.37	
LN metastasis				
Negative	55	63.2	16.42±6.27	0.001
Positive	32	36.8	21.58±7.74	
Distant metastasis				
Negative	67	77.0	16.14±5.36	<0.001
Positive	20	23.0	25.63±8.08	

characteristic (ROC) curves were constructed to determine the sensitivity and specificity of serum Gal-3 at optimal cut-off points in discriminating GC patients from patients with benign gastric lesions and healthy controls. Survival curves were estimated using the Kaplan-Meier method and compared by the log-rank test. In all analyses, *P*-values of less than 0.05 were considered to be statistically significant.

Results

The serum levels of Gal-3 before surgery were 18.32±7.25 ng/ml in patients with GC patients, 10.04±3.47 ng/ml in patients

with benign gastric lesions, and 10.56±3.63 ng/ml in healthy controls. Serum levels of Gal-3 in patients with GC were significantly higher than those in benign disease patients and those in healthy controls (*P*<0.001), but no difference was found between benign disease patients and healthy controls (*P*=0.635) (Figure 1).

Comparisons of serum levels of Gal-3 according to the clinicopathological factors are shown in Table 1. Serum Gal-3 levels were significantly correlated with lymph node metastasis (*P*=0.001) and distant metastasis (*P*<0.001). However, serum Gal-3 levels were not related to gender (*P*=0.204), age (*P*=0.269), tumor size (*P*=0.399), location (*P*=0.715), TNM stage (*P*=0.385),

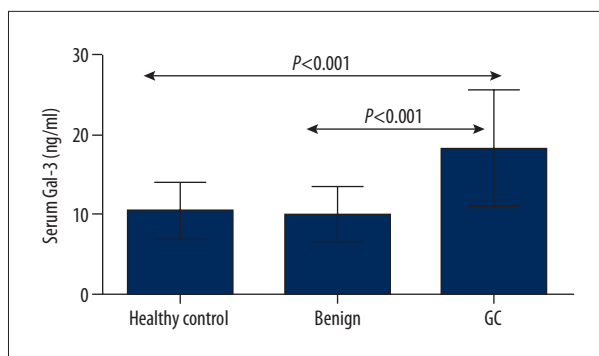


Figure 1. Comparison of serum Gal-3 levels in GC, benign gastric lesions, and healthy controls.

differentiation ($P=0.135$), or invasion depth ($P=0.273$). These results indicate that serum Gal-3 is related to GC metastasis.

The diagnostic performance of serum Gal-3 was evaluated using ROC analysis. When using benign disease patients and healthy controls as the comparison group for all cases, the area under the curve (AUC) values for serum Gal-3 were 0.843 (95% CI: 0.786–0.901) (Figure 2). The cutoff value for Gal-3, as calculated by ROC, was 13.30 ng/ml. The sensitivity and specificity of serum Gal-3 for GC were 77.0% and 82.7%, respectively.

Based on the median of serum Gal-3 (Gal-3: 16.40 ng/ml), we performed Kaplan-Meier analysis to estimate patient overall survival. During the follow-up period, 5 patients were lost and 82 patients were identified and finally included. The followed-up rate was 94.3%. The 5-year overall survival rate was 37.7% in the high Gal-3 level group and 46.3% in the low Gal-3 level group. The Kaplan-Meier survival analysis revealed that OS with high Gal-3 levels were not significantly different from those with low Gal-3 levels ($P = 0.099$, Figure 3).

Discussion

Although therapy is currently relatively effective in controlling early-stage GC, mortality is usually caused by recurrence and metastasis. Therefore, the identification of reliable biomarkers is critical for early diagnosis and prognostic evaluation, and for finding therapeutic molecular targets of GC. In the present study, we evaluated the serum levels of Gal-3 in patients with GC. Our study demonstrated that Gal-3 was involved in the progression of GC, and it could be a potential biomarker in GC.

As a multifunctional protein widely expressed by many types of human cells, Gal-3 overexpression and changes of sub- and inter-cellular localization are commonly seen in diverse types of cancer [31]. Notably, significantly increased serum levels of circulating Gal-3 have been observed in cancer patients, including those with colorectal cancer [22], lung cancer [23],

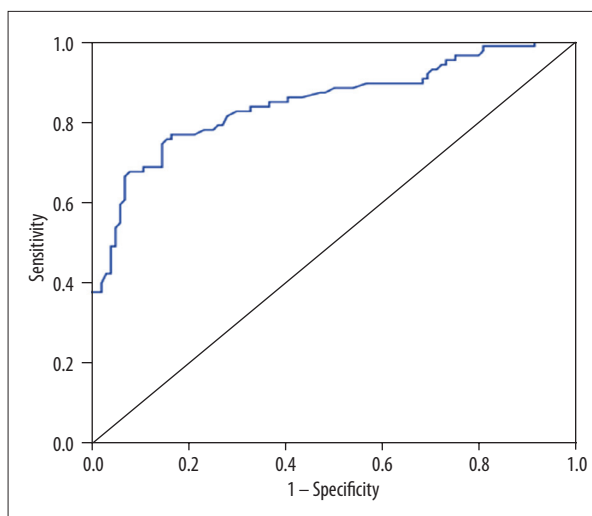


Figure 2. ROC analysis of serum Gal-3 levels for gastric cancer.

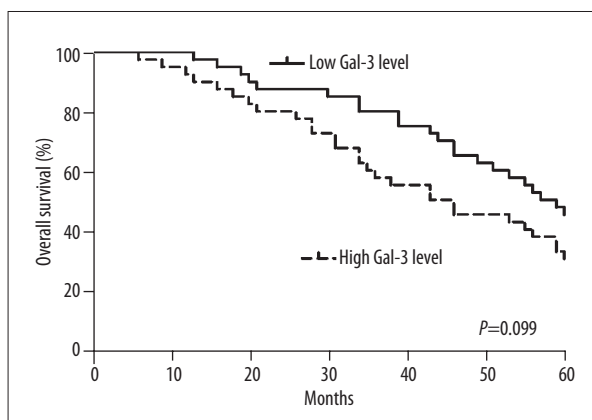


Figure 3. Kaplan-Meier survival curve of GC patients stratified by Gal-3 level.

pancreatic carcinoma [24], bladder cancer [10], thyroid cancer [25], prostate cancer [26], and head and neck squamous cell carcinoma [27]. However, the role of Gal-3 in human gastric carcinogenesis is still ambiguous. A previous study showed that positive galectin-3 expression was observed in 84% of gastric cancer cases [21]. Moreover, malignant gastric tissues expressed higher levels of Gal-3 than normal gastric tissues [32]. However, reduced Gal-3 expression has been found in GC tissue, and mainly detected in the cytoplasm and nuclei of the GC cells, while Gal-3 expression was mainly detected in the nuclei of the normal epithelial cell [30]. Therefore, we speculate that Gal-3 may be involved in the progression of GC, and then determined the serum Gal-3 levels in different subjects. In the present study, serum Gal-3 levels in GC patients were significantly higher than those in patients with benign gastric lesions and in healthy controls. Furthermore, at a cutoff point of 13.30 ng/ml, serum Gal-3 had a sensitivity of 77.0% and a specificity of 82.7% for the prediction of GC, suggesting that serum Gal-3 might be as an effective diagnostic marker.

To explore the important role of Gal-3 in the progression of GC, we analyzed the relationship between serum Gal-3 levels and clinicopathological factors of GC. Our results indicated that serum Gal-3 level was associated with lymph node metastasis and distant metastasis. Recently, several studies have reported Galectin-3 shows pleiotropic biological functions in cell growth, apoptosis induction, and tumor progression. Gal-3 on the cell surface downregulates cellular adhesion to the extracellular matrix proteins by interacting with integrins $\alpha 1\beta 1$ [33]. A previous study demonstrated that silencing of Gal-3 enhanced apoptosis induction with chemotherapeutic agents by reducing the expression of anti-apoptotic and/or cell survival molecules such as survivin, cyclin D1, and XIAP, and increasing the expression of pro-apoptotic XAF-1 [34]. Also, the increased circulation of Gal-3 in patients with cancer induces secretion of several metastasis-promoting cytokines from the blood vascular endothelium that enhances endothelial cell activities in metastasis [35]. Moreover, angiogenesis in tumors is a complex process and plays an important role in the progression of the disease and in the metastatic process [36]. Galectin-3 modulates VEGF- and bFGF-mediated angiogenesis by binding to integrin $\alpha v\beta 3$, and subsequently activating the signaling pathways that promote the growth of new blood vessels [37]. Additionally, Gal-3 promotes GC metastasis through up-regulation of fascin-1, an actin-binding protein [32]. Taken together, GC progression may be facilitated by changing the expression pattern of Gal-3, as this can modify the ability of cancer cells to penetrate into the extracellular matrix, to enter into blood vessels, and to generate secondary sites.

To further evaluate the role of serum Gal-3 levels in the prognosis of GC patients, we investigated the relationship between

serum Gal-3 level and overall survival rate of GC patients. Although serum Gal-3 was not identified as a significant prognostic factor, it had a tendency to be associated with poor prognosis. However, larger prospective studies are warranted to validate the diagnostic and prognostic value of serum Gal-3 level in GC patients.

Conclusions

In conclusion, we presented evidence that serum Gal-3 level was significantly elevated in GC patients, and serum Gal-3 level was significantly correlated with lymph node metastasis and distant metastasis. Due to its reasonable sensitivity and specificity for GC, Gal-3 could be useful as a potential circulating biomarker for GC diagnosis. In addition, Gal-3 shows a promising tendency as a prognostic marker in GC patients. Further studies should be conducted on larger cohorts to investigate correlations between Gal-3 and relevant clinicopathological parameters in defined subpopulations of patients.

Acknowledgments

We gratefully acknowledge the clinical data provided by the Department of Clinical Laboratory Medicine (No. 202 Hospital).

Conflict of interest

All authors state that there are no conflicts of interest or financial disclosures to declare.

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