Assessment of Galectins -1, -2, -3, and -8 Expression in Endometrial Carcinoma and Its Clinical Implications

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Abstract

Background and aim: Galectins are a type of animal lectins that play a crucial role in regulating various cellular functions that can promote cancer progression including endometrial carcinoma. The aim of this study is to explore the expression of m RNA of galectins 1, 2, 3, and 8 in endometrial cancer and determine their relation to the extent of the disease.

Methods: This case-control study was carried out at the Ain Shams University Maternity Hospital, as well as in the molecular biology laboratory located in the Zoology Department of the Faculty of Science at Ain Shams University. The study was conducted between October 2019 and January 2022. A total of 72 patients were scheduled for hysterectomy due to endometrial diseases. The study involved two groups. Group 1 comprised 60 women with endometrial malignancy, which includes atypical endometrial hyperplasia and/or endometrioid adenocarcinoma. Group 2 was the control group, which had 12 women with normal endometrial tissues. 58 tumor samples of endometrial pre-cancer and cancer lesions for mRNA expression using qRT-PCR.

Results: The present study included 58 women with endometrial carcinoma, out of which almost 57% had endometrioid adenocarcinoma. Among them, 37 women were in FIGO stage I and II. The study found that LGALS1, LGALS2, LGALS3, and LGALS8 were significantly over-expressed in endometrial carcinoma patients as compared to the control group. However, the expression of these genes did not differ significantly when compared in different FIGO staging or based on the presence or absence of lymph node metastasis. There was also no significant difference in their expression when comparing patients with either endometrioid or non-endometrioid adenocarcinoma of the uterus.

Conclusion: Our findings support the role of galectins in endometrial carcinogenesis, disease progression, and lymph node metastasis.

Running title: Galectins in Endometrial Carcinoma

Keywords: Endometrial carcinoma; galectins; mRNA; qRT-PCR; biomarkers; lymph node metastasis.

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Introduction

Endometrial cancer is the second most common gynecologic cancer in Egypt after ovarian cancer (1).It is the fourth leading cause of death in women due to gynecologic cancers globally, with 382,069 new cases and 89,929 deaths in 2018 (2) . The incidence rate of EC is increasing rapidly and projected to increase by more than 50% worldwide by 2040 (3).

Endometrial cancer is classified into endometrioid and non-endometrioid types. Molecular-based classification is now used for improved diagnosis and prognosis.

Galectins are a type of animal lectins that possess one or more carbohydrate recognition domains.. (4-8) Galectins are proteins that can specifically bind to sugar molecules and exert their effects either inside or outside cells. Numerous studies have strongly linked galectins to tumor progression, due to their impact on immune surveillance, angiogenesis, cell migration, tumor cell adhesion, and cellular response to chemotherapy (9). Galectins have been studied in various types of cancer including hematologic, gastrointestinal, breast, prostatic, and gynecologic cancers, such as ovarian, cervical, and endometrial cancer (10-15).

In this study, we investigated galectins mRNA expression in endometrial carcinoma patients to evaluate their potential as biomarkers to disease stage and prognosis.

Patients and Methods

This case-control study was carried out at the Ain Shams University Maternity Hospital, as well as in the molecular biology laboratory located in the Zoology Department of the Faculty of Science at Ain Shams University. The study was conducted between October 2019 and January 2022. A total of 72 patients were scheduled for hysterectomy due to endometrial diseases. The study was approved by the Department of Obstetrics and Gynecology and gained the approval of the Faculty of Medicine (FMASU 258/2021).

Sample size Justification: To determine the sample size, we used the STATA program. The type of error (alpha) was set to 0.05 and the power (1-p) to 0.9. A previous study by Sun and Dai in 2019 (17) found that the expression of galactin 9 among normal cases was 20%, compared to 10.8% among cases group. Based on these values, the sample size was calculated to be 20 cases and 20 controls. However, we increased the sample size to strengthen the study.

The study involved two groups. Group 1 comprised 60 women with endometrial malignancy, which includes atypical endometrial hyperplasia and/or endometrioid adenocarcinoma. Group 2 was the control group, which had 12 women with normal endometrial tissues.

The population sample under study were informed about research protocol and were asked to participate and informed consent was taken from each participant.

- All data from participating women were confidentially protected.

The inclusion criteria were the women eligible for hysterectomy due to endometrial cancer or benign causes, willing to participate, and consent.

Exclusion criteria: were women who received hormone therapy, radiotherapy, or chemotherapy before surgery. (unlikely with endometrial cancer) Or women refused to participate in our study.

The personal and demographic data were collected, including age, parity, body mass index (BMI), menopausal status, medical and surgical co-morbidities.

Operative data:

All intraoperative details were collected from eligible women. During the surgical procedure, all intraoperative findings and

extent of surgery were documented, followed by the immediate removal of the uterus. A 2*2 cm sample of the endometrial tissue was collected and preserved in TRIzol reagent, then stored at -80 C until the beginning of the RNA extraction process. The remaining excised tissues were sent for histopathology, where the final FIGO staging and grade of endometrial carcinoma and histological subtype were recorded.

The tissues were preserved in RNA Later (Qiagen, Germany) and stored at -80°C until the RNA purification step takes place.

RNA isolation, cDNA synthesis, and qRT-PCR

To extract the total RNA from the freshly collected tumours, TRIZOL reagent (Bioflux, China) was used according to the manufacturer's instructions. Subsequently, cDNAs were synthesized with Genedirex's MMLV reverse transcriptase. PCR was carried out using specific primers for five Galectins (1, 2, 3, and 8) and GAPDH genes, with the same primer sequences as previously published data (16) . The Cycle threshold (CT) data was extracted onto Excel sheets and normalized to GAPDH. The fold change analysis was conducted using the 2-ΔΔ Cycle threshold formula.

Determination and purification of RNA concentration

The concentration of isolated RNA from endometrial tissue sample was measured using Nano Drop by calculating the absorbance ratio at 260nm to 280nm. Pure RNA has a ratio of 2.0 and isolated RNA has a ratio of 1.6-2.0.

Reverse Transcription

The purified RNA from endometrial tissue sample was reversely transcriped to cDNA using MMLV reverse transcriptase (Genedirex Taiwan) method.

Real- Time PCR Analysis

The qRT-PCR was performed using SYBR Green Real-Time Master Mix (Genedirex, Taiwan). The forward and reverse primers for the studied genes LGALS1, LGALS2, LGALS3 and LGALS8 were utilized. The fold changes of the Galactin family members, namely LGALS1, LGALS2, LGALS3, and LGALS8 were calculated and compared with the control group.

Statistical analysis

The data analysis was performed using IBM© SPSS© Statistics version 26 (IBM© Corp., Armonk, NY) and MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

For non-normally distributed continuous variables, the median and interquartile range were used, and differences were compared using the Mann-Whitney test for two-group comparison or the Jonckheere-Terpestra trend test for multiple-group comparison. Normally distributed continuous variables were presented as mean and standard deviation and categorical variables as counts and percentages.

The Spearman rank correlation was used to test correlations between numerical variables. The correlation coefficient (Spearman's rho) was interpreted as follows: <0.2 = very weak, 0.2 to 0.39 = weak, 0.4 to 0.59 = moderate, 0.6 to 0.79 = strong, and ≥0.8 = very strong.

To evaluate the predictive value of galectins expression, a Receiver-operating characteristic (ROC) curve analysis was conducted. The area under the ROC curve (AUC) was interpreted as follows: AUC < 0.6 = fail, 0.6 to 0.69 = poor, 0.7 to 0.79 = fair, 0.8 to 0.89 = good, and ≥ 0.9 = excellent.

Finally, multivariable binary logistic regression analysis was used to calculate the adjusted odds ratio for relevant outcome measures. P-values less than 0.05 were considered statistically significant.

Results

In this study, 80 patients were assessed for eligibility, of which 72 were included. Eight patients were excluded due to issues with RNA extraction caused by storage or processing problems. The analysis was based on data collected from 72 women diagnosed with endometrial diseases planned for endometrial sampling or hysterectomy. These women were divided into two groups: the study group, consisting of 60 women with endometrial malignancy (endometrioid adenocarcinoma), and the control group, composed of 12 women with normal endometrial tissues at the proliferative stage removed for benign causes.

Table 1. shows the different demographic, clinical, FIGO staging, histopathological diagnosis, and the fold change in galectin expression in studied cases.

Fifty-eight endometrial cancer samples were included in the final statistical analysis. The histopathology examination of the removed specimens proved endometrioid adenocarcinoma in 56.9% of the samples (33 patients) while the rest were of non-endometrioid type (43.1%).

Fifty-eight samples of endometrial carcinomas were analyzed using qRT-PCR to evaluate the mRNA expression of four galectins. The obtained Cycle threshold values were normalized to GAPDH. Then, the fold change was calculated by comparing samples to twelve samples of normal endometrial tissue.

Even though endometrioid adenocarcinoma is the most common clinicopathological findings of endometrial carcinoma (20), the current study revealed that endometrioid adenocarcinoma was found in 56.9% of cases followed by the non-endometrioid type in 43.1% of cases with LN metastasis in 62.1%.

Accordingly, the four galectins -1, -2, -3, and -8 showed no statistically significant difference in expression between cases with lymph nodes metastasis and those with no metastasis (p values = 0.791, 0.501, 0.370, 0.501) respectively (table 2,3).

The expression of galectins was also checked in relation to FIGO staging. that there were no statistically significant differences between the studied groups regarding Galectin-1, 2, 3 and 8 expressions in patients with various FIGO stages of stage IC, IIB, IIC, IIIB, IIIC and IV (p values = 0.890, 0.441, 0.961, 0.750) respectively (Table 4).

There was no significant statistical difference in the expression of the four tested galectins in endometrioid and non- endometrioid endometrial cancer.

The prediction value of the tested galectins was assessed by ROC curve either for lymph node metastasis , or high FIGO staging (stages III and IV) and they showed poor prediction value (figure 1and 2). Similarly, they were examined for the predictive value for the presence of organ metastasis. All four galectins had poor predictive value (AUC = 0.513, 0.558, 0.526 or 0.554 for galectin-1, galectin-2, galectin-3 or galectin-8, respectively) [Tables 5-7, Figures 1-3] .

DISCUSSION

Endometrial cancer is an epithelial malignant tumor that originates in the endometrium. It is one of the most common malignant tumors of the female reproductive tract (17). Its incidence rate is still rising, and the number of new deaths is expected to increase by 17.4% by 2025 [18]

Galectins regulate numerous cell functions critical for cancer progression, including elevated cell proliferation, cell adhesion and migration, apoptosis and immune suppression. Furthermore, the cell-cell and cell-matrix interactions exhibited by galectins and their high affinity for specific oligosaccharides make galectins promising markers and/or therapeutic targets for cancer. The expression and function of galectins in

EC prognosis and progression have not been well investigated to date [19].

Interest in the galectin family and its role as a diagnostic and prognostic marker for endometrial cancer has been addressed in many studies [20].

This study aimed to explore the galectins family expression in endometrial carcinoma and its relation to the stage of the disease.

As part of this study, seventy-two endometrial samples were analyzed, including twelve benign endometrium samples which were used as controls. The study focused on sixty women who had either endometrial malignancy or endometrial hyperplasia with atypia and investigated the expression of galectins 1, 2, 3, and 8 in these patients. The results showed that LGALS1, LGALS2, LGALS3, and LGALS8 were significantly overexpressed in patients with endometrial carcinoma, as compared to the control group. However, there was no significant difference in the expression of these genes when comparing different FIGO staging or based on the presence or absence of lymph node metastasis. Additionally, there was no significant difference in their expression when comparing patients with either endometrioid or non-endometrioid adenocarcinoma of the uterus.

Comparison of our results to similar studies

Different studies were done exploring the expression of Galectins family and clinicopathological features in endometrial carcinoma, some of them agree and others differ from these current results.

Galectin-1 expression by tumours is associated with poor prognosis and the formation of metastasis through modulation of among others cell migration, adhesion and angiogenesis [21].

Another team, Sun et al. [17] conducted a prospective study to explore the expression of Galectin-1 and clinicopathological features in endometrial carcinoma. The study involved the collection of endometrial tissues from a total of 91 patients who were admitted and treated surgically for endometrial diseases and revealed that the rates of positive expression of Galectin-1 in normal endometrial tissue (NE), atypical endometrial hyperplasia (AH), and endometrial adenocarcinoma (EC) were 30, 70, and 90.2%, respectively which was significantly higher in EC and AH than that in NE ($p < 0.05$) indicating that Galectin-1 is involved in the occurrence and development of tumor cells, while high expression of Galectin-1 suggested a poor prognosis.

Other several studies have been published on galectins in endometrial cancer, with contradictory results. Galectin-1, but not galectin-3, has been shown to increase in EC compared with normal endometrium (22). Similarly, galectin-1 was found to be increased in endometrioid EC from well differentiated to undifferentiated carcinoma [23-24]

Regarding galectin-2 expression in cancerous vs. normal tissue, few reports are published which are scarce and unpredictable. Cada et al. (2009) (25) reported on reduced expression in basal cell carcinoma of the skin as compared to normal skin. Another large study on galectin-2 expression in numerous cancerous tissues and their normal counterparts was performed by Saal et al. (2005) (26). They observed a reduction in colon cancer and an increase in thyroid cancer. No changes were observed in stomach, lung, kidney or bladder cancer (26).. Subsequently a systematic review showed no changes in pancreatic, colon, skin or kidney cancer while galectin-2 levels were elevated in ovarian cancer as well as in the stroma of lung and bladder cancer but decreased in liver and breast cancer [27].

As regard Galectin-3, Boutas et al. (2021) (20) conducted a systematic review to describe the outcomes of some studies which examined the levels of Galectin-3 expression in endometrial carcinomas, the outcomes, and the prognosis of these carcinomas. Two of the studies concluded that high expression

of Galectin-3 is associated with a tumor's histological grade, type and depth. This enhanced nuclear Galectin-3 expression might assist in progression to atypia and neoplasia (28-29). The other three on the contrary concluded that malignant tumors had a decreased expression of Galectin-3 and that Galectin-3 played a suppressive role in tumor growth [23-30-31]. Moreover, Labropoulou et al. (2016) (28) observed that the Galectin-3 expression level increased from low to high-grade endometrial tumors. Thus, demonstrated a strong correlation between protein expression levels, poor prognosis and survival in patients with EC.

Also, Ege et al. (2010) [30] examined 64 individuals diagnosed with endometrial cancer who underwent hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphadenectomy and evaluated the relationship of Galectin-3 expression to clinicopathological findings. They reported decreased expression of epithelial and stromal Galectin-3 in endometrial carcinogenesis and demonstrated the possible interaction of Galectin-3 with ER, PR, c-erbB2, and Ki-67 indicating a down-regulation of Galectin-3 in the endometrial cancer group compared to healthy individuals which was in concordance with Van de Brule et al. (1996) [23] who observed a significant down-regulation of Galectin-3 expression in endometrial cancer cells compared with normal mucosa.

Brustmann et al. (2003) [29] evaluated the expression of Galectin-3 in 101 curettage specimens from normal, hyperplastic, and neoplastic endometrial tissues by performing immunostaining and suggested that Galectin-3 expression may reflect the progression to atypia and neoplasia in endometrial tissues and Galectin-3 expression increased from normal to hyperplastic, atypical, and cancerous states of endometrial tissues, reflecting the classification of non-atypical hyperplasia, atypical hyperplasia with relation to endometrioid adenocarcinoma, and high grade endometrial serous papillary and clear cell carcinomas.

As regard Galectin-8, to our knowledge, Galectin-8 expression was not examined in women with endometrial carcinoma although Nikzad et al. (2013) [32] reported expression of Galectin-8 on normal human endometrium. They used immunohistochemistry staining to demonstrate that galectin-8 was expressed at a very low concentration during the proliferative phase but showed a high expression throughout the luteal phase. The expression of galectin-8 was observed in luminal surface epithelium, glandular epithelium and stroma. The up-regulation of the expression of galectin-8 during luteal phase may suggest galectin-8 as one of the potential molecular markers of the endometrial receptivity. [32]

Consequently, the current study is the first study that explores galectin-8 immuno- expression status in endometrial carcinomas and its association with other clinicopathological findings.

Other studies investigated Galectin-8 expression in other cancer types. Nagy et al. [33] reported a marked decrease in immunohistochemical expression of galectin-8 occurred with malignancy development in human colon tissue. Compared to normal tissues, galectin-8 expression appears to be increased in breast cancer, larynx cancer [34] and a subset of cutaneous T cell lymphomas, and decreased in skin cancer [25] as well as in several cancers of the digestive tract including pancreas and liver and.

Other Galectins family (Galectin-7, Galectin-9) may have role in EC and associations between galectin immuno- expression and grade, stage and poor differentiation of endometrial carcinomas but, unfortunately, their expressions in our study was not done due to limited resources and unavailability of kits.

Strengths and Limitations of Study

This study has several strengths, including its prospective study design, its location at a single tertiary care center, and having no

patients lost to follow-up during the study period. It is the first study to investigate the expression of galectin-2 and galectin-8 in endometrial carcinomas and its relation to the disease's stage.

However, there are also some limitations worth mentioning. Firstly, the study had a relatively small sample size compared to previous studies, and it was not a multicentric study. This represents a significant risk of publication bias. Secondly, the follow-up period for patients after surgery was relatively short, and there were no observations on patient survival and recurrence. Thirdly, the study was conducted during the pandemic covid-19, which limited the availability of patients.

Recommendation for Further studies: While informative, the number of samples per group was too small for conclusive observations and additional studies are required using larger patient groups. However, greater inclusive studies are undoubtedly of great value for estimating the diagnostic and prognostic values of this galectins in endometrial tumors.

CONCLUSION

Based on current evidence, Galectin-1, 2, 3 and 8 expressions in patients with endometrial carcinomas had no role for prediction of EC prognosis, LN metastasis and FIGO Stage.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical statements

The study was approved by the clinical research ethical committee. A written consent was taken from each patient or their legal guardian.

Consent for publication

Not applicable.

Competing interest

Authors declare no competing interest.

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Authors' contribution

Mahmoud M. Abdelfattah, Amira A Ghonim and Hany Naeem performed the experiments. Radwa Mansour Mohamed, Ahmed Sherif Abdelhamid, and Mohamed A M F Kortam supervised samples collection and data collection. Reham Helwa was involved in experimental design, data interpretation, and writing. All Authors revised and approved this manuscript.

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Table 1. Characteristics of the study population

Variable	Value
Age (years), mean ± SD (range)	50.2 ± 8.4 (30.0 to 76.0)
Age at menarche (years), mean ± SD (range)	11.7 ± 0.8 (10.0 to 13.0)
BMI (kg/m^2), mean ± SD (range)	28.4 ± 3.8 (21.0 to 36.0)
Parity, N (%)	
Nullipara	3 (5.2%)
Multipara	55 (94.8%)
Menopausal status, N (%)	
Premenopausal	43 (74.1%)
Postmenopausal	15 (25.9%)
Comorbidities, N (%)	
Medical	53 (91.4%)
Surgical	44 (75.9%)
Histopathology of D & C biopsy, N (%)	
Normal	5 (8.6%)
Endometrial hyperplasia	8 (13.8%)
Endometrial Carcinoma	45 (77.6%)
Optimal debulking, N (%)	58 (100.0%)
Metastasis, N (%)	
LN metastasis	36 (62.1%)
Organ metastasis	52 (89.7%)
FIGO classification, N (%)	
Stage IC	21 (36.2%)
Stage IIB	10 (17.2%)
Stage IIC	6 (10.3%)
Stage IIIB	10 (17.2%)
Stage IIIC	7 (12.1%)
Stage IV	4 (6.9%)
Histopathology, N (%)	
Non-Endometrioid	25 (43.1%)
Endometrioid	33 (56.9%)
Galectins expression (fold change), median (IQR) (min, max)	
Galectin-1	15.66 (0.95, 113.38) (0.01, 3136.63)
Galectin-2	1.21 (0.28, 4.59) (0.01, 140.07)
Galectin-3	1.26 (0.31, 4.53) (0.01, 168.90)
Galectin-8	7.41 (1.09, 32.22) (0.06, 3848.29)

IQR = interquartile range, max = maximum, min = minimum, N = number, SD = standard deviation

Table 2. Galectins expression in patients with or without LN metastasis

	No LN metastasis (N=22)		LN metastasis (N=36)		Mann-Whitney U test		
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value**
Galectin-1 (fold change)	17.51	0.95 to 113.38	13.74	0.96 to 133.75	379.500	-0.264	0.791
Galectin-2 (fold change)	1.77	0.28 to 4.76	1.01	0.28 to 4.43	354.000	-0.673	0.501
Galectin-3 (fold change)	2.08	0.50 to 4.53	1.14	0.29 to 4.53	340.000	-0.897	0.370
Galectin-8 (fold change)	8.39	1.26 to 32.00	6.63	0.99 to 33.26	354.000	-0.673	0.501

IQR = interquartile range, U = Mann-Whitney U statistic, Z = Z-statistic

Table 3. Galectins expression in patients with or without organ metastasis

	No organ metastasis (N=6)		Organ metastasis (N=52)		Mann-Whitney U test		
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value**
Galectin-1 (fold change)	14.13	0.95 to 223.63	15.66	0.96 to 105.70	152.000	-0.102	0.919
Galectin-2 (fold change)	1.77	0.65 to 2.66	1.15	0.28 to 4.68	138.000	-0.460	0.646
Galectin-3 (fold change)	1.07	0.63 to 4.38	1.26	0.29 to 5.28	148.000	-0.204	0.838
Galectin-8 (fold change)	3.43	1.26 to 7.26	8.62	1.06 to 33.26	139.000	-0.434	0.664

IQR = interquartile range, U = Mann-Whitney U statistic, Z = Z-statistic

Table (4): Galectins expression in patients with various FIGO stages

	Stage IC (N=21)		Stage IIB (N=10)		Stage IIC (N=6)		Stage IIIB (N=10)		Stage IIIC (N=7)		Stage IV (N=4)		Jonckheere-Terpstra trend test		
Variable	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	J-T	Z	P-value
Galectin-1 (fold change)	39.26	0.43 to 169.48	4.70	3.62 to 27.19	14.86	0.67 to 34.42	9.66	0.59 to 223.63	13.88	0.19 to 985.70	36.76	10.06 to 77.63	645.500	-0.139	0.890
Galectin-2 (fold change)	1.21	0.22 to 2.38	3.46	0.28 to 48.50	0.32	0.14 to 2.45	1.33	0.66 to 2.07	0.70	0.27 to 19.03	2.81	1.07 to 8.81	711.000	0.770	0.441
Galectin-3 (fold change)	1.27	0.42 to 2.87	5.20	0.57 to 62.25	0.23	0.15 to 3.86	0.77	0.34 to 3.03	0.50	0.08 to 32.67	2.35	1.32 to 6.01	652.000	-0.049	0.961
Galectin-8 (fold change)	5.70	1.25 to 13.74	12.69	2.89 to 212.31	26.27	0.76 to 34.30	7.24	1.91 to 38.85	1.05	0.46 to 136.24	13.98	5.59 to 26.31	678.500	0.319	0.750

IQR = interquartile range, J-T = Jonckheere-Terpstra statistic, Z = Z-statistic

Table 5. Receiver-operating characteristic (ROC) curve analysis for prediction of LN metastasis using galectins expression

	Marker			
ROC metric	**Galectin-1**	**Galectin-2**	**Galectin-3**	**Galectin-8**
Area under ROC curve (AUC)	0.521	0.553	0.571	0.552
Standard Error (SE)	0.081	0.080	0.079	0.078
95% Confidence interval	0.386 to 0.654	0.417 to 0.684	0.434 to 0.700	0.416 to 0.683
z statistic	0.259	0.659	0.892	0.672
Significance level P (AUC=0.5)	0.796	0.510	0.373	0.501
Youden (J) index†	0.104	0.230	0.205	0.139
Associated criterion	>1.952	≤1.414	≤3.031	≤0.323
Sensitivity (%)	69.4	63.9	75.0	13.9
Specificity (%)	40.9	59.1	45.5	100.0

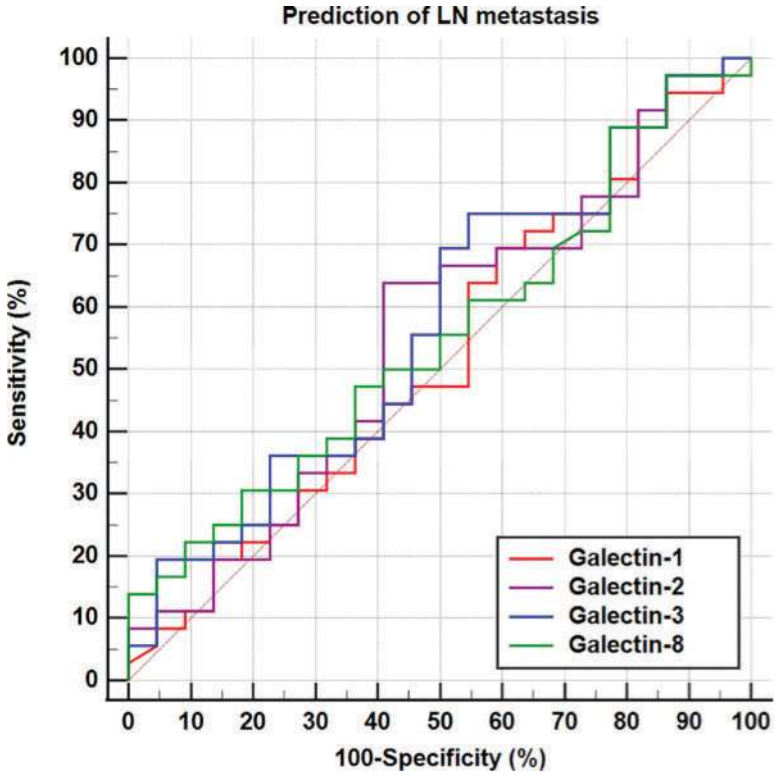

Figure 1. Receiver-operating characteristic (ROC) curves for prediction of LN metastasis using galectins expression. All four galectins had poor predictive value (AUC = 0.521, 0.553, 0.571 or 0.552 for galectin-1, galectin-2, galectin-3 or galectin-8, respectively).

Table 6. Receiver-operating characteristic (ROC) curve analysis for prediction of organ metastasis using galectins expression

	Marker			
ROC metric	**Galectin-1**	**Galectin-2**	**Galectin-3**	**Galectin-8**
Area under ROC curve (AUC)	0.513	0.558	0.526	0.554
Standard Error (SE)	0.140	0.117	0.119	0.107
95% Confidence interval	0.378 to 0.646	0.421 to 0.688	0.390 to 0.658	0.418 to 0.685
z statistic	0.092	0.493	0.216	0.509
Significance level P (AUC=0.5)	0.927	0.622	0.829	0.611
Youden (J) index†	0.250	0.263	0.199	0.372
Associated criterion	>1.075	≤1.682	≤0.574	>7.260
Sensitivity (%)	75.0	59.6	36.5	53.9
Specificity (%)	50.0	66.7	83.3	83.3

†. Youden (J) index = (Sensitivity + Specificity) - 1

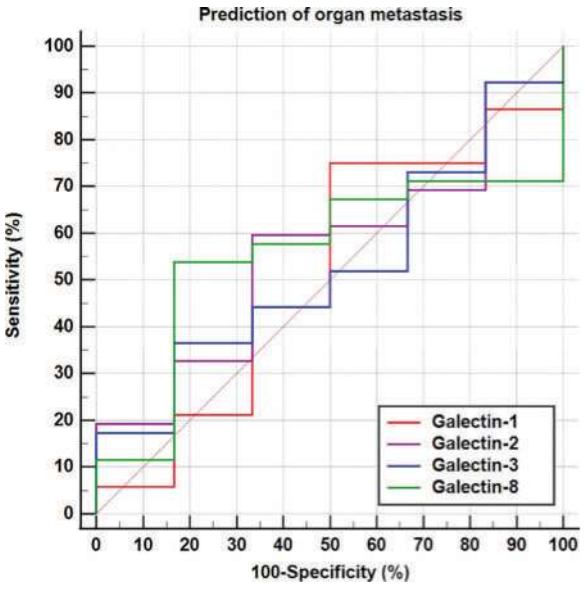

Figure 2. Receiver-operating characteristic (ROC) curves for prediction of organ metastasis using galectins expression. All four galectins had poor predictive value (AUC = 0.513, 0.558, 0.526 or 0.554 for galectin-1, galectin-2, galectin-3 or galectin-8, respectively).

Table 7. Receiver-operating characteristic (ROC) curve analysis for prediction of FIGO stage III/IV using galectins expression

	Marker			
ROC metric	**Galectin-1**	**Galectin-2**	**Galectin-3**	**Galectin-8**
Area under ROC curve (AUC)	0.501	0.560	0.515	0.526
Standard Error (SE)	0.082	0.077	0.080	0.080
95% Confidence interval	0.367 to 0.635	0.423 to 0.690	0.380 to 0.648	0.391 to 0.659
z statistic	0.016	0.776	0.184	0.329
Significance level P (AUC=0.5)	0.988	0.438	0.854	0.742
Youden (J) index†	0.109	0.263	0.109	0.115
Associated criterion	>0.190	>0.409	≤0.090	≤152.219
Sensitivity (%)	81.0	85.7	19.1	95.2
Specificity (%)	8.1	40.5	91.9	16.2

†. Youden (J) index = (Sensitivity + Specificity) - 1

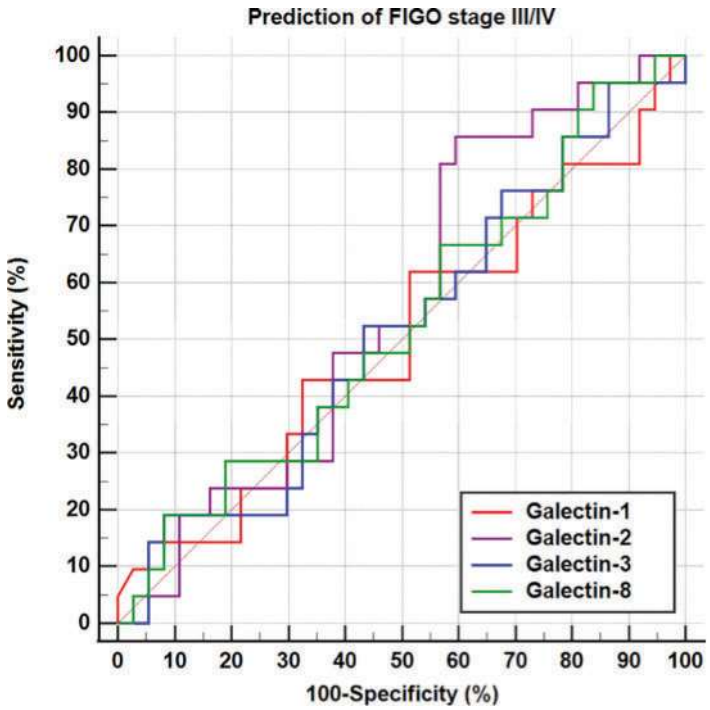

Figure 3. Receiver-operating characteristic (ROC) curves for prediction of FIGO stage III/IV using galectins expression. All four galectins had poor predictive value (AUC = 0.501, 0.560, 0.515 or 0.526 for galectin-1, galectin-2, galectin-3 or galectin-8, respectively).