

## Evaluation of Galactin-3 Levels in Patients with Colorectal Cancer and Control Subjects

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### Abstract

**Objective:** To assess the serum levels of galectin-3(GAL-3) in patients with colorectal cancer and healthy subjects. For this purpose, a prospective analysis was performed on serum specimens from 40 patients with colorectal cancer and 40 healthy subjects.

**Methods:** Enzyme-Linked Immune Sorbent Assay (ELISA) was used to measure levels of galectin-3. Serum levels of GAL-3 were compared between patients with colorectal cancer and healthy control.

**Results:** The (mean  $\pm$  SD) GAL-3 level of healthy controls was  $4.26 \pm 5.8$  (ng/ml) and in the CRC patients was  $5.46 \pm 6.3$  (ng/ml). The level of GAL-3 in CRC was slightly increased compared to control group, but no statistically significant differences were observed when comparing GAL-3 serum levels between patients with colorectal cancer and healthy control ( $p$  values = 0.38). Hence, in the results of ROC curve, the surface under the GAL-3 curve according to the larger test result indicates more positive test (AUC = 0.58) indicates that GAL-3 assay has not sensitivity and specificity as biomarker for diagnosis of the colorectal cancer.

**Conclusion:** We concluded that the measurement of GAL-3 levels cannot be considered as a prognostic biomarker for colorectal cancer, nevertheless further basic and clinical research is needed to clarify role of GAL-3.

**Keywords:** Colorectal cancer; Galectin-3

**Abbreviations:** CRC: Colorectal Cancer; GAL-3: Galectin-3; ELISA: Enzyme-Linked Immunosorbent Assay

### Introduction

Colorectal cancer is caused by the uncontrolled growth of epithelial cells in the colon and rectum layers of the gastrointestinal system; it is the third most common cancer in the world leading to death. Thus, identifying a new biomarker is necessary for early diagnosis of CRC<sup>[1]</sup>. Galectins are endogenous immune regulatory lectins which constitute a galactoside-binding protein family of 15 members<sup>[2]</sup>. Galectin-3 (Gal-3) is the only member of galectin represented in chimera-type, and due to this feature, it is capable of attaching to various cells and molecules and exerting significant effects in many physiological and pathologic processes<sup>[3]</sup>. Gal-3 is usually located in the cytoplasm; however, it has been identified in the nucleus, on the cell surface and in the extracellular environment as well. In the nucleus, through the association with transcription factors or their stability, they alter the expressed genes and participate in mRNA splicing process<sup>[4]</sup>. Several studies have been conducted on the role of galectins as an index in regulating the functional characteristics of cancer cells, such as adhesion, attack, and metastasis<sup>[5]</sup>. High level expression of Gal-3 in cancer cells increases intercellular and cellular interactions with the environment and helps promote the spread of cancer to other tissues<sup>[6]</sup>. Gal-3 is widely expressed in the human gut

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including the large intestine and rectum and in recent years, association between Gal-3 and colon cancer in terms of diagnosis and prognosis were studied and various results were obtained<sup>[7]</sup>. The current study was designed to assess and compare serum levels of Gal-3 in patients with colorectal cancer and healthy controls.

## Materials and Methods

### Patients and Samples

Colorectal cancer patients (n = 40, 20 men and 20 women), hospitalized in the Imam Khomeini Educational Hospital and Tuba Clinic, Mazandaran University of Medical Sciences in Sari, Northern Iran, from November 2017 and Jan 2019, participated in this study. The patients were within the age range of 30 to 70 years. After consultation with a gastroenterologist, the diagnosis of the patients was verified. The control group consisted of 40 healthy volunteers (8 males and 32 females), aged 30–70 years. To reduce and control other interveners, the selection of controlled and studied groups was matched to the range of age, region, and demographic conditions. A metal-free sterile tube was used to collect a 5-ml sample of venous blood. Then, the blood was put to clot. Afterwards, for serum extraction, centrifuging was done at 3000 rpm for 10 min. The serum samples with no indication of hemolysis were aliquoted into Eppendorf tubes and kept at -80°C before assaying.

### Inclusion criteria

Patients aged 30 to 70 years, CRC patients diagnosed by consultation with a gastroenterologist and they had not been under chemotherapy and radiotherapy treatment yet.

### Exclusion criteria

Patients aged above 70 years and under 30 years; patients undergoing chemotherapy, radiotherapy; smoking cigarette; having a history of other cancers and malignant and autoimmune disease. In this study, ELISA based on the Biotin double antibody sandwich technology was used to measure human Galactin-3 in sera. Prior to performing the assay, to defrost the serum samples thoroughly, they were put at room temperature for roughly 30 min. The human Galactin-3 ELISA kit (Bioassay Technology Laboratory – E1951Hu – 96 Test) was used to specify Galactin-3 in duplicate. The assays were carried out according to the manufacturer's instructions. In brief, after providing five dilutions of the basic standard, 50 µl of standard and streptavidin-HRP were added to the standard solution well; in sample well, 40 µl of sample and 10 µl of Galactin-3 antibodies were added and in blank well only 50 µl of chromogens and stop solution were added. Then, the wells gently were shaken to mix them completely, which were then incubated at 37°C for 60 minutes. Washing solution was prepared, each well was filled with it and was drained after 30 seconds standing, and this procedure was repeated five times and blots the plate. Then, for color development, 50 µl of chromogenic solution A and B was added to each well as well; after shaking them completely, they were incubated at 37°C for 10 minutes and then 50 µl of stop solution was added to each well to stop the reaction. ELISA reader (Awareness Technology INC Star Fax-2100) was used to read data at 450 nm wavelength. Galactin-3 concentrations were specified

by estimating based on the standard curve created by plotting the absorbance of the standards vs. matching concentrations. All protocols involving patients and control subjects were confirmed by the Ethics Committee of Payame Noor University with the code number of (IR.PNU.REC.1397.036).

### Statistical analysis

To analyze data, the SPSS software package (version 21) was applied. The results were expressed as means ± standard deviations (mean ± SD). T-test was applied to compare the colon cancer patients and control groups in terms of the serum Gal-3 levels. A p value < 0.05 for the variable difference between groups was set to be significant statistically.

## Results

The demographic characteristics are shown in Table 1. Galactin-3 serum levels in colorectal cancer patients and controls are demonstrated in Table 2. Patients (n = 40, 20 women and 20 men) and 40 healthy subjects (32 women and 8 men) meeting the inclusion criteria took part in the study. The mean age of patients was 60 ± 9.42 years (range 30–70 years), and the average age of control group was 43 ± 13.4 years (range 30–70 years). The (mean ± SD) GAL-3 level in healthy controls was 4.26 ± 5.8 (ng/ml) and in the CRC patients was 5.46 ± 6.3 (ng/ml). The level of GAL-3 in CRC was slightly increased compared to control group, but no significant difference was found in this comparison (P = 0.38). The area under curve (AUC) was used to express the results of Receiver Operating Characteristic (ROC) curve analysis (Figure 1,2).

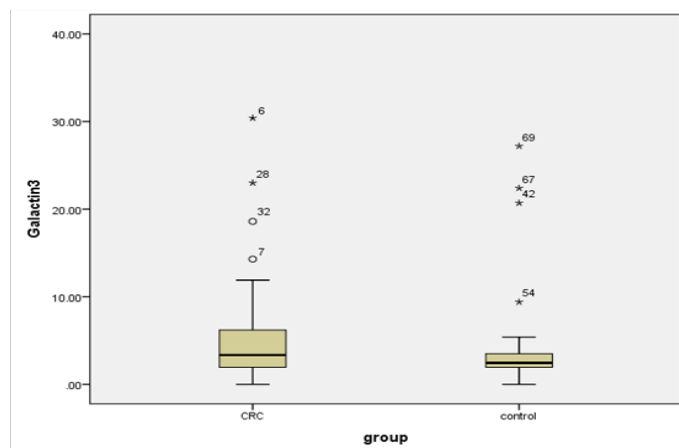
**Table 1:** Demographic features of colon cancer patients and controls.

		Control group	Cancer group
number		40	40(24CC+16CRC)
Gender	female	32	20
	male	8	20
Age, Y (mean ± SD)		43 ± 13.4	60 ± 9.42
Weight (kg) (mean ± SD)		69.38 ± 8.16	73.32 ± 11.63
Height (cm) (mean ± SD)		164.48 ± 6.9	164.24 ± 7.21
BMI (kg/m <sup>2</sup> ) (mean ± SD)		25.61 ± 2.31	27.22 ± 4.3

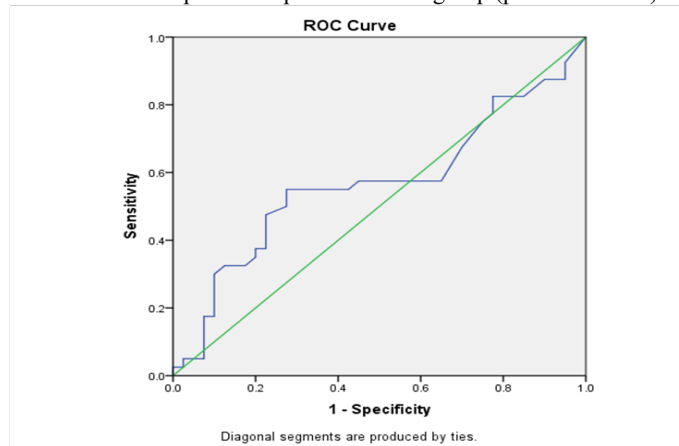
**Table 2:** Galactin-3 serum levels in colorectal cancer patients and controls.

	Healthy control Mean ± SD	colorectal cancer patients Mean ± SD	p values*
Galactin-3 (ng/ml)	4.26 ± 5.8	5.46 ± 6.3	0.38 ( Mann-Whitney U test)

\*p values for case-control comparisons from Mann-Whitney U test where appropriate.



**Figure 1:** Levels of GAL-3 in colorectal cancer patient's and normal human's serum by Enzyme-linked immunosorbent assay (ELISA). T-test used to compare to respective normal group (p values = 0.38).



**Figure 2:** Analysis GAL-3 of sensitivity and specificity in the diagnosis of colorectal cancer by ROC curve. Data from ELISA method for GAL-3 (Considering the larger test result indicates more positive test). ROC curve analysis shows Area under Receiver Operating Characteristic (AUROC) 0.56.

## Discussion

The biological role of galectin-3 in cell growth, differentiation, adhesion, malignant transformation, and apoptosis has been identified. Altered expression of galectin has been associated with malignancy, progression, and invasion in several human tumors and its altered expression correlates with the type and stage of tumor progression. Accordingly, the serum level of Gal-3 may be an alarming biomarker for malignancy and metastasis<sup>[4]</sup>. In this study, we have determined serum Gal-3 concentrations in patients with colorectal cancer compared with the levels in healthy individuals. The level of Gal-3 in CRC was slightly increased compared to control group, but no statistically significant differences were observed when comparing Gal-3 serum levels between patients with colorectal cancer and healthy control.

Levels of Gal-3 in patients with gastric, lung, bladder, thyroid, and prostate were significantly higher than those in benign disease patients and healthy controls, however, in other cancers, such as breast and ovary cancer, higher than those in benign disease patients and healthy controls<sup>[8,9,11]</sup>. The pattern of Gal-3 sera level in colorectal cancers is still unknown, because some investigators have found increasing its in CRC where as others have observed the opposite results. In a study in 1997, the

pattern of Gal-3 expression in colorectal cancer progression was specified reporting that there is a link between colorectal tumor progression and reduced galectin 3 expression in early stages and increases in later phases of tumor progression. Considering that in our study Gal-3 levels were slightly higher than control group, it could be concluded that our study confirms this finding<sup>[10]</sup>. In another study in 2000, Iurisci et al. showed that patients with metastatic colorectal carcinomas had significantly higher Gal-3 serum levels than those with non-metastatic disease. They also revealed that serum levels of Gal-3 were higher in patients with later stage tumors and distant metastasis compared to those with localized tumors at earlier stages. Differences of our result may be attributed to the different stages and the number of samples tested in our study<sup>[11]</sup>. In another study in 2005, the relationship between Gal-3 expression and clinic-pathological factors was investigated; they, reported that the incidence of lymph node and distant metastasis in Gal-3 positive cancer was significantly higher than that in Gal-3 negative cases; thus, they concluded that immunohistochemical detection of elevated levels of Gal-3 is a powerful prognostic marker in colorectal cancer<sup>[12]</sup>. Barrow et al. (2012) reported concurrent determination of galectin-3/-4 levels in the sera of patients with colorectal cancer; their result showed high specificity and high sensitivity in distinguishing colorectal cancer patients with metastases from those with no metastases as well as from healthy individuals<sup>[13]</sup>. Another study in 2015 demonstrated that Gal-3 and its binding protein levels were not significantly different between patients in early and advanced stages<sup>[14]</sup>. In general, we concluded that the measurement of Gal-3 levels for early detection of colon cancer might be considered as a prognostic biomarker for colorectal cancer. Despite these results, we suggest additional studies be performed with a larger sample size for better statistical evaluation.

## Conclusion

Serum Gal-3 levels in colorectal cancer patients were slightly higher compared to the healthy controls. Higher Gal-3 levels were correlated with biochemical and clinical features in colorectal cancer patients.

**Some limitation to be noted:** Firstly, our sample size was small. Secondly, we were unable to assess the stage of tumor progression.

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**Author Contribution:** DQ, designed the experiments, RA performed the experiments, and RA analyzed the results and wrote the manuscript.

**Competing Interests:** The authors state that they have no conflict of interests.

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