Clinics in Oncology

Research Article
Published: 09 Jan, 2018

# The Importance of Galectin-3 and MUC1 in the Metastatic Spread of the Gastric Carcinoma

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## Abstract

**Objective:** Gastric cancer mortality ranks forefront among cancer-related deaths. In order to be able to decrease cancer-related deaths, many steps of carcinogenesis, and cancer biology should be understood. It has been determined that MUC1 is a natural ligand of galectin-3 in cancer cells, and binding of galectin-3 to MUC1 increases cell-surface polarization of MUC1 with a process which might be related to the development of metastases. The purpose of his study is to evaluate the association between the expressions of MUC1, and galectin-3 with other prognostic factors in primary tumor, and synchronous lymph node metastasis of the primary tumor in patients diagnosed as gastric carcinoma using immunohistochemical methods.

**Material and Method:** Samples of 125 primary gastric cancer, and synchronous lymph node metastases of the primary tumor were evaluated as for the association between expressions of galectin-3, and MUC1 with other prognostic parameters, and survival.

**Results:** Decrease in the expressions of galectin-3 and MUC1 in 105 cases with synchronous lymph node metastases was detected relative to the primary tumor. Correlations were detected between the level of galectin-3 expression, depth of tumoral invasion (p=0.015), and recurrence (p=0.002) in the primary tumor, and also between galectin-3 expression, Lauren's classification (p=0.028), lymphovascular invasion (p=0.054), neural invasion (p=0.016), recurrence (p=0.005), and metastasis (p=0.015) in the synchronous lymph node metastasis. In the primary tumor, correlations were detected between the level of MUC1 expression, and Lauren's classification (p=0.033), and recurrence (p=0.033), and MUC1 expression, and Lauren's classification (p=0.028), lymphovascular invasion (p=0.054), neural invasion (p=0.016), and recurrence (p=0.005) in the synchronous lymph node metastasis. In the primary tumor, and synchronous lymph node metastasis, any correlation was not detected between the expressions of galectin-3, and MUC1, and survival in the primary tumor, and also between the expression of galectin-3, and disease-free survival in the synchronous lymph node metastasis. However in the primary tumor as the level of MUC1 expression increased the probability of recurrence also increased (p=0.037). In the synchronous lymph node metastasis, level of MUC1 expression did not affect DFS (p=0.189).

**Conclusion:** In the gastric carcinoma, and synchronous lymph node metastasis, individual determination of galectin-3, and MUC1 expression levels, may be determinative factor as for the pathobiological behavior of the gastric carcinoma, and its prognosis. However further studies which will evaluate metastatic and/or recurrent tumoral tissue in addition to primary tumor tissue are needed.

**Keywords: Gastric carcinoma; MUC1; galectin-3; metastasis; prognosis**

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**Received Date**: *27 Nov 2017*
**Accepted Date:** *28 Dec 2017*
**Published Date:** *02 Jan 2018*

***Citation:***
*Eliyatkin NO, Aktas S, Erkul Z, Yalcın E, Comut E, Zengel B, et al. The Importance of Galectin-3 and MUC1 in the Metastatic Spread of the Gastric Carcinoma. Clin Oncol. 2018; 3: 1401.*

## Introduction

Gastric carcinoma ranks 4. Among the most prevalently seen cancer types in the world, and it is the second most frequent cause of cancer-related mortalitty [1]. The steps of carcinogenesis in gastric cancer, and biology of gastric cancer should be very well understood in order to be able to decrease mortality rates, and develop new successful and effective treatment strategies. The first step in the progression of cancer is the degradation of extracellular matrix by the release of tumor cells. Later on, at the critical stage of carcinogenesis, metastases develop as disseminated tumor cells adhere to vascular endothelium of distant organs. This process is thought to be mediated via mechanical properties of cancer cells, and synchronous specific expression of various adhesion molecules, and/or ligands to enable them to attach to molecules on the surface of cancer, and endothelial cells. Mucins are epithelial glycoproteins with heavy molecular weight. Miscellaneous types of mucins have been defined. They divide into two different groups based on their morphologic structure, and functions as secreted type ve membrane-associated type. Membrane-associated type mucins are associated with epithelial cell-cell interactions. Mucin 1 (MUC1), is a bulky transmembrane mucin protein of membrane-associated type which is expressed on the apical surface of many secretory epithelial cells. In many cancer types's 10-fold increase in MUC1 expression has been reported [2]. The association between this expression, and increased metastatic potential, and worse prognosis has been described. Cancer-associated MUC1 loses its apical membrane polarization, and it is expressed all over the cell surface. Over expression of MUC1 inhibits E-cadherin-related cell-cell, and integrin-related cancer-matrix interactions, and increases tumoral cell release from the area of primary tumor. However galectin-3 is a galactoside-binding protein expressed by many cell types. It may be found in the cell, and extracellular space, associated with cell surface, and circulation. Extracellular galectin-3 bound to cell surface acts as an adhesion molecule during cell -cell interaction, and it is associated with metastases. In various studies performed on cell cultures, it has been determined that in human cancer cells MUC1 is a natural ligand of galectin-3, and with binding of galectin-3 to MUC1, surface polarization of MUC1 increases, and this increase might be associated with the development of metastases [3].

The purpose of this study is to evaluate the relationship between MUC1, and galectin-3 expressions, and other prognostic parameters in primary tumors, and synchronous lymph node metastases of the tumor in patients diagnosed as gastric carcinoma in Izmir Bozyaka Training and Research Hospital between the years 2003, and 2013. Therefore, expressions of MUC1 and galectin-3 in cases with gastric carcinoma may clarify molecular mechanism of metastasis, and predict potential metastases which will occur later, and determine the possibility of their becoming an important target agent in the development of preventive treatment strategies.

## Methods

### Patients vs Tissue Samples

The study was approved by the Ethics Committee of Clinical Researches of İzmir Bozyaka Training and Research Hospital. The patients who were diagnosed as gastric carcinoma based on histopathological examination of surgical resection materials between the years 2003, and 2013 in Izmir Bozyaka Training and Research Hospital were registered electronically. Archived H&E stained preparations of the patients who had complete clinical, and

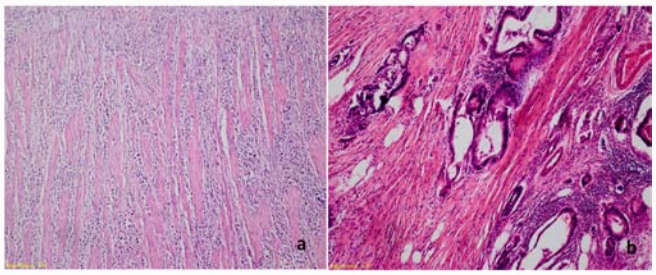


**Figure 1**:

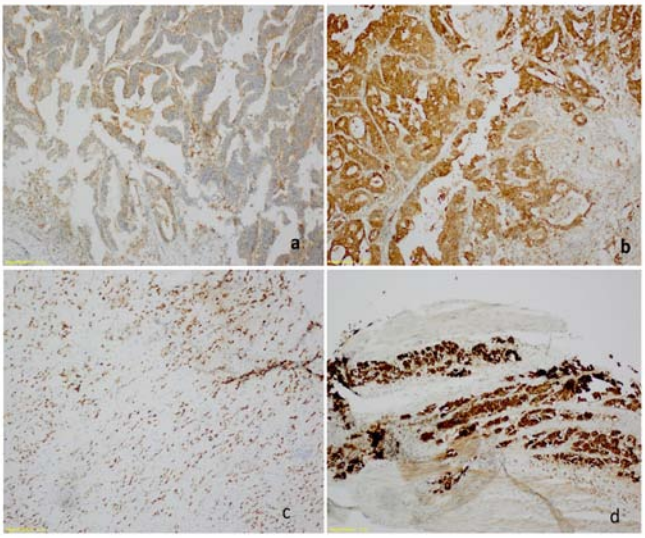


**Figure 2**:

histopathological data, and received surgical, and chemotherapeutic treatment modalities based on a standard procedure, then followed up by the attending physician were re-evaluated. Archived tissue blocks of lymph node metastases were chosen if adequate primary tumor tissue and lymph node metastasis were available. Patients with adequate tumor tissue, and paraffin blocks were included in the study. Size, location, histological type of the tumor, depth of invasion, lymph node metastasis, and perinodal invasion were evaluated. Besides, lymphovascular invasion, neural invasion, lymphocytic response to tumor and state of the serosal margins were evaluated. Histological type of the tumor was determined based on WHO 2010 classification, and other prevalently used Lauren's classification (Figure 1). The tumor stage was classified according to the 7th edition of the TNM Classification of the International Union against Cancer (UICC). Among the patients, 42 were females and 83 male. Their age ranged from 29 to 91 years, with a mean age of 62.29 years. The average period of postoperative follow-up period was 37, 79 months (0.23-182.01).

### Tissue microarray (TMA)

H&E stained prepararions of the patients included in the study which contained primary tumor tissue, and (if available) representative tumoral areas for lymph node metastases were chosen for sampling. From tumoral areas detected in archived paraffin blocks of these selected preparations, 2 mm-diameter tissue cores per donor block were punched out and transferred to a recipient block with a maximum of 24 cores using a tissue microarrayer (Histopathology, Japan). Thin sections (4 μm) were consecutively cut from the tissue micro-arrays and transferred to polylysine-coated glass slides. H&E

**Table 1:** Galectin-3 and MUC-1 expression in the primary tumor and the synchronous lymph node metastasis.

| Groups | n | Galectin-3 expression | | | |
|---|---|---|---|---|---|
| | | - | + | ++ | +++ |
| Tumor tissue | | 6 | 22 | 37 | 60 |
| Lymph node metastasis | | 13 | 13 | 39 | 40 |
| **Groups** | **n** | **MUC-1 expression** | | | |
| | | - | + | ++ | +++ |
| Tumor tissue | 125 | 4 | 21 | 39 | 61 |
| Lymph node metastasis | 105 | 13 | 19 | 35 | 38 |
| **Groups** | **n** | **Galectin-3 expression** | | | |
| | | Low expression | | High expression | |
| Tumor tissue | 125 | 28 | | 97 | |
| Lymph node metastasis | 105 | 26 | | 79 | |
| **Groups** | **n** | **MUC-1 expression** | | | |
| | | Low expression | | High expression | |
| Tumor tissue | 125 | 25 | | 100 | |
| Lymph node metastasis | 105 | 32 | | 73 | |

staining was performed for confirmation of tumor tissue.

### Immunohistochemistry

Immunostaining of Galectin-3 and MUC1was performed by the streptavidin–biotin peroxidase method by tissue microarray. Serial 5-µm sections were obtained, and these slides were baked overnight at 60°C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat-induced epitope retrieval in the microwave (in 10 mM/L citrate buffer, pH 6.0, for 40 min, followed by cooling at room temperature for 20 min) and blocked for endogenous peroxidase and biotin. Mouse anti-human Galectin-3 (Novo Castra, UK) and MUC-1 (Novo Castra, UK) antibodies were used at 1: 100 dilutions to detect the respective proteins, with anti-mouse Envison-PO (DAKO, USA) as the secondary antibody. Binding was visualized with 3, 3'- diaminobenzidine (DAB) and counterstaining with Mayer's hematoxylin was performed to aid orientation. Immunoreactivity for MUC1 was localized in the cytoplasm and membrane of the tumor cells, while Gal-3 showed a cytoplasmic pattern. One hundred cells were randomly selected and counted from five representative fields of each section blindly by two independent observers (NE, ZE) and the percentages of positive cells in the total counted were graded semi-quantitatively using a four-tier scoring system: negative (−), 0–5%; weakly positive (+), 6–25%; moderately positive (++), 26–50%; and strongly positive (+++), 51–100%. After, the levels of the expression of MUC1, and galectin-3 were separated to two groups: low (negative and weakly positive) expression and high (moderately positive and strongly positive) expression.

### Statistical analysis

The categorical variables were presented in a descriptive way in tables containing absolute frequencies. Continuous variable of normal distribution was described using the mean. Expression of gal-3 and MUC1was assessed with various clinic-pathological parameters using the $\chi 2$ test. Survival rates were calculated by the Kaplan-Meier method. The difference between the survival curves was analyzed by the log-rank test. Starting time was the day of surgical resection of gastric cancer. Differences were considered significant when the P value was less than 0.05.

## Results

Galectin-3 and MUC1 expression in the primary tumor and the lymph node metastasis of 125 patients with gastric cancer was investigated by immunohistochemical analysis and the levels of the expression of MUC1, and galectin-3 were separated to two groups: low (negative ve weakly positive) expression and high (moderately positive and strongly positive) expression as defined above (Figure 2-4) (Table 1).

In 105 cases with synchronous lymph node metastasis of primary gastric carcinoma, galectin-3 levels of expression in primary tumor, and its metastasis were compared, using dependent 2-group nonparametric Wilcoxon Test, and a statistically significantly difference was found between levels of expression (p=0.001 Wilcoxon test) which demonstrated lower galectin-3 expression level in lymph node metastasis relative to that in the primary tumor. A statistically significant correlation was not found between the groups with low, and high levels of galectin-3 expression (p=0.166). When we compared the groups as for levels of MUC1 expression, a statistically significant difference was detected between levels of expression (p=0.0001 Wilcoxon test). Similar to the finding for galectin-3, MUC1 expression levels in lymph node metastasis were lower than those found in the primary tumor. However contrary to lack of intergroup correlation for galectin-3 expression levels, a statistically significant correlation was found between groups with lower, and higher expression levels of MUC1 (p=0.005).

A statistically significant correlation was detected between galectin-3 expression level in the primary tumor, and tumor invasion depth (low –grade tumor T, and high-grade tumor T) (p=0.015), and probability of recurrence (p=0.002). In cases of synchronous lymph node metastasis, a statistically significant correlation was detected between galectin-3 expression, and Lauren's classification (p=0.028), lymphovascular invasion (p=0.054), neural invasion (p=0.016), probability of recurrence (p=0.005), and incidence of metastasis (p=0.015) (Table 2).

In cases of primary tumor, a statistically significant correlation was detected between MUC1 expression level, and Lauren's classification (p=0.033), and recurrence (p=0.033). In cases of synchronous lymph

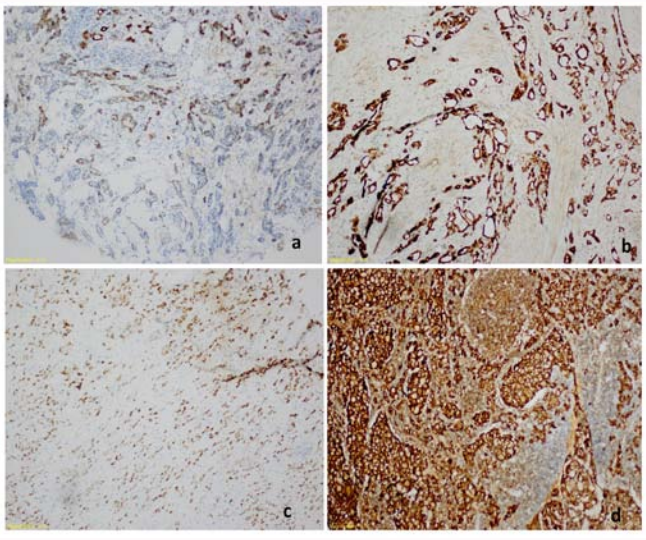


**Figure 3**:

**Table 2:** Relation between Galectin-3 expression and clinicopathological features in the primary tumor and the synchronous lymph node metastasis.

| Gal-3 expression | | | | | | |
|---|---|---|---|---|---|---|
| Clinico-pathologic feature | Primary tumor, n | | | Lymph node metastatis, n | | |
| | Low | High | P-value | Low | High | P-value |
| Gender | | | | | | |
| Female | 8 | 34 | 0.345 | 6 | 33 | 0.068 |
| Male | 28 | 63 | | 20 | 46 | |
| Tumor invasion | | | | | | |
| T1/T2 | 7 | 7 | 0.015 | 2 | 2 | 0.256 |
| T3/T4 | 21 | 90 | | 24 | 77 | |
| Lauren's classification | | | | | | |
| Diffuse | 11 | 47 | 0.362 | 10 | 42 | 0.028 |
| Intestinal | 16 | 42 | | 16 | 30 | |
| Lymphovascular invasion | | | | | | |
| $Lv^-$ | 9 | 25 | 0.329 | 8 | 11 | 0.054 |
| $Lv^+$ | 19 | 72 | | 18 | 68 | |
| Neural invasion | | | | | | |
| - | 15 | 37 | 0.108 | 14 | 22 | 0.016 |
| + | 13 | 60 | | 12 | 57 | |
| Nodal status | | | | | | |
| $pN^-$ | 7 | 13 | 0.12 | | | |
| $pN^+$ | 21 | 84 | | | | |
| UICC staging | | | | | | |
| 0-1 | 8 | 20 | 0.259 | 2 | 6 | 0.636 |
| 2-4 | 20 | 77 | | 24 | 73 | |
| Recurrence | | | | | | |
| Hepatic/periton | 4 | 25 | 0.527 | 2 | 27 | 0.176 |
| Others | 2 | 18 | | 4 | 16 | |
| Recurrence | | | | | | |
| - | 22 | 54 | 0.002 | 20 | 36 | 0.005 |
| + | 6 | 43 | | 6 | 43 | |
| Metastasis | | | | | | |
| - | 23 | 66 | 0.11 | 22 | 47 | 0.015 |
| + | 5 | 31 | | 4 | 32 | |
| Ex | 13 | 66 | *0.032* | 14 | 59 | 0.042 |
| Alive | 15 | 31 | | 12 | 20 | |

node metastasis a statistically significant correlation was found between MUC1 expression and Lauren's classification (p=0.028), lymphovascular invasion (p=0.054), neural invasion (p=0.016), and likelihood of recurrence (p=0.005) (Table 3).

In Pearson correlation analysis the correlation between age, and disease-free survival (DFS), and overall survival (OS) was evaluated. In patients younger than 60 years, median DFS, and OS were found as 43.94, and 45.94 months, respectively. However in patients older than 60 years, DFS, and OS were 28.85, and 30.95 months, respectively which might be related to increased DFS, and OS in patients aged more than 60 years.

Patients with improved OS rate as detected in Kaplan-Meier analysis, and lower galectin-3 expression in primary tumor tissue as assessed by Log-Rank test, had a higher quality of life without any statistically significant intergroup difference (p=0.071). In the group with synchronous lymph node metastasis lower, or higher galectin-3 expression levels did not apparently effect overall survival rates (p=0.637). The 0-0.5 % of the patients who had negative (-) galectin-3 expression in the primary tumor tissue, had a worse disease progression, while at higher expression levels more improved disease progression was detected. (p=0.053). However in 51-100 % of the cases with strongly positive (+++), galectin-3 expression had a worse prognosis. A significant difference was not detected as for overall survival rates between groups with lower, and higher MUC1 expression levels in the primary tumor tissue (p=0.801). However, disease progression worsened as MUC1 expression levels in the primary tumor tissue increased. (p=0.048). MUC1 expression levels in the synchronous lymph node metastasis did not affect prognosis (p=0.220). Besides, grouping based on lower, and higher MUC1

**Table 3:** Relation between MUC-1 expression and clinicopathological features in the primary tumor and the synchronous lymph node metastasis.

| MUC-1 expression | | | | | | |
|---|---|---|---|---|---|---|
| Clinico-pathologic feature | Primary tumor, n | | | Lymph node metastatis, n | | |
| | Low | High | P-value | Low | High | P-value |
| Gender | | | | | | |
| Male | 10 | 32 | 0.298 | 13 | 26 | 0.391 |
| Female | 19 | 68 | | 19 | 47 | |
| Tumor invasion | | | | | | |
| T1/T2 | 3 | 11 | 0.562 | 1 | 3 | 0.644 |
| T3/T4 | 22 | 89 | | 31 | 70 | |
| Lauren's classification | | | | | | |
| Diffuse | 17 | 41 | 0.033 | 22 | 30 | 0.029 |
| Intestinal | 6 | 52 | | 9 | 37 | |
| Lymphovascular invasion | | | | | | |
| $Lv^-$ | 9 | 25 | 0.195 | 6 | 13 | *0.554* |
| $Lv^+$ | 16 | 75 | | 20 | 60 | |
| Neural invasion | | | | | | |
| - | 10 | 42 | 0.521 | 7 | 29 | 0.058 |
| + | 15 | 58 | | 25 | 44 | |
| Nodal status | | | | | | |
| $pN^-$ | 5 | 15 | 0.366 | | | |
| $pN^+$ | 20 | 85 | | | | |
| UICC staging | | | | | | |
| 2-Jan | 7 | 21 | 0.307 | 2 | 6 | 0.538 |
| 3 | 18 | 79 | | 30 | 67 | |
| Recurrence | | | | | | |
| Hepatic/periton | 7 | 22 | 0.343 | 9 | 20 | 0.505 |
| Others | 3 | 17 | | 7 | 13 | |
| Recurrence | | | | | | |
| - | 15 | 61 | 0.551 | 16 | 40 | 0.404 |
| + | 10 | 39 | | 16 | 33 | |
| Metastasis | | | | | | |
| - | 19 | 73 | 0.257 | 21 | 48 | 0.58 |
| + | 9 | 27 | | 11 | 25 | |
| Ex | 13 | 66 | 0.143 | 22 | 51 | 0.541 |
| Alive | 12 | 34 | | 10 | 22 | |

expression levels had not any significance with respect to prognosis. (p=0.687).

In Kaplan-Meier analysis of DFS using log-rank test, galectin-3 expression level (-, +, ++, +++) in the primary tumor tissue was not statistically significant (p=0.050). Patients lower galectin-3 expression levels had a higher quality of life, albeit lack of any significant difference. A significant difference was not detected between groups with lower, and higher galectin-3 expression levels regarding prognosis (p=0.068). Galectin-3 expression levels (-, +, ++, +++) in the synchronous lymph node metastases did not affect DFS (p=0.836). Higher, and lower galectin-3 expression levels also had not any prognostic significance (p=0.548). Increases in MUC1 expression in the primary tumor was associated with higher recurrence rates (p=0.037) (Figure 5). Besides, lower, and higher MUC1 expression levels had not any impact on DFS (p=0.937). MUC1 expression levels in the synchronous lymph node metastasis also did not affect DFS (p=0.189) (Figure 6). Lower, and higher expression levels had not any significance for DFS (p=0.714).

## Discussion

In this study, in a patient group diagnosed as gastric carcinoma the correlation between expressions of galectin-3, and MUC1 in the primary tumor, and its synchronous lymph node metastasis with other prognostic parameters and their impact on DFS, and OS were

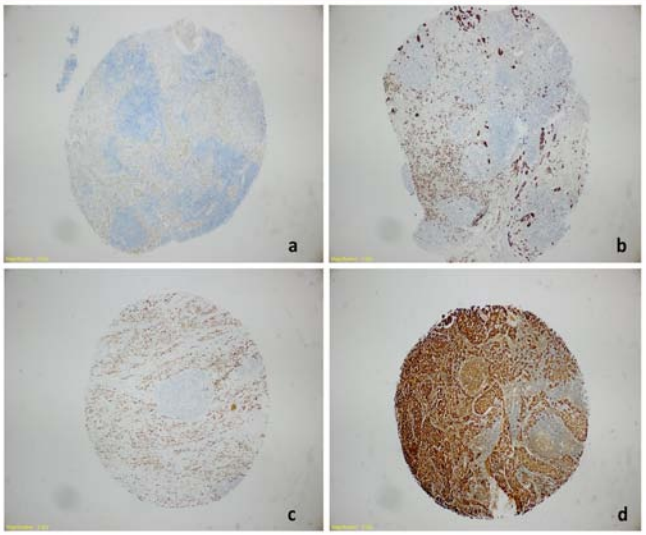


**Figure 4**:

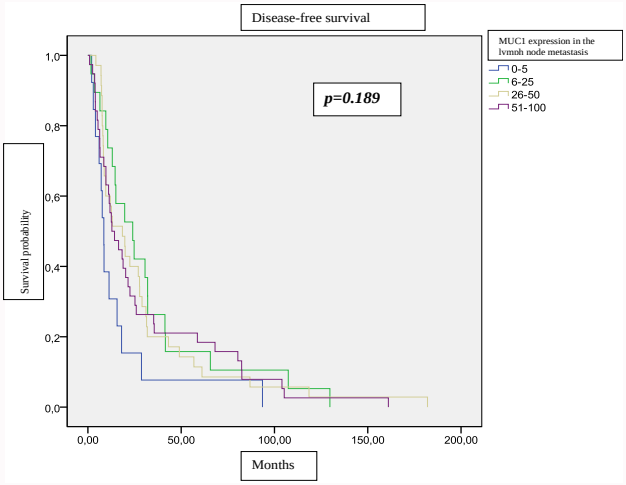


**Figure 6**: Kaplan–Meier curve of disease-free survival of patients according to MUC1 expression levels in the synchronous lymph node metastasis (p=0.189).

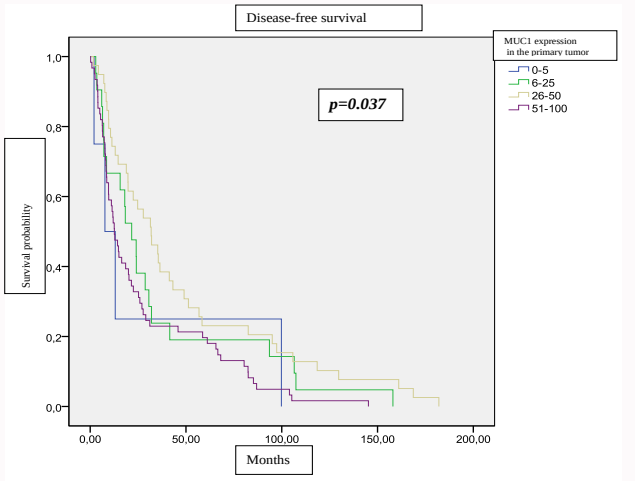


**Figure 5**: Kaplan–Meier curve of disease-free survival of patients according to MUC1 expression levels in the primary tumor tissue (p=0.037).

investigated. In various cancer types, expressions of galectin-3 and MUC1 have been investigated in only primary tumor tissue, and diverse outcomes have been reported. In some studies, comparisons have been made also with adjacent non-tumoral tissue. In a study where levels of galectin-3 were evaluated in cell line, gastric carcinoma, and adjacent non-tumoral tissue, the association between decreased galectin-3 expression and presence of distant metastasis, and in vitro higher invasive phenotype was determined [4]. In our study level of galectin-3 expression level was analyzed in terms of recurrence, and metastasis, and significantly decreased galectin-3 levels were observed in synchronous lymph node metastasis when compared with the primary tumor. Okada et al. detected higher galectin-3 expression levels in cancer tissue when compared with normal tissue. However in the same study the authors concluded that decreased galectin-3 expression in the tumor tissue was associated with poor prognosis characterized by lymph node metastasis, advanced stage, and/or poorly differentiated tumor [5]. However detected that decreased galectin-3 expression in cases of pancreas ductal adenocarcinoma was associated with advanced stage, and increased number of lymph node metastases [6]. In another study, increased galectin-3 expression had been correlated with poorly differentiated carcinoma, and tumor progression [7]. This outcome was not in accordance with our study findings. Where as in the literature controversial outcomes have been indicated about clinicopathological significance of galectin-3 expression of patients with colorectal, gastric, ovarian, oral cavity or thyroid cancers [8-12]. Immunohistochemical evaluation galectin-3 expression in 57 samples of gastric cancer tissue, galectin-3 was determined as an unreliable biomarker of prognosis [13]. However in this study, galectin-3 expression was scored in only two subgroups as follows: 1 (negative or weak), no staining or less than 50 % of tumour cells are stained, and 2 (moderate or strong) when ≥50 % of the tumour cells are stained. Besides cytoplasmic and/or nuclear staining were/was considered as positive reaction. As is the case with many studies, we also evaluated only cytoplasmic/membranous staining as an evidence of positivity, and we didn't take nuclear staining into consideration which might account for our diverse outcomes. In some studies it has been demonstrated that apart from galectin-3 expression levels, its localization patterns also play a role in cancer progression [14]. Found that nuclear galectin-3 immunoreactivity is significantly stronger in the diffuse type tumors relative to intestinal type tumors [9]. Though the role of galectin-3 in carcinogenesis has not been fully defined yet, cellular localization of galectin-3 may indeed play an important role in malignant transformation. One of the probable mechanisms may be related to the regulation of signal pathways [15]. Galectin-3 is down regulated by tumor suppressor gene p53 [16]. Besides, antiapoptotic activity of galectin-3 has been demonstrated [15].

In the literature, some studies have evaluated serum galectin-3 levels using ELISA method. Cheng et al. found significantly higher galectin-3 levels in patients with gastric cancer when compared with cases with benign gastric diseases, and healthy controls. They also concluded that serum galectin-3 levels were associated with lymph node metastasis (p=0.001), and distant metastasis (p<0.001) [17]. However its use as a screening test seems to require conduction of larger series.

Increased levels of MUC1 in various cancer types have been described [18-19]. MUC1, acts as an anti-adhesion molecule, and

facilitates release of tumor cells from tumoral tissues in malignancies. In malignant cells increased levels of MUC1 expression may suppress extracellular domains, increase survival times of tumor cells, and induce development of invasion, and metastasis. Similarly, in a study where MUC1 expression was evaluated using tissue microarray method, higher MUC1 expression levels were detected in gastric carcinoma when compared with non-tumoral gastric mucosa, and positive correlation was demonstrated between UICC staging, depth of invasion, lymphatic, and venous invasion, and lymph node metastasis [20]. In the study depth of invasion was defined as Tis-T1, and T2-T4, while UICC staging was described as 0-1, and 2-4, however in our study depth of invasion was found as T1-T2, and T3-T4, while UICC stages were grouped as 1-2, and 3-4. Therefore, we thought that different outcomes might be obtained from other studies. However, in this study where Lauren's classification was performed based on the criteria similar to ours, comparable relevant outcomes were obtained (p=0.002) also acquired similar results [21]. However Wang et al. [22] and Kocer et al. [23], could not detect any difference between these diffuse and intestinal types of carcinoma as for MUC1 expression [22-23]. This discordance might stem from scarce number of study participants or heterogeneity of the study population. In our study decreased overall survival rates were observed as the level of MUC1 expression increased (p=0.048). In a study where the state of MUC1, and MUC4 expressions were evaluated in early stage gastric cancer, higher levels of MUC1 expression were detected in well-differentiated tumors. As a concluding remark the authors stated that MUC1 was correlated with lymph vascular invasion without any association with survival rates. Since this study population consisted of only early-stage gastric cancer patients, according to their interpretation MUC1 was not associated with survival rates [24]. The same research team performed a study on advanced-stage gastric cancer patients, detected higher MUC1 expression levels in well-differentiated gastric adenocarcinoma which might affect survival rates [25]. In our study, most of the patients were in advanced stage, and OS decreased as expression levels of MUC1 increased in primary tumor tissue (p=0.048). Since when we grouped patients as those with lower, and higher MUC1 expression levels, we couldn't detect any significant intergroup difference as for OS (p=0.801) we thought that numerical evaluation (ie. Ki-67 proliferation index) of MUC1 expression might be more meaningful. MUC1 expression levels in synchronous lymph node metastases did not affect prognosis. Increased likelihood of recurrence in line with increased MUC1 expression levels in the primary tumor (p=0.037) suggest that MUC1 expression might be an important determinant of DFS. However as a striking finding, levels of MUC1 expression in the synchronous lymph node metastasis did not influence DFS (p=0.189). In our study MUC1 expression was evaluated both in the primary tumor, and synchronous lymph node. Since we haven't encountered similar type of study in our literature review, conduction of comparable studies should be performed.

In normal tissues MUC1 acts as a preventive barrier. However in neoplastic tissues MUC1 is underglycosylated, and in normal cells their suppressed epitopes are disclosed. This characteristic feature transforms tumor-related MUC1 into a target molecule by means of a process mediated by antibodies, toxins or radionucletides or it allows discovery of a vaccine which targets tumor-related MUC1 [26]. Crucial role of over expression of tumor-related MUC1 in the metastasis, and progression of epithelial ovarian cancer has been reported, and thus it has been considered as an ideal treatment target in the control of metastasis, and recurrence [26]. Increased MUC1 expression is associated with invasion, and metastasis. Especially, extracellular domain promotes progression of cancer, and it is effective like an ant adhesion molecule. It also induces detachment of tumor cells from tumor tissue, and causes formation of micro metastases.

In a meta-analysis of 23 different studies consisting of 3245 cases, the prognostic importance of MUC1 in various carcinomas (cholangiocarcinoma, breast carcinoma, colorectal carcinoma gallbladder carcinoma, and gastric carcinoma) was evaluated, and negative predictive value of positive MUC1 staining for OS was detected. In subgroup analysis of the gastric cancer patients, the increased MUC1 expression was found to be more effective on OS compared to other groups [27]. In their in vitro studies, Zhao et al, demonstrated that interaction between cancer cells which express galectin-3, and MUC1 in the circulation (cells carrying galectin-3 ligand TF) promoted development of metastases. They also displayed that galectin-3 disrupts protective shield of the cell surface [28].

## According to the Results of the Present Study

In gastric carcinoma, when galectin-3, and MUC1 expression levels in the primary tissue, and synchronous lymph node metastasis were grouped in four separate scores, lower levels of galectin-3, and MUC1 expression were detected in the metastatic tissue. Whereas, when categorized as groups with lower, and higher expression levels, since only MUC1 expression levels were statistically significantly different, we thought that evaluation of immune expression should be made and indicated numerically as percentages would be more meaningful rather than in groups of lower, and higher expression levels.

In the group where galectin-3 expression was negative (-) in the primary tumor tissues of 0-5 % of the patients, decreased overall survival times were detected, while OS times decreased as MUC1 expression levels increased in the primary tumor tissue. Levels of MU1 expression in the synchronous lymph node metastases did not affect prognosis. Cases with lower galectin-3 expression in the primary tumor tissue had longer disease-free survival times, without any statistically significant intergroup difference. Level of galectin-3 expression in the synchronous lymph node metastases did not affect DFS. As the level of MUC1 increases in the primary tumor, probability of recurrence also increases. However, MUC1 expression levels in the synchronous lymph node metastases did not affect DFS. A correlation existed between MUC1 expression in primary tumor tissue and synchronous lymph tissue and recurrence rates.

In this study, in cases with gastric carcinoma determination of galectin-3, and MUC1 expression levels in the primary tumor tissue, and its synchronous lymph node metastasis separately, may play a determinative role in the pathobiologic behavior, and prognosis of gastric carcinoma Nonetheless, further studies which will evaluate these biomarkers both in the metastatic, and/or recurrent tumor tissue in addition to the primary tumor tissue are needed.

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