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Galectin 1, 3, 8, and 9 as Potential Biomarkers for COVID-19 Infection

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Abstract

Objectives: This study aimed to assess the significance of these non-invasive biomarkers (Galectin-1-, 3, 8, and 9) in predicting early diagnosis of moderate state of COVID-19 infected patients and correlation with other parameters.

Methods: A prospective case–control study was carried out between February 2023 and February 2024 involving 80 patients diagnosed with moderate COVID-19 infection from multiple private clinics, alongside 40 healthy controls. Serum levels of Galectin-1, Galectin-3, Galectin-8, and Galectin-9 were measured in both groups using commercially available human ELISA kits.

Results: Galectin-1 illustrated no statistically significant difference in the median levels of galectin-1 between COVID-19 patients and controls ($P = 0.1321$) while Galectin-3, Galectin-8, and galectin-9 were demonstrated a higher significant difference in the median levels of galectin-3, Galectin-8, and galectin-9 in COVID-19 patients compared to controls ($P = 0.0306$, $P = 0.004$, and $P = 0.0001$ respectively).

Conclusions: The results recommend that galectin-3, 8, and 9 having a limited to moderate discriminatory ability and may have a possible important role to be a biomarker for moderate state of COVID-19 infection.

Keywords: COVID-19, SARS-CoV-2, biomarkers, galectins, galectin 1, galectin 3, galectin 8, galectin 9

Introduction

The coronavirus disease 2019 (COVID-19) is a worldwide pandemic disease caused by virus known as SARS-CoV-2 has been known as a chief global health problem and socioeconomic disaster.[1] These clinical manifestations of this disease ranged from asymptomatic infection, mild and moderate symptomatic state including fever, cough, dyspnea, diarrhea, and vomiting.[2,3] The last state is a severe fatal disease requiring mechanical ventilation and hospitalization especially in patients with chronic diseases like asthma.[4] During infection, there is a complex immune cells activation and interaction driven by antigen presenting cells, macrophages, neutrophils, lymphocytes, and released cytokines and chemokines.[5] There are some predictor risk factors that contribute in exacerbation of inflammation leading to hospital admission and worse outcome like gender, age, and chronic diseases.[6] Other factors that contribute in COVID-19 inflammation and pathogenesis like galectin which is a protein consists of fifteen members present at different tissues playing an important role in regulation inflammatory and immune responses via cell adhesion, migration, and proliferation.[7] They are divided into three groups according to molecular structure of the protein and the most frequent form is Galectin-1 and Galectin-3 which synthesized by macrophages, T cells and B cells.[8] Galectin-1 is an immune modulator through its anti-inflammatory effect.[9] Regarding Galectin-3 belongs to a family known as carbohydrate-binding proteins involved in exacerbation of many inflammatory disease, malignancy, and autoimmune diseases.[10] Other Galectin members is galectin-8 protein which expressed on normal tissues belongs to tandem repeat and function as a matrix protein enhancing cell migration and clustering via interaction with cell surface integrin inhibit cell adhesion and interact with T cell ligands induced proliferation, protection of cells from stimuli, modulate the function of neutrophils, modulate autoimmunity, and antimicrobial effect.[11] Moreover, Galectin-9 is an immunomodulator β-galactoside binding lectin that had an important role in many microbial infections via its location in the nucleus, cytoplasm, and cell membrane mediating interaction with microbes, regulating signals among cells, growth, differentiation of the cells. Level of Galectin-9 is increased in many diseases like viral, parasitic, autoimmune diseases, cardiovascular diseases, and tumor.[12]

The aim of this study was to assess the significance of these non-invasive biomarkers (Galectin-1-, 3, 8, and 9) in predicting early diagnosis of moderate state of COVID-19 infected patients and correlation with other parameters.

Patients and Methods

A prospective case-control study was done from February 2023 to February 2024, involving 80 patients who infected with moderate state of COVID-19 infection attending many privet clinics and compared with forty healthy controls. The research was approved by Ethical and Scientific committee of Al-Kindy College of Medicine-University of Baghdad No. 5 date 14-12-2023. Written informed consent was obtained from all participants prior to enrollment and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. The inclusion criteria were patients who had a positive nasopharyngeal swab for COVID-19 done by real time -polymerase chain reaction (RT-PCR). Those patients were complained from fever, reproductive cough, shortness of breath, tiredness, and weakness while the exclusion criteria were patients who had other diseases of the respiratory system, and negative RT-PCR test.

Five mLs of venous blood were aspirated from both patients and control groups. Complete Blood counts (CBC) were determined using an automated hematology analyzer (China). Other tests like biochemical test including C reactive protein (CRP) measured by turbidimetry, and coagulation D-Dimer test assessed by Enzyme linked fluorescence assay, were performed according to instruction of the manufacture's leaflets. Serum of both groups were quantified for Galectin-1, Galectin-3, Galectin-8, and Galectin-9 using human ELISA kit (Catalog No. YLA0676HU, YLA1505HU, and YLA1337HU respectively) (Biont-Chaina) depend on sandwich enzyme linked immune sorbent assay principle. The standard curve was generated by plotting the optical density for standards at 450nm versus the known concentration.

Statistical Analysis

Data of this study were analyzed using Excel, Medcalc, and statistical package for the social sciences (SPSS) version 25. Descriptive statistics were employed to summarize demographic variables and clinical characteristics of the patients. The Chi-square test was used to compare categorical variables between groups. Student's t-test was applied for comparing continuous variables with normal distribution, while the Mann-Whitney test was used for non-normally distributed data. Receiver operating characteristic (ROC) curve, area under the curve, specificity, sensitivity, positive predictive value, negative predictive value, and accuracy analysis was conducted to assess the diagnostic performance of specific biomarkers. Pearson and Spearman correlation coefficients were calculated to evaluate the relationships between different variables. An appropriate false discovery rate (FDR) correction were applied to all correlation analyses to control for multiple testing. Statistical significance was set at $P \leq 0.05$.

Results

This study included 80 moderate COVID-19 infected patients and forty healthy controls. There was a statistically significant difference in gender distribution between the two groups (patients and control), with a higher proportion of males in the control group (57.4%) compared to the patient group (36.4%) ($P = 0.03$). Regarding age variable, COVID-19 patients were significantly older compared to the control group (43.7 ± 1.85 years vs. 34.5 ± 3.13 years, $P = 0.00$). Other parameters demonstrated no significant differences in smoking status, the presence of chronic diseases like diabetes mellitus and hypertension, or vaccination status (two doses of Pfizer vaccine) between the two groups. Though, COVID-19 patients displayed significantly higher weight (64.6 ± 1.0 kg vs. 60.1 ± 2.4 kg, $P = 0.04$) and BMI (23.98 ± 0.34 kg/m² vs. 22.11 ± 0.72 kg/m², $P = 0.00$) compared to the control group Table 1.

Hematological Parameters

COVID-19 patients displayed significantly lower lymphocyte counts in patients' group (22.6 ± 1.2%) compared to the control healthy group (27.24 ± 1.92%, $P = 0.03$), indicating lymphopenia in complete blood count. No significant differences were exhibited in neutrophil and platelet counts between the two patients and control groups Table 2.

Inflammatory Markers

A markedly elevated C-reactive protein (CRP) level was detected in COVID-19 patients (25.54 ± 1.1 mg/dl) compared to controls (0.3 ± 0.01 mg/dl, $P = 0.00$), indicating a marked, and pronounced inflammatory response in COVID-19 patients. Othe marker was D-dimer was also significantly higher in COVID-19 patients (305.32 ± 26.72 μg/ml) compared to controls (0.2 ± 0.02 μg/ml, $P = 0.00$), suggesting increased coagulopathy in patients infected with COVID-19.

Serological Markers

Anti-COVID-19 IgG levels were significantly higher in COVID-19 patients (1.3 ± 0.66 mIU/ml) compared to healthy controls (11 ± 0.2 mIU/ml, $P = 0.00$), confirming the presence of activation humoral immune response to COVID-19 infection. No significant difference was found regarding anti-COVID-19 IgM levels between the patients and control groups.

The distribution of median galectin levels between moderate COVID-19 infected patients and control group was shown in Table 3, Galectin-1illustrated no statistically significant difference in the median levels of galectin-1 between COVID-19 patients and controls ($P = 0.1321$) while Galectin-3, Galectin-8, and galectin-9 were demonstrated a

Table 1. **Demographic variables of COVID-19 infected patients compared with healthy control group**

Variables	COVID-19 patients group no. = 80		Healthy control group no. = 40		95% CI Confidence interval	*P*-value
	No.	%	No.	%		
Gender (Male)	29	36.4	23	57.4	0.3432–0.5268	0.03*
Gender (Female	51	63.6	17	42.6	0.4731–0.6568	
Age (years)X ± SEM	43.7 ± 1.85 (14–81)		34.5 ± 3.13 (11–77)		2.4484–15.9934	0.00**
Smoking (+ve)	60	75.0	30	75.0	0.6627–0.8247	1.00
Smoking (-ve)	20	25.0	10	25.0	0.1755–0.3372	
Diabetes mellitus and hypertension (+ve)	21	26.25	10	25.0	0.1828–0.3463	3.54
Diabetes mellitus and hypertension (-ve)	59	73.75	30	75.0	0.6538–0.8171	
Pfizer vaccine (BNT162b2)(+ve)	56	70	30	75	0.6272–0.7951	0.42
Pfizer vaccine (BNT162b2)(-ve)	24	30	10	25	0.2049–0.3728	
Weight (Kg) X ± SEM	64.6 ± 1.0(41–82)		60.1 ± 2.4(35–91)		0.1125–8.9475	0.04**
Height (m) X ± SEM	1.64 ± 0.0 (1.5–1.9)		1.64 ± 0.1 (1.53–1.76)		–0.14463–0.16463	0.89
BMI (Wt/m²) X ± SEM	23.98 ± 0.34 (16.0–30.0)		22.11 ± 0.72 (13.5–9.3)		0.4950–3.2450	0.00**

*Significant (Chi² test). **Significant (student's t-test).

Table 2. **Comparative analysis of laboratory parameters between COVID-19 patients and healthy controls**

Tests	COVID-19 patients no. = 80 X ± SEM	Healthy control no. = 40 X ± SEM	95% CI Confidence interval	**P*-value
Neutrophils 10^9/L	7.92 ± 0.4 (3.4–20.2)	7.3 ± 0.3 (2–8)	−0.5780 to 1.8180	0.30
Lymphocytes %	22.6 ± 1.2 (4.8–50.7)	27.24 ± 1.92 (7–45)	−8.9422 to −0.3378	0.03**
Platelets 10^9/L	240.48 ± 7.6 (163–438)	245.02 ± 12.382 (122–410)	−17269.0076 to 17259.9276	0.99
C-reactive protein mg/dl	25.54 ± 1.1 (12–43)	0.3 ± 0.01 (0.2–0.4)	22.1529 to 28.3271	0.000**
D-Dimer µg/ml	305.32 ± 26.72 (101–1521)	0.2 ± 0.02 (0.2–0.5)	230.1315 to 380.1085	0.000**
Anti-COVID-19 IgG mIU/ml	1.3 ± 0.66 (0.11–35.10)	11 ± 0.2 (10–17)	−11.5731 to −7.8269	0.000**
Anti-COVID-19 IgM mIU/dl	1.69 ± 1.0 (0.14–53.1)	0.2 ± 0.01 (0.1–0.4)	−1.3165 to 4.2965	0.29

*Student's *t*-test. **Significant.

Table 3. **Distribution of Galectin levels between COVID-19 patients and healthy controls group**

	Galectin-1 ng/ml patients no. = 80	Galectin-1 control no. = 40	Galectin-3 patients no. = 80	Galectin-3 control no. = 40	Galectin-8 patients no. = 80	Galectin-8 control no. = 40	Galectin-9 patients no. = 80	Galectin-9 control no. = 80
Lower limit	0.179	0.509	1.68	0.2	0.07	0.101	2.1	9.58
Upper limit	7.973	9.994	260.18	96.0	1.469	0.884	199.1	146.8
Q1	0.613	0.898	12.46	6.86	0.1	0.181	11.25	23.58
Q3	1.737	1.701	28.51	22.26	0.256	0.370	29.88	36.47
IQR	1.124	0.803	16.05	15.4	0.147	0.189	18.62	12.89
Median	1.134	1.494	17.54	12.86	0.184	0.246	18.24	28.76
95% CI	−0.580 to 0.051		0.47 to 9.82		−0.109 to −0.021		−16.5 to −6.43	
P-value*	0.1321		0.0306*		0.004*		0.0001*	

*Mann- Whitney test.

higher significant difference in the median levels of galectin-3, Galectin-8, and galectin-9 in COVID-19 patients compared to controls ($P = 0.0306$, $P = 0.004$, and $P = 0.0001$ respectively).

Pearson correlation analysis was conducted to assess the relationship between galectin levels (galectin-1, galectin-3, galectin-8, and galectin-9) and demographic variables (age, weight, height, and BMI) (Table 4). There was a strong positive correlation among Galectin-1, galectin-3, galectin-8, and galectin-9 ($P = 0.01$)(Figures 1, 2, and 3), weight with galectin-3 and galectin-8 ($P = 0.01$), age with weight and BMI ($P = 0.01$), and weight with BMI ($P = 0.01$), while moderate positive correlations between Galectin-1 with age, weight, and height ($P = 0.05$), Galectin-3 with age and weight ($P = 0.05$), Galectin-8 with age and weight ($P = 0.05$), and Galectin-9 with weight and BMI ($P = 0.05$). Additionally, Galectin levels showed weak or no correlations with most laboratory parameters, including inflammatory markers (CRP, ESR), hematological parameters (neutrophil, lymphocyte, platelet levels), and coagulation markers (D-dimer) while there were a negative correlation was observed between galectin-1 and D-dimer ($P = 0.05$) Figure 4 and Table 5. Spearman's correlation analysis was conducted to assess the relationship between different galectin levels (galectin-1, galectin-3, galectin-8, and galectin-9) and other non-parametric variables like gender, smoking, and vaccination status and showed no significant difference (Table 6). An appropriate false discovery rate (FDR) correction were applied to all correlation analyses to control for multiple testing, and adjusted *P*-value showed significant. Table 7 summarizes the performance of Galectin-1, Galectin-3, Galectin-8, and Galectin-9 in predicting moderate state of COVID-19 infection based on area under the curve (AUC), cut-off value, sensitivity, specificity, and positive and negative predictive values (PPV and NPV) along with accuracy.

Receiver Operating Characteristic (ROC) curves were made to evaluate the diagnostic performance of galectin-1, galectin-3, galectin-8, and galectin-9 in predicting COVID-19 infection. Among the four galectins, Galectin-9 has the highest AUC (0.727) followed by Galectin-8 (0.662), Galectin-3 (0.622), and Galectin-1 (0.585). An AUC greater than 0.7 is generally considered acceptable, while an AUC greater than 0.8 is considered good. Therefore, Galectin-9

Table 4. **Pearson correlation between Galectins levels (1,3,8,9) and other demographic variables**

Variables	Galectin-1 ng/ml patients no. = 80	Galectin-3 patients no. = 80	Galectin-8 patients no. = 80	Galectin-9 patients no. = 80	Age (years)	Weight (Kg)	Height M	BMI Kg/M²
Galectin-1 ng/ml patients no. = 80	1	0.527**	0.594**	0.585**	0.227*	0.158	0.19	0.041
Galectin-3 patients no. = 80		1	0.871**	0.858**	0.234*	0.248*	0.114	0.183
Galectin-8 patients no. = 80			1	0.935**	0.202	0.286*	0.082	0.24*
Galectin-9 patients no. = 80				1	0.195	0.299**	0.132	0.219
Age (years)					1	0.447**	0.286*	0.289**
Weight (Kg)						1	0.453**	0.799**
Height (M)							1	−0.17
BMI (kg/m²)								1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

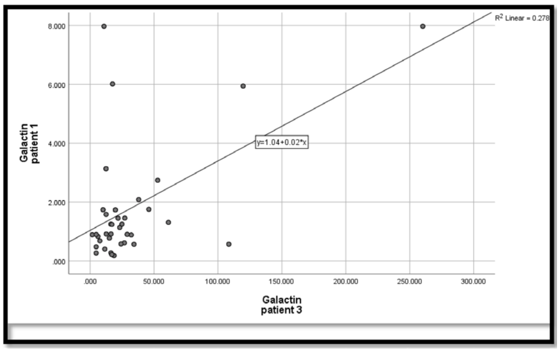

Fig.1 **Positive correlation between Galectin-1 and Galectin-3 levels in COVID-19 infected patients (R^2 linear = 0.0278) (P = 0.01).**

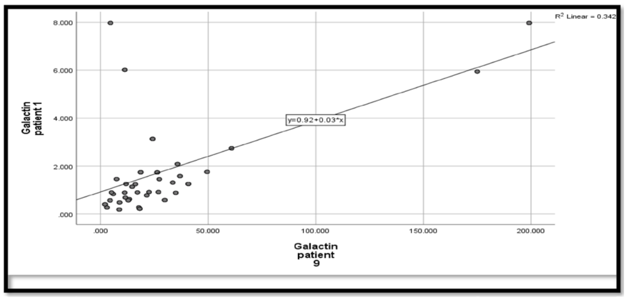

Fig. 3 **Positive correlation between Galectin-1 and Galectin-9 levels in COVID-19 infected patients (R^2 linear = 0.342) (P = 0.01).**

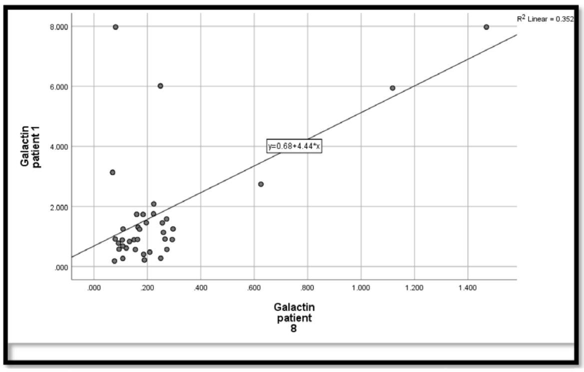

Fig. 2 **Positive correlation between Galectin-1 and Galectin-8 levels in COVID-19 infected patients (R^2 linear = 0.352) (P = 0.01).**

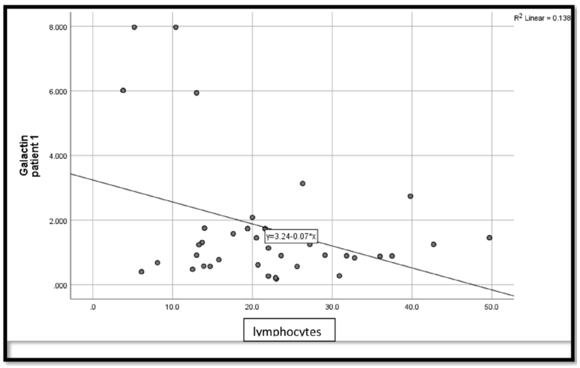

Fig. 4 **Negative correlation between Galectin-1 and lymphocytes levels in COVID-19 infected patients (R^2 linear = 0.138) (P = 0.01).**

shows the most promise as a biomarker based on AUC. There is a difference between sensitivity and specificity. Preferably, a biomarker should have both high sensitivity (true positives) and high specificity (true negatives). Regarding Galectin-1 has a moderate sensitivity (67.5%) but a relatively low specificity (52.5%) which means miss some true cases (low sensitivity) while low specificity may misclassify some healthy controls as positive (Figure 5). Galectin-3 shows similar tendencies to Galectin-1 with a moderate sensitivity (76.2%) and low specificity (50%) (Figure 6) while Galectin-8 has a balanced performance with a moderate sensitivity (62.5%) and a good specificity (67.5%) which may be a better choice than others (Figure 7). Galectin-9 has the highest AUC but a very low sensitivity (56.2%) and an extremely high specificity (97.5%) (Figure 8). This high specificity is necessary whereas the low sensitivity means it might miss a significant portion of true cases. This limits its usefulness and importance as biomarker.

Tabl 5. **Pearson Correlation between Galectins levels (1,3,8,9) and laboratory variables**

Variable	Galectin-1 ng/ml patients no. = 80	Galectin-3 patients no. = 80	Galectin-8 patients no. = 80	Galectin-9 patients no. = 80	Anti COVID-19 IgG antibodies	Anti COVID-19 IgM antibodies	CRP	ESR	Neutrophil level	Lymphocyte level	Platelets level	D-Dimer
Galectin-1 ng/ml patients no. = 80	1	0.527**	0.594**	0.585**	−0.076	−0.114	0.059	−0.111	−0.179	−0.371**	0.09	−0.221*
Galectin-3 ng/ml patients no. = 80		1	0.871**	0.858**	−0.016	−0.078	0.022	−0.089	0.096	−0.19	0.04	0.047
Galectin-8 ng/ml patients no. = 80			1	0.935**	−0.045	−0.035	0.117	0.044	0.093	−0.092	0.14	−0.022
Galectin-9 ng/ml patients no. = 80				1	0.013	−0.065	0.158	−0.006	0.016	−0.127	0.247*	−0.065
Anti COVID-19 IgG antibodies					1	0.802**	−0.146	0.024	−0.085	−0.086	−0.064	−0.053
Anti COVID-19 IgM antibodies						1	−0.061	0.088	−0.114	0.02	0.007	−0.035
CRP							1	0.00	−0.032	−0.059	0.038	0.2
ESR								1	−0.176	0.397**	−0.106	−0.012
Neutrophil level									1	0.262*	−0.162	−0.13
Lymphocyte level										1	0.09	−0.093
Platelets level											1	−0.006
D-Dimer												1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 6. **Spearman's correlation between Galectin levels (1,3,8,9) levels and other variables**

Variable	Gender	Smoking	Vaccination
Galectin-1 ng/ml patients no. = 80	−0.048	−0.05	−0.043
Galectin-3 ng/ml patients no. = 80	0.128	0.01	−0.199
Galectin-8 ng/ml patients no. = 80	−0.192	−0.068	0.171
Galectin-9 ng/ml patients no. = 80	0.175	0.155	0.034
Gender	1	0.766**	0.074
Smoking		1	0.063
Vaccination			1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Discussion

This cross-sectional study has identified older age, gender, weight, and BMI as a risk factor for COVID-19 infection which in align with previous studies that link age, overweight, and obesity as one of the major risk factors for infection, and severe COVID-19.[13,14] Obesity and overweight are associated with chronic inflammation and immune dysregulation due to Increased leptin levels which might contribute to susceptibility to viral infections.[15] The results also corroborate lack of significant differences in smoking status, chronic diseases (diabetes mellitus, hypertension), while regarding the differences in vaccination between two groups may be related to timing of sample collection. blood samples from patients were collected during the acute phase of infection, a period in which circulating IgG levels may not yet have peaked, whereas controls may have been sampled at a later time point following vaccination or prior infection, allowing for higher detectable IgG titers. Moreover, waning immunity and immune

Table 7. **Area under the curve, cut-off, sensitivity, and specificity of the Galectin-1, Galectin-3, Galectin-8, and Galectin-9**

Test result variables	Cut-off value	Sensitivity	Specificity	Area under the curve	SEM	*P*-value	95% Confidence Interval	+ PV	- PV	Accuracy
Galectin-1	≤1.455	67.5	52.5	0.585	0.052	0.106	0.491–0.674	74	44.7	0.212
Galectin-3	<12.032	76.2	50	0.622	0.055	0.029	0.528–0.708	75.3	51.3	0.2625
Galectin-8	≤0.209	62.5	67.5	0.662	0.051	0.002	0.57–0.745	79.4	47.4	0.300
Galectin-9	≤18.681	56.2	97.5	0.727	0.045	0.001	0.638–0.804	97.8	52.7	0.5375

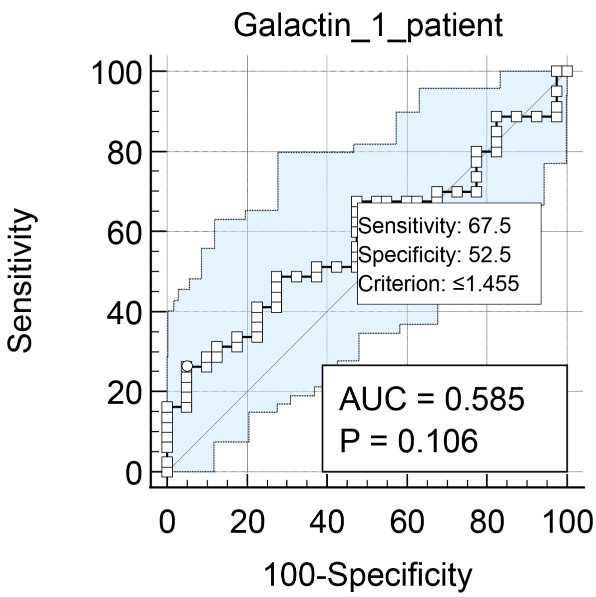

Fig. 5 **Receiver operating characteristic (ROC) curve plot of Galectin-1 for predicting COVID-19 (Area under curve = 0.585 (95% CI = 0.491 to 0.674) (*P* = 0.106).**

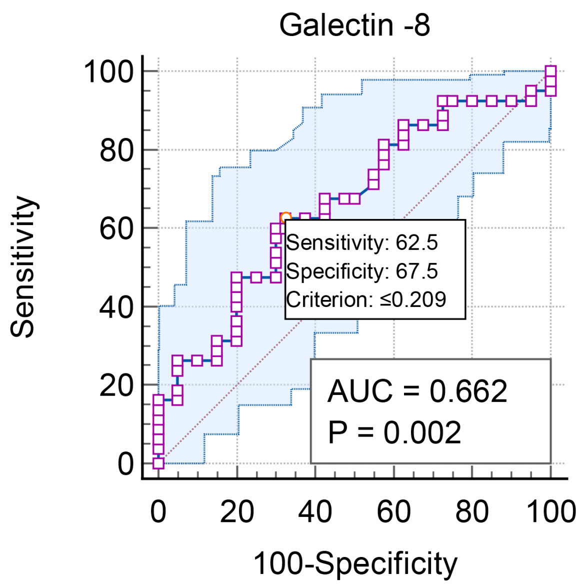

Fig. 7 **Receiver operating characteristic (ROC) curve plot of Galectin-8 for predicting COVID-19 (Area under curve = 0.662) (95% CI = 0.570 to 0.745) (*P* = 0.002).**

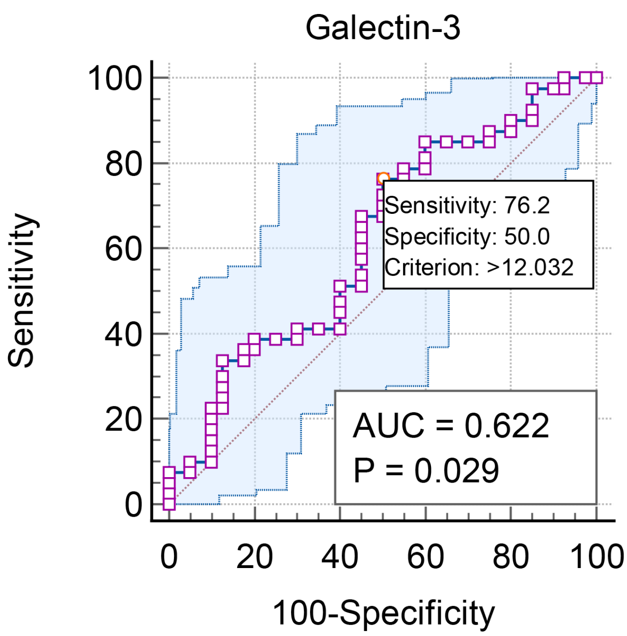

Fig. 6 **Receiver operating characteristic (ROC) curve plot of Galectin-3 for predicting COVID-19 (Area under curve = 0.622) (95% CI = 0.528 to 0.708) (*P* = 0.029).**

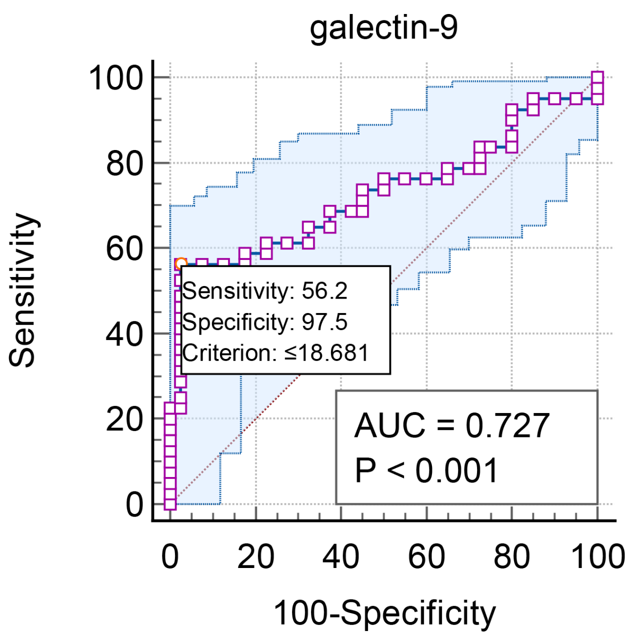

Fig. 8 **Receiver operating characteristic (ROC) curve plot of Galectin-9 for predicting COVID-19 (Area under curve = 0.727) (95% CI = 0.638 to 0.804) (*P* = 0.001).**

redistribution that in acute COVID-19 has been associated with transient immune dysregulation and redistribution of antibodies and immune complexes, which may result in lower measurable circulating IgG levels in patients compared with clinically stable controls. Assay-related factors that potential variability related to assay sensitivity, calibration, and detection thresholds, which may influence quantitative IgG measurements, particularly when comparing acutely ill patients with healthy individuals. These findings are inconsistent with previous research linking smoking (tobacco), chronic diseases, and vaccination to increased risk, hospital admission, and severity of COVID-19 infection.[16] Smoking can increase the expression of cathepsin B, and Furin (cell protease) that cleave the S1/S2 site of virus spike protein leading to viral spread.[17] These differences may be due to small sample size, cross-sectional design of the study in a specific geographical area and association cannot be inferred, and incidental. The study demonstrated a significant lymphopenia, raised CRP, and D-dimer levels in COVID-19 infected patients which are consistent with former researches.[18,19] This supports the dysregulation in the immune response due to virus particles may destroy the cytoplasmic components of lymphocytes causing apoptosis and increased in coagulopathy via elevated serum D-Dimer which is due to multifactorial factors.[20,21] These two biomarkers may help to assess the severity of the disease and prognosis. Other parameters showed absence of significant differences in neutrophil and platelet counts suggests that these hematological parameters might not be as specific biomarkers for moderate COVID-19 infection in this patient's group. This was in disagreement with other studies that exhibited especially in severe cases where the virus interact with leukocytes can intensify inflammatory state and thrombosis and this virus induced platelet activation may participate in thrombus formation and inflammatory responses in COVID-19 patients.[22] According to immunologic markers, significant increase in anti-COVID-19 IgG antibodies in patients group confirms the development and stimulation of a humoral immune response against the virus while absence of significance in anti-COVID-19 IgM levels might be due to the time, and period elapsed since infection in the study patients' group. This was in agreement with other studies that reported stimulation of IgG formation against spikes of COVID-19 virus.[23]

The main cornerstone of this study is assessment of different Galectin levels which reported a significant elevated levels of galectin-3, galectin-8, and galectin-9 compared to healthy controls. These findings suggest a potential important role for these galectins which are a family of carbohydrate binding proteins that involved in the host immune response, inflammation, and pathogenesis of COVID-19. These results were in accordance with previous results reported elevated level of Galectin-3 in severe cases of COVID-19 which implicate in inflammation, fibrosis, pathogenesis, modulate immune response, and serve as prognostic, predictive and therapeutic biomarkers monitoring the course of the disease and its sequelae.[24,25] Galectin-3 binds to its receptors on the cell surface of innate immune cells acting as alarmin stimulating secretion of TNF-α, IL-1β, IL-6, IL-12.[26] Other Galectin which is less studied in previous researches is Galecti-8 belongs to a family of animal lectins that showed a significant intriguing elevation in COVID-19 patients indicating its important role in inflammatory process, cells adhesion, immune regulation, proliferation, apoptosis, immune response, and COVID-19 pathogenesis.[27] Galectin-8 induces in paracrine and autocrine method enhancing the expression and secretion of many cytokines and chemokines like RANKL, IL-6, IL-1β, MCP-1es. It also binds to following receptors MRC2/uPAR/LRP1, integrins, and CD44 triggers FAK, ERK, Akt, and the JNK signaling pathways, leading to induction of NF-κB that promotes cytokine expression. This is known as vicious cycle leads to development cytokine storm which is a main contributor to the poor prognosis of infection.[28] Other Galectin is Galectin-9 has been associated with immune regulation, viral immunopathogenesis, and inflammation. The marked elevation of galectin-9 in COVID-19 patients in this study suggests a potential involvement in the disease pathogenesis. This was coincided with other researches who mentioned elevated level of Galectin-9 enhancing viral attachment, and replication in epithelial cells of respiratory tract induced pro-inflammatory cytokines secretion like IL-6, IL-8, IL-17, EIF2, and TNFα.[29] However, Galectin-1 showed a non-significant difference with control group which contract the results of other articles which showed elevated level of Galectin-1 in advance state of COVID-19 infection.[30,31] These differences may be due to moderate state of COVID-19 infected patients, sample characteristics, and size. These molecules might act synergistically to influence the progress of disease. So, further studies with larger sample sizes is necessary to elucidate its potential involvement.

Person's correlation indicated a strong positive correlation between galectin-1, galectin-3, galectin-8, and galectin-9, suggesting a possible collective shared regulatory immune mechanism or all of them had a common stimulus inducing and influencing their production. This is consistent with previous findings of other studies on the interrelationship of galectin family members and effect on immune response that become an important immune modulating, and biomarkers for the predicting, diagnosis, outcome, and prognosis of COVID-19 especially in lung fibrosis, and hopeful molecular targets for the progress of new therapeutic agents to treat COVID-19.[32] The positive correlation between galectin-3, galectin-8, and increased weight reinforces and support the association between obesity and increased levels of these immune inflammatory markers. This finding aligns with previous and former studies strongly linking increasing weight with elevated Galectin-3 leading to chronic inflammation and its possible role in COVID-19 pathogenesis.[33] The moderate positive correlation between age and Galectin-3 levels suggests a possible age-related increase in this biomarker which in accordance with Fontana Estevez FS. et al. 2022, finding a strong association of Galectin-3 and age dependent mechanisms of fibrosis and apoptosis.[34] Concerning the correlation between different Galectin levels and lack of significant with most laboratory parameters suggests that these galectins might not be directly involved in the pathogenesis acute phase response or other inflammatory processes assessed by these biomarkers. The negative correlation between galectin-1 and D-dimer is interesting and warrants further investigation. It suggests a potential inverse relationship between galectin-1 and coagulation activation. These findings are depended on correlational analysis and do not suggest causation. So further studies are needed to clarify the primary mechanisms involved in these relationships. There are limited studies are available specifically on the specific correlation between different galectin levels and other laboratory parameters in COVID-19 patients. However, previous studies have investigated the relationship between Galectin-3 and coagulation parameter (D-Dimer) in other diseases like Migraine which reported a positive correlation between Galactie-3 and D-dimer.[35] Other positive correlation was reported between galectin-3 and inflammatory marker CRP in heart failure.[36] Other non-parametric parameters showed no significant correlation with different Galectin levels suggesting might not had a major role in the context of COVID-19 infection in this study which may be due to small sample size, which limit the statistical power. Limited research is existing on the correlation between galectin levels and smoking and demonstrated that smoking induces Gal-3 synthesis and secretion that modulates the pathological signaling pathways in epithelial cells of the lung implicating Gal-3 as a new diagnostic biomarker in COVID-19 infected patients.[37]

The ROC curve analysis shows that Galectin-9 and Galectin-8 proves the best diagnostic performance for predicting COVID-19 infection with an AUC = 0.727, and 0.662 respectively and a statistically significant difference ($P = 0.000$, and 0.0018 respectively). This suggests that Galectin-9, and Galectin-8 has good discriminatory ability in differentiating between COVID-19 patients and healthy controls. Other biomarker is Galectin-3 also demonstrated a hopeful AUC of 0.622 with statistical significance is ($P = 0.029$), suggesting a possible predictive biomarker. Galectin-1 exhibit lower AUC values (0.585) and lack statistical significance ($P = 0.106$), indicating a limited diagnostic utility and efficacy for these biomarkers. So, the galectins investigated are now explicitly framed as adjunctive or exploratory biomarkers, potentially useful in combination with other clinical or laboratory parameters rather than as independent diagnostic tools. This was in agreement with other results reported by Nikitoppulou I. et al. 2023[38] who showed increased in these parameters in critical and severe cases and this study also found an increase in these biomarkers but in moderate state of COVID-19 infection. Other research found an elevated in these biomarkers and considered them as valuable and useful biomarkers for early diagnosis and prognosis of lung fibrosis post-COVID-19 infection and may be used as hopeful molecular targets for the development of new therapeutic drugs to treat COVID-19.[32] Iwasaki-Hozumi H. et al. 2023[39] reported Galectin-9 Is a Surrogate biomarker for defining the Severity of COVID-19 infection and monitoring the therapeutic effects of using monoclonal antibodies (Tocilizumab) in the treatment disease. Overall, the results suggest that Galectin-1,3,8, and 9 might be the most promising biomarkers considering its balanced sensitivity and specificity. Though, further research with larger sample sizes, and pay in consideration the confounding factors like age, gender, weight, and body mass index because galectins are known to be influenced by metabolic status, obesity, and age, due to these differences represent major confounding factors.

Potentially combining these biomarkers with other diagnostic tools is necessary to improve the accuracy of disease prediction. It is important to note that these results depend on this study and other factors are important like cost-effectiveness of these tests should be considered when assessing these galectins as biomarkers. The galectins investigated are now explicitly framed as adjunctive or exploratory biomarkers, potentially useful in combination with other clinical or laboratory parameters rather than as independent diagnostic tools. A biologically unexpected observation is reported in which anti-COVID-19 IgG levels are higher in controls than in patients .

Limitation of the Study

Further research with longitudinal designs is essential to found the sequential relationship between these demographic factors and COVID-19 infection. Galectin alterations may be influenced by demographic and metabolic factors and that further well-matched, longitudinal studies, and multivariate regression analyses adjusting for age, sex, and BMI are needed to confirm COVID-19–specific effects. Absence of patients' follow-up to assess the dynamic, and active changes in laboratory parameters during the course of COVID-19 infection and explore the effects and underlying mechanisms linking galectin levels to disease pathogenesis. A relatively small sample size might have influenced the statistical power to detect weak or no correlations.

Conclusions

The study population exhibited demographic characteristics consistent with previous findings on COVID-19 risk factors. The higher prevalence of older age, male gender, and obesity among COVID-19 patients highlights the importance of targeted prevention and management strategies for these high-risk groups. However, further research is needed to elucidate the complex interplay between demographic factors and COVID-19 susceptibility. The present study demonstrated significant differences in levels of Galectin-3, Galectin-8, and galectin-9 in COVID-19 patients compared to healthy controls. The present study revealed significant correlations between galectin levels and demographic variables, particularly weight and age. The present study revealed limited correlation between different Galectin levels and laboratory parameters in COVID-19 patients which play an important role in modulating inflammatory and coagulation processes. The results suggest. The results recommend that galectin-3,8, and 9 having a limited to moderate discriminatory ability and may have a possible important role to be a biomarker for moderate state of COVID-19 infection. So several observed associations require confirmation in larger, independent cohorts.

Conflict of Interest

None.

Funding

None.

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