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ORIGINAL ARTICLE

Preliminary study of serum Galectin-1 in breast cancer carcinogenesis [Izmir Oncology Group (IZOG) study]

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Summary

Purpose: *Galectin-1 is a lectin involved in the carcinogenesis of many cancers. In the present study, we aimed to investigate the importance of galectin-1 in breast cancer carcinogenesis and its relationship with tumor development.*

Methods: *Patients who were diagnosed with new breast cancer and a healthy volunteer population were included in the study. Preoperative and postoperative (1 month following visit at the medical oncology outpatient clinic) serum samples were collected from breast cancer patients and the healthy volunteer control group.*

Results: *There was no statistically significant difference between patients' age, height, weight and body mass index (BMI) ($p>0.05$). The mean galectin-1 value of the preoperative group was 2.16±0.69 ng/ml, in the postoperative group; 1.75±0.31 ng/ml, and the healthy control group 1.64±0.40 ng/ml. A comparison of mean galectin-1 values between the groups showed that the highest galectin-1 level was found in the preoperative patients. When the mean serum galectin-1 levels of preoperative and postoperative patients were compared, a statistically significant difference was found between the two groups ($p<0.001$). Furthermore, a comparison of the control group and preoperative patients also revealed a statistically significant difference between the groups ($p<0.001$). When the control group and postoperative patients were compared, no statistically significant difference was found between them ($p=0.16$).*

Conclusion: *Serum galectin-1 levels were higher in breast cancer patients than in the healthy control group. In addition, postoperative galectin-1 levels of breast cancer patients tended to decrease. This suggests that serum galectin-1 levels are important in breast carcinogenesis and positively correlated with the presence of tumors.*

Key words: *galectin-1, breast cancer, carcinogenesis, non-metastatic*

Introduction

In addition to being the most common type of cancer in women, breast cancer is also the leading cause of death for women. Primary etiological factors that are considered to intensify breast cancer development include genetic factors, diet, reproductive factors, and hormonal imbalances [1,2].

Galectins are members of the lectin protein family. These galactoside-binding proteins were first identified by Barondes et al in 1994. Galectins are known to be involved in cellular events such as cell proliferation, cell cycle control and apoptosis, as well as tumor invasion [3].The first discovered

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galectin-1 is coded by the LSGALS1 gene localized at human 22q12 chromosome [4].Galectin-1 is a homodimeric protein consisting of 14.5 kDa subunits and contains two β-galactoside binding sites [5]. Although Galectin-1 has both intracellular and extracellular functions, its defining carbohydrate-binding role presents to be primarily extracellular [1].Galectin-1 plays a key role in HRAS and MAPK signaling pathways. Galectin-1 contains a plasma membrane binding protein that allows its interaction with GTP/HRAS. It triggers cell proliferation and mitogenesis through activated pathways RAS, RAF/ERK/MEK and IP3K/MTOR [6].Galectin-1 is demonstrated to be associated with a series of mechanisms including cellular aggregation, proliferation, tumor-induced angiogenesis and metastasis, and it contributes to cancer pathogenesis [6-11]. Galectin-1 expression is also shown to be associated with tumor progression in cervical carcinoma [12], oral squamous cell carcinoma [13], ovarian carcinoma [14], renal carcinoma [15], lung carcinoma [16], bladder carcinoma [17] and breast cancer [18].

In this context, the present study aimed to investigate the importance of galectin-1 in the tumor progression of breast carcinogenesis and its relationship with tumor presence by assessing preoperative and postoperative galectin-1 level in newly diagnosed operable patients with breast cancer and then comparing those levels with a control group.

Methods

Patient eligibility criteria

The present study included a healthy volunteering control group and a group of patients newly diagnosed with breast cancer who were planned for operation at the medical oncology outpatient clinic in Ataturk Training and Research Hospital, Katip Celebi University, in 2017-2018. Prior to study entry, we applied to our University's ethical board for interventional clinical trials and had their approval. A written informed consent form was obtained from all the patients included in the study. The inclusion criteria were set as follows: non-metastatic female patients aged 18-80 years, having no other malignancy except for breast cancer, no recently administered radiation or chemotherapy, no history of infection or trauma, and no neurodegenerative disease.

The subjects twice provided preoperative and postoperative (part of the first checkup after operation at the medical oncology outpatient clinic) venous blood samples in one biochemical tube. The healthy volunteers in the control group provided, for one time, venous blood samples in one biochemical tube.

Anthropomorphic measurements

We measured age, height and body weight of the patients that referred to the medical oncology outpatient clinic. The body mass index (BMI) was calculated by dividing the patient's weight by the square of her height (kg/m^2), and the World Health Organization reference interval was taken as basis [19].

Sample collection and storage

Blood samples were collected from the patients and healthy controls into venous blood gel tubes (Vacusera brand serum separator tube, 5 ml) at the sitting position, between 8:00-9:00 a.m., following 8-12 h of fasting. The blood samples were incubated for half an hour and then centrifuged at 3000 rpm for 10 min. Separated serum samples were portioned into safe-locked Eppendorf tubes and stored at -20°C until the tests were assayed. Patients' serum galectin-1 levels were determined with ELISA (enzyme-linked immunosorbent assay) kits used for scientific research. Patients' sera were solved at room temperature, and they were made homogeneous by using a vortex. These tests were assayed by employing the working procedures contained in the ELISA kits and performing necessary dilutions. At the end of the study, results were obtained by multiplying the data with an appropriate dilution factor. Serum galectin-1 was measured with a Fine Test (Wuhan Fine Biological Technology Co., Ltd. C6-323 Biolake, No. 666 Gaoxin AVE. Eastlake High-tech Development District, Wuhan, Hubei, China) ELISA kit. The intra- and inter-assay coefficient of variation (CV) of galectin-1 were <8% and <10%, respectively.

Statistics

The data were evaluated by using SPSS 16.0 for Windows package program. We performed a matched *t*-test to compare preoperative and postoperative galectin-1 levels of the oncological patients, Student's t-test to compare galectin-1 levels between the control and patient groups, and Mann-Whitney U test and Pearson's correlation analysis to associate the rates between the groups. $P<0.05$ was taken to indicate statistical significance.

Results

The present study included 39 healthy controls and 39 newly diagnosed non-metastatic patients with breast cancer, who were planned for operation. The mean age of the patients and the control group were 56.37±11.34 and 52.25±13.76, respectively. Mean body weight of the patient group was 72.94±13.4 kg and healthy group was 66±2 kg. Mean height measurements of the patients and the control group were 159.34±5.4 cm and 162.75±5.5 cm, respectively. Lastly, mean BMIs of patient group was 28.77±5.2 kg/m^2 and and healthy group was 24±1.64 kg/m^2. There was no statistically significant difference between the two groups in terms of age, weight, height or BMI (p=0.5, 0.31, 0.24, 0.07 respectively). Table 1 presents the clinical characteristics of the study population.

Table 1. Clinical characteristics of the study group

	Patient (n=39) mean ± SD	*Healthy controls (n=39) mean ± SD*	*p value*
Age, years	56.37±11.34	52.25±13.76	>0.05
Weight, kg	72.94±13.4	66±2	>0.05
Size, cm	159.34±5.4	162.75±5.5	>0.05
BMI, kg/m^2	28.77±5.2	24 ±1.64	>0.05

Table 2. Demographic characteristics of breast cancer patients

Characteristics	*Patients n (%)*
Age, years (mean±SD)	56.37±11.34
Surgical treatment	
Modified radical mastectomy	8 (20.5)
Simple mastectomy	3 (7.7)
Breast sparing surgery	28 (71.8)
Stage (TNM)	
I	16 (41.1)
II	14 (35.8)
III	9 (23.1)
Histologic grade	
I	2 (5.1)
II	25 (64.1)
III	12 (30.8)
Hormone receptor status	
ER/PR Positive	30 (76.9)
ER/PR Negative	3 (7.7)
ER Negative/PR Positive	1 (2.5)
ER Positive/PR Negative	5 (12.9)
Cerb-B2	
Positive	13 (33.3)
Negative	26 (66.7)
Ki-67	
0-10	13 (33.3)
10-20	13 (33.3)
>20	13 (33.3)
HER-2	
Positive	6 (15.3)
Negative	33 (84.7)

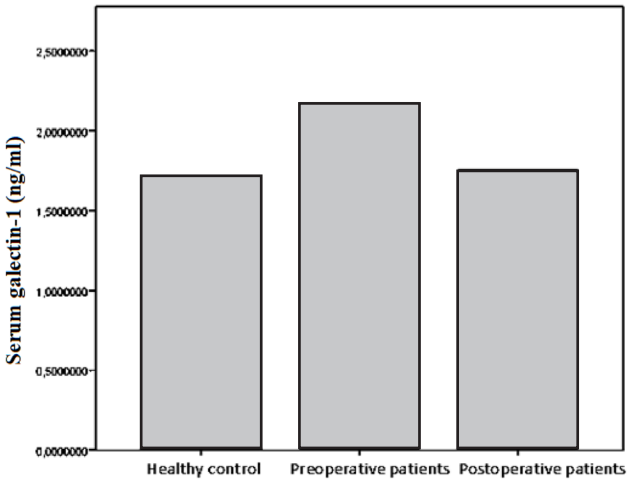

Figure 1. Distribution of serum galectin-1 levels between groups.

In terms of disease stage, 16 patients (41.1%) had stage 1, 14 (35.8%) stage 2 and 9 (23.1%) stage 3. As to mean galectin-1 levels with respect to the disease stage, stage-1 was 1.91±0.47 ng/ml, stage-2 2.44±0.91 ng/ml, and stage 3 2.19±0.48. There was no significant difference between the patients and control group in terms of galectin-1 levels by disease stage (p=0.11). Regarding the distribution of patients by histological grade, 2 (5.1%) patients had grade 1, 25 (64.1%) grade 2, and 12 (30.8%) grade 3. An examination of mean galectin-1 by histological grade showed 3.11±1.52 ng/ml in grade 1, 2.07±0.80 ng/ml in grade 2, and 2.19±0.42 in grade 3 (Table 2). Again, no significant difference was detected between the two groups in terms of grade (p=0.12). Breast cancer cases did not present a significant difference between their pathological Ki-67 and venous blood preoperative galectin-1 level (p=0.31).

As revealed by a comparison of mean galectin-1 levels between the groups, the preoperative patient group had the highest galectin-1 level. Preoperative and postoperative mean serum galectin-1 levels were 2.16±0.69 ng/ml and 1.75±0.31 ng/ml, respectively (Figure 1). There was a significant difference between preoperative and postoperative patient groups in terms of galectin-1 levels (p<0.001). A comparison of the control group with preoperative patients showed mean serum galectin-1 levels to be 1.64±0.40 ng/ml and 2.16±0.69 ng/ml, respectively. The difference between these two groups was significant (p<0.001). When the control group was compared with the postoperative patient group, the mean serum galectin-1 levels were 1.64±0.40 ng/ml and 1.75±0.31 ng/ml, respectively. There was no significant difference between these two groups (p=0.16) (Table 3).

Table 3. Serum galectin-1 levels in breast cancer patients and healthy controls

	Pre-operativepatient (n=39) mean ± SD	*Post-operativepatient (n=39) mean ± SD*	*p value*
Galectin-1 (ng/ml)	2.16±0.69	1.75±0.31	<0.001
	Pre-operative patients (n=39) mean ± SD	*Healthy controls (n=39) mean ± SD*	*p value*
Galectin-1 (ng/ml)	2.16±0.69	1.64±0.40	<0.001
	Pre-operative patients (n=39) mean ± SD	*Healthy controls (n=39) mean ± SD*	*p value*
Galectin-1 (ng/ml)	1.75±0.31	1.64±0.40	0.16

Discussion

Galectin-1 has been shown to be associated with carcinogenesis in different types of cancer. In the present study, we investigated the importance of galectin-1 in the progression of breast cancer development and its relationship with tumor presence. The study included newly-diagnosed and operable patients with breast cancer, and the serum galectin-1 level was assessed for 3 groups including the preoperative, postoperative and healthy control groups. When the preoperative and postoperative patient groups were compared as part of the study, galectin-1 level was significantly higher in preoperative than in postoperative patients. In addition, serum galectin-1 levels were observed to be significantly higher in the preoperative patient group compared to the healthy control group.

Although there are few studies in the literature investigating the galectin-1 level in breast cancer, available studies seem to investigate the expression of galectin-1 by immunohistochemical methods. In the present study, serum galectin-1 level was assayed by the ELISA method. In other cancers, some studies checked the galectin-1 level by an immunohistochemical method and others by the ELISA. In a study by Jung et al, the galectin-1 expression in the tumor tissue extracted after curative surgery was evaluated by an immunohistochemical method in 105 breast cancer cases (16 carcinoma in situ, 89 invasive ductal carcinoma). The samples presented no galectin-1 expression in the cancer tissue, while this expression was high in the cancer-associated stromal tissue. In addition, the same study detected a positive correlation between tumor stage and galectin-1 level. In our study, however, there was no significant relation between galectin-1 level and stage or histological grade. Another study by Zhu et al investigated the immunohistochemical expression of galectin-1 in breast cancer tissue and normal breast tissue. The results showed that carcinoma-associated fibroblasts isolated from breast tumor tissues synthesized more galectin-1 when compared with matched normal breast tissue fibroblasts [20].

Besides breast cancer, Chen et al studied serum galectin-1 levels in 140 patients with epithelial ovarian cancer and 70 healthy individuals by the ELISA method. In those patients, serum galectin-1 levels decreased in the postoperative period compared to the preoperative period. Serum galectin-1 levels were also significantly higher in patients with metastatic disease than those with localized tumors. This trend points to an association between increased serum galectin-1 levels, metastasis formation and tumor burden. Moreover, the same study found that galectin-1 level was increased in patients with recurrent ovarian cancer. After debulking surgery, galectin-1 serum levels were observed to be decreased in the same patient group with recurrent cancer [21]. In this sense, and in the light of the available data, we think that there may be a positive correlation between tumor presence and serum galectin-1 level. Another study conducted by Punt et al on patients with cervix cancer investigated the galectin-1 expression in 160 patients by an immunohistochemical method. The galectin-1 level was found to be high in patients with cervix cancer, but it was not evaluated postoperatively. In addition, similar to our findings, there was no significant relation between galectin-1 level and tumor stage [22].

Previous studies on different types of cancer have shown that patients with cancer have higher levels of galectin-1 than healthy controls [15,23-26]. For instance, in a study conducted by Kaneko et al in a group of renal cell carcinoma patients, a higher serum galectin-1 level was found in cancer patients than in healthy controls [15]. Likewise, Bosch et al also reported a high serum galectin-1 level in patients with pancreatic ductal adenocarcinoma [23]. Moreover, Arcolia et al reported that the level of immunohistochemical galectin-1 in the malignant thyroid tissue was again higher than

that of healthy controls [24]. These findings are consistent with our study.

The limitations of our prospective study include the absence of an immunohistochemical evaluation and short follow-up. In conclusion, in our study, the galectin-1 level was found significantly higher in preoperative breast cancer patients compared to the levels of galectin-1 in the postoperative period and in the healthy control group. This suggests that there may be a positive correlation between tumor presence and serum galectin-1 levels. In the light of these data, we believe that galectin-1 may play an important role in breast carcinogenesis and may be used as a guiding marker for patient follow-up or evaluation of treatment response. Further studies with a higher number of controls are required to shed light on this matter.

Conflict of interests

The authors declare no conflict of interests.

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