Life Sciences 231 (2019) 116577

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Assessment of serum galectin-3, methylated arginine and Hs-CRP levels in type 2 diabetes and prediabetes

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ARTICLE INFO

Keywords:
Galectin-3
Methylated arginines
Type 2 diabetes
Prediabetes

ABSTRACT

Purpose: Galectin-3 is associated with the process of inflammation and fibrosis. The aim of this study was both to evaluate of galectin-3, methylated arginines and hs-CRP in subjects with type 2 diabetes and prediabetes and to investigate a relation between serum galectin-3, methylated arginines and hs-CRP levels.

Methods: In this study, all subjects were defined as the control group, type 2 diabetes ($n = 84$) by fasting plasma glucose and prediabetes ($n = 34$) by 75-g oral glucose tolerance test. Also, participants with type 2 diabetes were divided into as group I (HbA1c $\leq 7\%$, $n = 40$) and group II (HbA1c $\geq 7\%$, $n = 44$). The analysis of serum methylated arginines levels was analyzed by tandem mass spectrometry. Galectin-3 levels were determined via chemiluminescent microparticle immunoassay (CMIA).

Results: Serum galectin-3, ADMA, L-NMMA and SDMA levels were significantly lower in the control group (13.3 ± 3.42; 0.630 (0.13–1.36); 0.176 (0.02–0.53); 0.115 (0.04–0.26), respectively) compared to diabetic subjects (15.71 ± 4.22; 0.825 (0.23–2.80); 0.366 (0.08–1.41); 0.1645 (0.06–0.47), $p = 0.002$, $p = 0.01$, $p = 0.001$ and $p = 0.006$, respectively). Galectin-3 was positively correlated with hs-CRP ($r = 0.295$ $p = 0.001$), L-NMMA ($r = 0.181$ $p = 0.022$), HbA1c ($r = 0.247$ $p = 0.002$), neopterin ($r = 0.160$ $p = 0.045$) and FPG ($r = 0.207$ p = 0.001) respectively. Also, there was positively correlated ADMA with FPG ($r = 0.192$ $p = 0.016$) and eAG ($r = 0.235$ $p = 0.003$).

Conclusions: Thus, galectin-3 might be a useful prognostic marker in the population with prediabetes and diabetes. Moreover, it can be a marker showing the condition of developing complications in diabetic patients.

1. Introduction

Diabetes that rapidly growing over the world and threatening society is a global health problem. According to the 2017 reports of International Diabetes Federation (IDF), a total number of adults with diabetes (20–79 years) are about 425 million around the world and it is predicted that the number of diabetes will increase about 629 million by 2049 [1]. Macrovascular and microvascular complications occur in long-term follow-up of diabetic patients. Finally, diabetes-related mortality and morbidity are inevitable [2–4].

It is important to find out a biomarker at an early stage to prevent mortality and morbidity in diabetes. In particular, there is a need for potential biomarkers related to artery disease with type 2 diabetes. From this point, most studies show that AGE-R3 (galectin-3) is a scavenger receptor for AGEs and has an important regulatory role in many biological functions by interacting with some other molecules in the cell [5].

Galectin-3 is a member of the beta-galactoside-binding lectin family [6]. Galactin-3 consists of two separate structural regions which affinity for β-galactosides and a carbohydrate recognition domain (CRD) with consecutive amino acid chains [5,7]. C-terminal CRD has the role of lectin-glycoconjugate interactions and consists of approximately 130 amino acids. N-terminal domain, with a unique short end (approximately 30 amino acids) continuing with proline-glycine-alanine-tyrosine-rich (PGAY) repeat motif, plays a role peptide-peptide associations [5].

Galectin-3 is found both inside and outside the cell. It plays a role most important processes by binds to some ligands via CRD and N-terminal domain. For instance, intracellular galectin-3 acts modulating cell proliferation and differentiation by binding to various proteins

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https://doi.org/10.1016/j.lfs.2019.116577
Received 21 May 2019; Received in revised form 1 June 2019; Accepted 14 June 2019
Available online 15 June 2019
0024-3205/ © 2019 Published by Elsevier Inc.

Table 1
Clinical characteristic of normal distribution parameters among groups.

	Control (n:41)	Prediabetes (n:34)	Group I (n:40)	Group II (n:44)	P
Galectin-3 (ng/mL)	13.3 ± 3.42	14.28 ± 3.45	15.71 ± 4.22	15.65 ± 3.31	a = 0.249, b = 0.007, c = 0.002, d = 0.119, e = 0.079, f = 0.942
Age (years)	41.29 ± 10.22	48.76 ± 8.96	54.76 ± 8.70	52.65 ± 10.51	a = 0.001, b = 0.001, c < 0.001, d = 0.005, e = 0.08, f = 0.326
BMI (kg/m^2)	27.07 ± 4.67	29.23 ± 3.58	29.64 ± 5.13	30.86 ± 5.68	a = 0.015, b = 0.009, c < 0.001, d = 0.67, e = 0.064, d = 0.194
C-peptid (ng/mL)	2.30 ± 0.73	2.74 ± 0.74	2.88 ± 1.02	2.53 ± 1.20	0.151
eAG (mg/dL)	108.0 ± 8.24	118.07 ± 11.29	133.26 ± 15.16	219.53 ± 55.49	a = 0.499, b = 0.002, c = 0.000 d = 0,156, e = 0.000, f = 0.000
HOMA-IR	2.03 ± 0.21	2.70 ± 0.19	4.26 ± 0.58	5.46 ± 0.71	a = 0.779, b = 0.007, c = 0.000 d = 0.139, e = 0.001, f = 0.305

HOMA-IR: Homeostatic Model Assessment Insulin Resistance, *eAG*: Estimated Average Glucose. *BMI*: Body Mass Index, *LDL-C*: low-density lipoprotein cholesterol, *TC*: total cholesterol.
Values of P < 0.05 was considered significant and descriptive statistics are presented as mean ± standard deviation.
Group I are diabetics have regular blood sugar, Group II diabetics have unregulated blood sugar.
[a]Control-Prediabetes, [b]Control-Group I, [c]Control-Group II, [d]Prediabetes-Group I, [e]Prediabetes-Group II, [f]Group I-Group II.

Table 2
Demographic and clinical parameters of all groups.

	Control (n:41)	Prediabetes (n:34)	Group I (n:40)	Group II (n:44)	P
Hs-CRP (mg/dL)	0.1300 (0.02–1.75)	0.1350 (0.04–0.82)	0.1550 (0.03–1.23)	0.3850 (0.05–2.20)	a = 0.886, b = 0.247, c < 0.001, d = 0.234, e < 0.001, f = 0.002
ADMA (μmol/L)	0.630 (0.13–1.36)	0.535 (0.17–2.14)	0.565 (0.23–2.06)	0.825 (0.23–2.80)	a = 0.840, b = 0.380, c = 0.01, d = 0.558, e = 0.03, f = 0.01
SDMA (μmol/L)	0.115 (0.04–0.26)	0.112 (0.05–0.34)	0.1115 (0.05–0.31)	0.1645 (0.06–0.47)	a = 0.523, b = 0.536, c = 0.006, d = 0.892, e = 0.025, f = 0.021
L-NMMA (μmol/L)	0.176 (0.02–0.53)	0.256 (0.08–0.92)	0.30 (0.08–0.93)	0.366 (0.08–1.41)	a = 0.075, b < 0.001, c = 0.001, d = 0.389, e = 0.179, f = 0.395
Citrulline (μmol/L)	90.00 (18.8–230.0)	107.00 (45.2–455.0)	97.20 (34.7–285.0)	131.0 (22.9–441.0)	a = 0.135, b = 0.425, c = 0.002, d = 0.422, e = 0.144, f = 0.019
Arginine (μmol/L)	52.00 (12.60–148.00)	49.15 (22.8–300.0)	53.00 (21.8–284.0)	82.1 (28.7–272.0)	a = 0.386, b = 0.400, c = 0.001, d = 0.944, e = 0.016, f = 0.017
Arginine/ADMA ratio	87.4 (24.3–344.0)	95.2 (43.5–200.6)	86.65 (38,40-203,60)	125.15 (50.2–228.8)	a = 0.259, b = 0.640, c = 0.002, d = 0.058, e = 0.009, f < 0.001
SDMA/ADMA	0.20 (0.10–0.30)	0.20 (0.10–1.30)	0.20 (0.04–0.70)	0,20 (0.10–0.50)	0.748
TMA (μmol/L)	0.859 (0.24–2.09)	0.956 (0.42–3.14)	0.945 (0.56–3.00)	1.377 (0.42–3.69)	a = 0.636, b = 0.118, c = 0.002, d = 0.334, e = 0.025, f = 0.117
Neopterin (nm/L)	5.98 (2.39–20.19)	6.745 (0.68–12.10)	5.495 (2.69–14.81)	4.76 (0.18–8.59)	a = 0.078, b = 0.194, c = 0.001, d = 0.004, e < 0.001, f = 0.016
Fasting insulin (μIU/mL)	8.05 (2.25–32.30)	10.15 (2.78–19.61)	9.99 (1.18–37.31)	9.915 (3.15–47.50)	0.085
FPG (mg/dL)	92.0 (80.0–103.0)	108.00 (92.0–125.0)	117.00 (88.00–226.00)	172.00 (36.0–388.0)	a < 0.001, b < 0.001, c < 0.00, d = 0.025, e < 0.001, f < 0.001
HbA1c (%)	5.50 (5.0–5.9)	5.70 (5.00–6.90)	6.30 (5.20–62.00)	8.90 (7.10–14.30)	a = 0.003, b < 0.001, c < 0.001, d < 0.001, e < 0.001, f < 0.001
TG (mg/dL)	100.0 (40.0–321.0)	132.00 (36.0–336.0)	138.0 (4.6–619.0)	170.0 (40.0–627.0)	a = 0.145, b = 0.083, c < 0.001, d = 0.642, e = 0.001, f = 0.007
UACR		10.00 (4–48)	17.00 (4–275)	22.0 (2–777)	0.065
DBP (mmHg)	80.00 (64–80)	72.00 (9–100)	74.00 (70–100)	74.0 (65–110)	a = 0.002, b = 0.470, c = 0.580, d = 0.036, e = 0.031, f = 0.780
SBP (mmHg)	120.00 (100–140)	113.00 (90–148)	120.00 (90–150)	124.00 (90–160)	0.164

Values of P < 0.05 was considered significant and descriptive statistics are presented as median (Min-Max).
Hs-CRP: high-sensitive C reactive protein, *DBP*: diastolic blood pressure, *SBP*: systolic blood pressure, *FPG*: fasting plasma glucose, *HbA1c*: hemoglobin A1c, *TG*: triglycerides, *HDL-C*: high density lipoprotein cholesterol, *ADMA*: asymmetric dimethylarginine, *L-NMMA*: N^G-monomethyl L-arginine, *SDMA*: symmetric dimethylarginine, *TMA*: total methylarginine, *UACR*: microalbumin/creatinine ratios, Group I are diabetics have regular blood sugar, Group II diabetics have unregulated blood sugar.
[a]Control-Prediabetes, [b]Control-Group I, [c]Control-Group II, [d]Prediabetes-Group I, [e]Prediabetes-Group II, [f]Group I-Group II.

[8,9]. Extracellularly, galectin-3 plays a regulatory role in some events such as cell adhesion and inflammatory function through CRD. Recently, galectin-3 has been shown to be a prognostic marker in acute as well as in chronic heart failure (CHF) [9,10].

The studies demonstrated that galectin-3 levels have been associated with the physiopathology of diabetic patients. Insulin levels in galectin-3 knockout mice have been found higher in the healthy group compared to the diabetic group [11]. It has been shown the overexpression of galectin-3 prevented the beta cell destroyed because of the effect of pro-apoptotic cytokines [12]. Furthermore, strong evidence for galectin-3 have been shown that it is involved in the pathogenesis of diabetic complications via receptor function for advanced glycation end products (AGEs) and developing lipoxidation end products (ALEs) [5]. However, the role of galectin-3 is still unclear in diabetes and there is a need for studies. Our aim determine the association of galectin-3, methylated arginines and high sensitive CRP (hs-CRP) in diabetic and prediabetic patients.

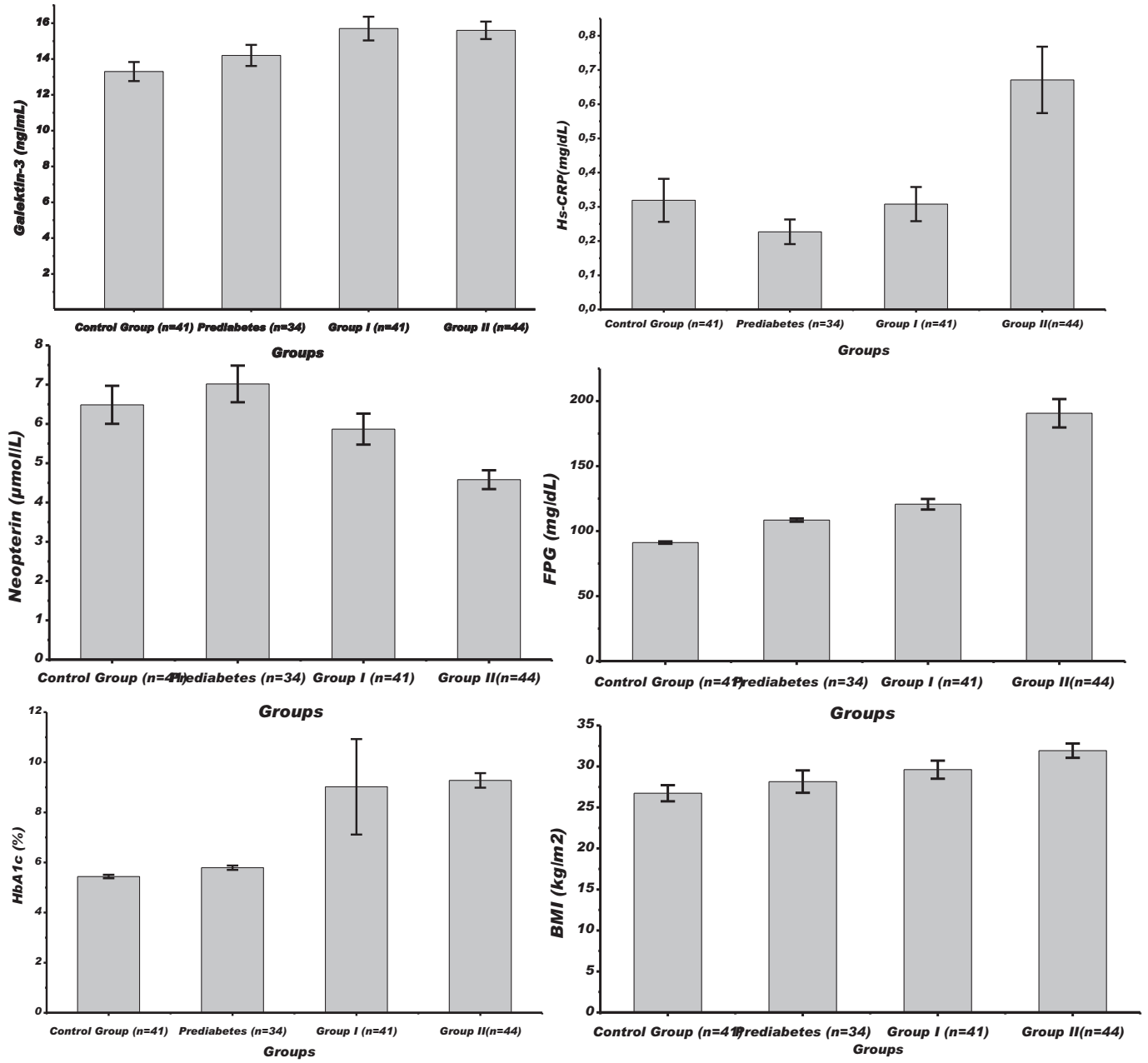

Fig. 1. Serum ADMA, L-NMMA, SDMA, citrulline, arginine, total methylarginine, arginine/ADMA ratio and SDMA/ADMA levels in participants including control, prediabetes, group I and group II.

2. Materials and methods

2.1. Subjects

159 participants (98 females and 61 males) admitted to outpatient Endocrinology Clinic at Selcuk University Medical Faculty in Konya, Turkey was enrolled to this study. Subjects with chronic diseases, malignancy, and thyroid diseases were excluded from the study. Homeostatic model assessment for insulin resistance (HOMA-IR), Quantitative insulin-sensitivity check index (QUICKI) and McAuley index was calculated [13]. The subjects in this study enrolled 41 control who had normal values of FPG and OGTT, 34 prediabetes by 2-h 75-g oral glucose tolerance test (OGTT, 140–199 mg/dL) and 88 type 2 diabetic by fasting plasma glucose (FPG, 100–125 mg/dL). Also, the participants with type 2 diabetes were divided into as group I (A1c $\leq$7%, n = 40) regulated type 2 diabetics and group II (A1c $\geq$7%, n = 44) unregulated type 2 diabetic. This study is confirmed by Selcuk University Faculty of Medicine Ethics Committee (Number: 2014/327; Date: 02/12/2014) and all subjects were informed about this study, and written consent of each patient was received.

2.2. Laboratory procedures

Blood samples were drawn into serum separator tubes by venipuncture from each subject after fasting for at least 8 h without the caloric intake. After centrifuging the samples were aliquoted and stored at −80 °C further until analysis. The samples were separated by centrifugation at 4000 × g for 10 min at 4 °C. When blood samples were collected via venipuncture, serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and fasting plasma glucose (FPG) levels were measured with the ARCHITECT c16000 clinical chemistry analyzer.

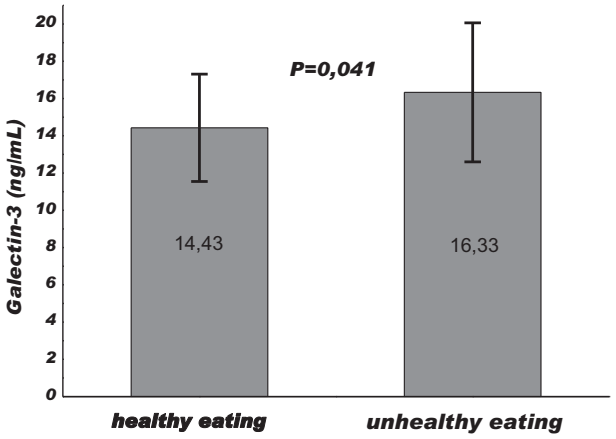

Fig. 2. Serum galectin-3 levels in participants unhealthy and healthy eating.

C-peptid and fasting insülin levels were measured by a Cobas e601 auto-analyzer (Roche Diagnostics, Germany). Hemoglobin A1c (HbA1c) was also measured by glycohemoglobin analyzer (G7 HPLC Analyzer, Tosoh Bioscience, USA) Serum samples were carefully separated and stored at −80 °C to study serum galectin-3, methylated arginines, and neopterin levels until the day of study.

The concentration of high-sensitivity CRP was analyzed using a high-sensitivity immunoturbidimetric assay on the Architect C8000 analyzer (Canada). 100 µL of serum sample for the neopterin analysis were added 50 µL of TCA (5%). The vortexed samples were centrifuged at 10000 rpm for 10 min at the end of incubation and were detected at 353 nm of excitation and 438 nm of emission by Agilent 1200 Series Fluorescence Detector [14].

Furthermore, insulin resistance was estimated using homeostatic model assessment (HOMA-IR). The HOMA index was calculated as (Fast Plasma Glucose (mmol/L) × Insulin (µIU/mL))/22.5 [15] and estimated average glucose (eAG) is calculated by the formula 28.7 × A1C − 46.7 = eAG (mg/dl) [16].

2.3. Methylated arginines levels measurement

The analysis of serum Asymmetric dimethylarginine ADMA, Symmetric dimethylarginine (SDMA) and L-N^G-monomethyl arginine (L-NMMA) levels was performed by using the Shimadzu LC-20AD chromatography system coupled with Applied Biosystems MDS SCIEX (ABSCIEX, Canada) API 3200 Triple-Quadrupole Mass Spectrometry in electrospray ionization (ESI) positive mode by Phenomenex Luna C18 column (Catalog Number:00G-4252-E0) with a modified method [17]. Shortly, 100 µL of internal Standard (deuterated7-ADMA) were purchased from Cambridge Isotope Laboratories (Catalog No: DLM-7476-5, USA) which was dissolved in HPLC grade methanol (Sigma Aldrich Cat. No: 34860-30L-R) were added to 200 µL each serum sample previously separated the sample centrifuged. The mixture was centrifuged at 13000 ×*g* for 10 min at 4 °C, so that proteins were precipitated to isolate from this mixture. The supernatant was taken into glass tube and dried under nitrogen (N2) at 65 °C for 20 min. 200 µL of a freshly prepared butanol (Sigma Aldrich Cat No: B7906-500ML) solution including 5% (*v*/v) acetyl chloride (Sigma Aldrich Cat No: 320129-1KG) for the derivatization process was added into the dried sample extract and this mixture incubated at 65 °C during 25 min. These samples were quickly dried under nitrogen gas at 65 °C. The specimens were dissolved in 100 µL of water–methanol (90:10, *v*/v) mixture including 0.1% (*v*/v) formic acid (Sigma Aldrich Cat No: 5438040100) and mixed then 40 µL was injected into LC-MS/MS system. 50 µM standards of ADMA, SDMA, L-NMMA, Arginine and citrulline were infused to device for optimal multiple reaction monitoring parameters were to determine thus preliminary fragmentation studies were performed. For this application method, the intra-day coefficient of variation (CV) and the inter-day CV were 8.6% and 10.1%, respectively. The limit of detection and the limit of quantification were below or equal than the lowest calibration point: LOD was 0.01 µM for all compounds in serum. The LOQ was 0.05 µM for ADMA, SDMA, L-NMMA, citrulline, and arginine.

2.4. Serum galectin-3 levels measurement

Prior to analysis, galectin-3 standard tubes were inverted about 10 times. Galectin-3 levels were quantitatively determined with a chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT i2000SR (Abbott Diagnostics, Abbott Park, IL, USA).

2.5. Statistical analyses

The data were evaluated using the IBM SPPS v21 program. In our study statistically; Values of $p < 0.05$ was considered significant and the data are expressed as mean ± standard deviation or median (interquartile range) in the evaluation of the data. Normality of the variables was evaluated by the one-sample Kolmogorov-Smirnov and Shapiro-Wilk tests. Differences between groups were tested using the One-Way ANOVA for parametric data and the Mann–Whitney *U* test for non-parametric data. Correlation between clinical parameters was assessed by Pearson's test.

3. Results

Demographic and biochemical findings of prediabetes, diabetes (group I and II) and the control group were expressed in Tables 1 and 2. No differences were observed with respect to total cholesterol, C-peptid, HDL-C, LDL-C, SDMA/ADMA, fasting insulin, systolic blood pressure and microalbumin/creatinine ratios between groups. BMI levels in the control group were significantly lower (27.07 ± 4.67) than the other groups, but BMI did not differ between prediabetes (29.23 ± 3.58) and diabetes (groups I and II). The age in the control group was lower than compared to other groups. While the mean age of the prediabetics (48.76 ± 8.96) was lower than compared to group II, it did not differ between prediabetes and group I. No differences with respect to age were observed between group I (54.76 ± 8.70) and group II (52.65 ± 10.51) (Table 1).

Serum galectin-3 levels of the control (13.3 ± 3.42) were significantly lower than that of group I (15.71 ± 4.22, $p = 0.007$) and group II (15.65 ± 3.31, $p = 0.002$), whereas galectin-3 levels did not differ between other groups ($p > 0.05$) (as shown in Fig. 1 and Table 1).

Serum galectin-3 levels in participants with healthy eating (14.42 ± 2.88) were significantly higher than compared to unhealthy eating (16.33 ± 3.732) (as shown in Fig. 2).

The ADMA ($p = 0.01$, $p = 0.03$ and $p = 0.01$, respectively), SDMA ($p = 0.006$, $p = 0.025$ and $p = 0.021$, respectively), arginine/ADMA ratio ($p = 0.002$, $p = 0.009$ and $p < 0.001$, respectively) and hs-CRP values ($p < 0.001$, $p < 0.001$ and $p = 0.002$, respectively) in group II were significantly higher than compared to other groups ($p < 0.05$). Furthermore, group I and II had significantly higher L-NMMA values than the control group, but there was no significant difference between the other groups (as shown in Fig. 3 and Table 2).

Finally, serum neopterin values in group II ($p = 0.001$, $p < 0.001$ and $p = 0.016$, respectively) were significantly higher than compared to the levels of other group. Also, neopterin values in prediabetes was significantly higher than in group I diabetes ($p = 0.004$).

There was a positive correlation between galectin-3 with hs-CRP ($r = 0.295$ $p = 0.001$), L-NMMA ($r = 0.181$ $p = 0.022$), BMI ($r = 0.277$ $p = 0.001$), HbA1c ($r = 0.247$ $p = 0.002$), neopterin ($r = 0.160$ $p = 0.045$), age ($r = 0.350$ $p = 0.000$) and FPG ($r = 0.207$ $p = 0.001$) in the subjects. Furthermore, galectin-3 levels have a positive correlation with eAG ($r = 0.247$ $p < 0.002$) and HOMA-IR ($r = 0.185$

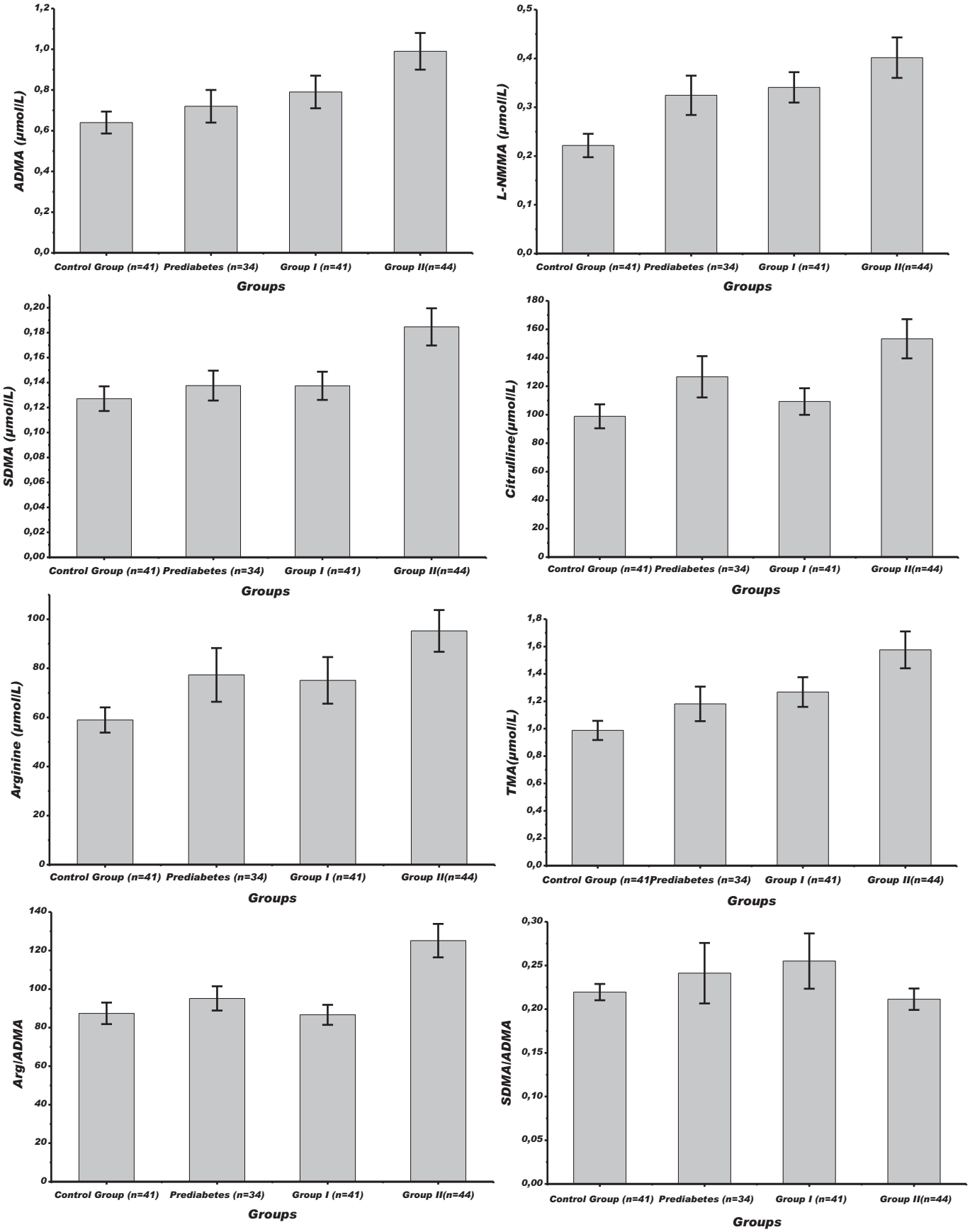

Fig. 3. Serum ADMA, L-NMMA, SDMA, citrulline, arginine, total methylarginine, arginine/ADMA ratio and SDMA/ADMA levels in participants including control, prediabetes, group I and group II.

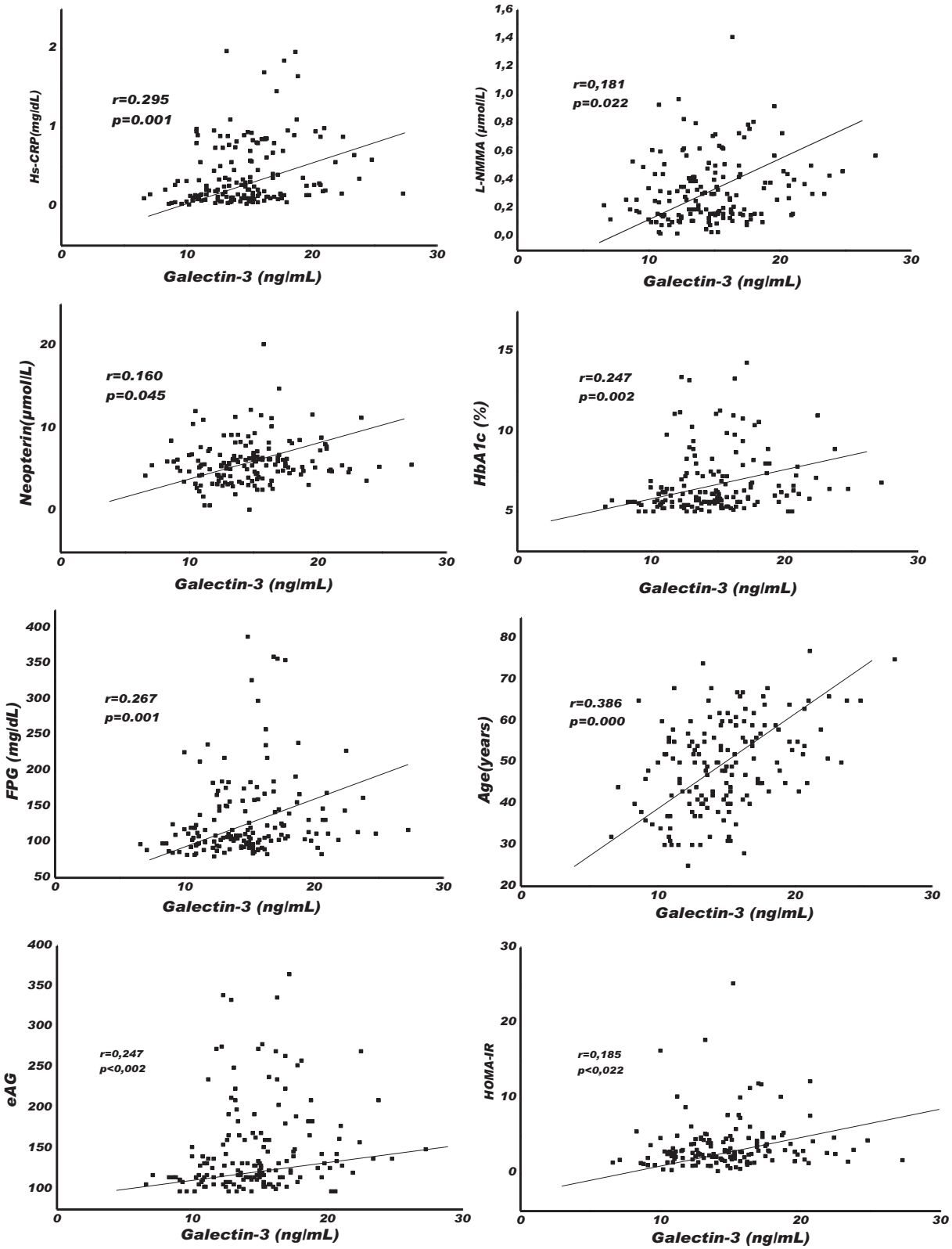

Fig. 4. Positive correlation of serum galectin-3 with hs-CRP, L-NMMA, neopterin, HbA1c, FPG, age, eAG and HOMA-IR in the subjects were calculated by simple regression analysis.

$p = 0.005$). However, there were no correlations between galectin-3 and ADMA ($p > 0.05$) (as shown in Figs. 4 and 5).

There was positively correlated ADMA with FPG ($r = 0.192$ $p = 0.016$), HbA1c ($r = 0.235$ $p = 0.003$) and eAG (r = 0.235 p = 0.003). Furthermore, L-NMMA was positively correlated with FPG ($r = 0.155$ $p = 0.053$) (as shown in Fig. 5).

Finally, there was positively correlated serum hs-CRP with FPG ($r = 0.441$ $p = 0.000$), HbA1c ($r = 0.400$ p = 0.000), eAG (r = 0.441 p = 0.000) and HOMA-IR ($r = 0.314$ p = 0.000) (as shown in Fig. 6).

Duration of diabetes (months) of individuals was positively

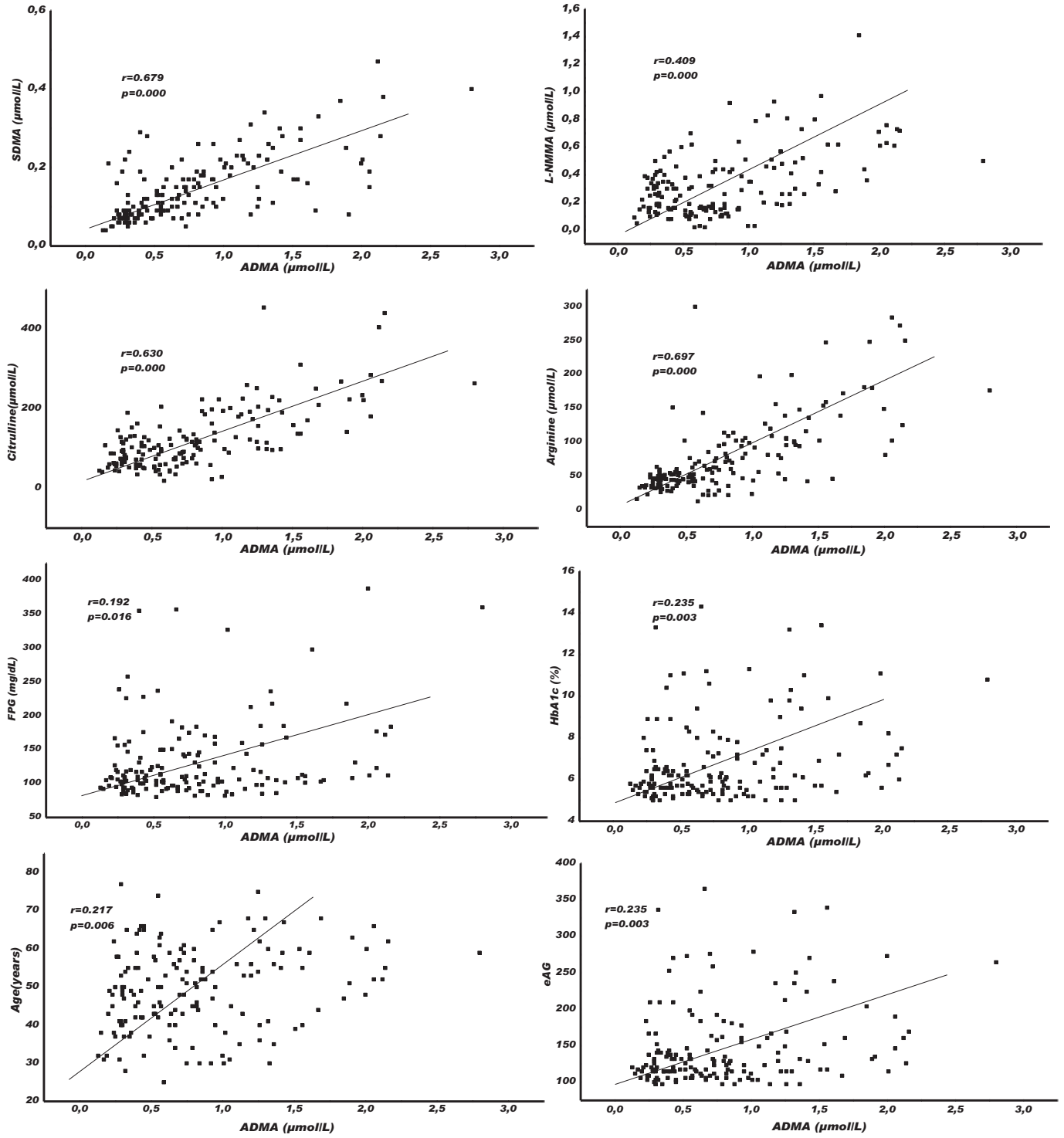

Fig. 5. Positive correlation of serum ADMA with SDMA, L-NMMA, citrulline, arginine, HbA1c, FPG, age, and eAG in the subjects were calculated by simple regression analysis.

correlated with ADMA ($r = 0.313$ $p = 0.006$), SDMA ($r = 0.321$ $p = 0.004$), citrulline ($r = 0.280$ $p = 0.014$) and arginine ($r = 0.343$ $p = 0.033$) (as shown in Fig. 7).

4. Discussion

Our data provide evidence that the serum galectin-3 level in group I and group II was higher comparison to both prediabetics and the control group. Originally, galectin-3 levels in diabetes were significantly higher than in other groups. Moreover, galectin-3 was correlated with HbA1c, BMI and FPG in the subjects. Thus, galectin-3 is a useful prognostic marker of diabetes in clinical practice and diabetes could be identified with high sensitivity and specificity. The effect of galectin-3 on the development of type 2 diabetes and prediabetes has remained unclear. In this study, galectin-3 levels in group I and group II was significantly higher than compared to both prediabetics and the control group. As a result, levels of galectin-3 have been reported higher than in patients with type 2 diabetics depending on the diabetic complication

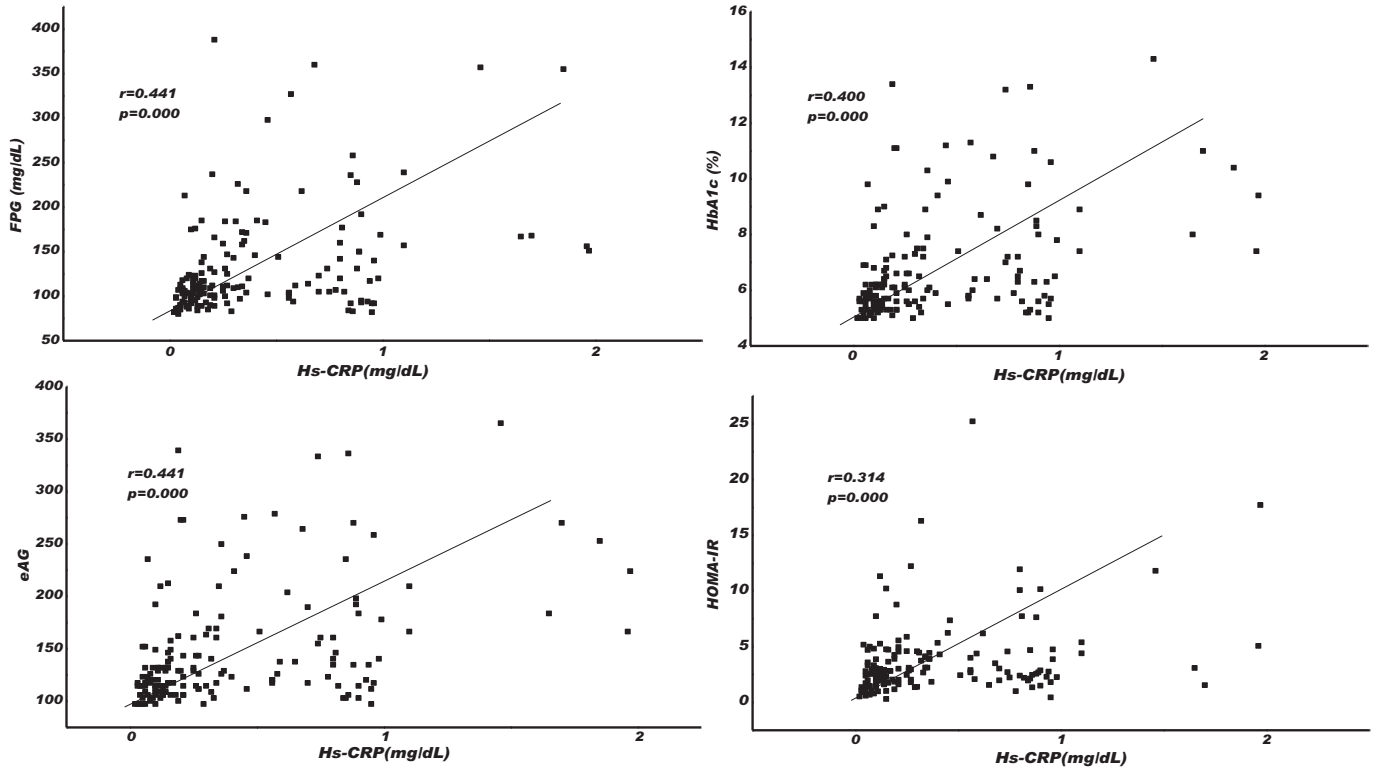

Fig. 6. Positive correlation of serum hs-CRP with HbA1c, FPG, eAG and HOMA-IR in the subjects were calculated by simple regression analysis.

[18].

Similarly, several studies reported that galectin-3 level in type 2 diabetics was significantly higher that of other groups and serum galectin-3 levels were related to FPG and BMI [19,20]. These studies suggested that galectin-3 might generally be both an independent predictor and a useful prognostic marker of diabetes in the population.

Galectin-3 is not exactly the cause of this relationship with diabetes, but both our findings and the results of some studies explain slightly this problem. There is a positive correlation between galectin-3 and hs-CRP levels. Hs-CRP levels were also observed statistically higher in diabetic patients. The association of galectin-3 with CRP supports that galectin-3 is also a key component of the defense mechanism against microbial infections and increases in the onset of acute and chronic inflammation. This finding is consistent with the literature findings. For example, Weigert et al. found that there was a positive correlation between galectin-3 and hs-CRP levels [20]. Misra et al. and Asegaonkar et al. have also found higher levels of hs-CRP in diabetes subjects than the controls [21,22]. In an animal, experiment reported that CRP and IL-6 were significantly increased in the serum of galectin-3 deficient mice which supplied a high-fat diet compared to the diet-matched wild type mice [23]. In our findings, hs-CRP was correlated a positive with galectin-3 and both markers were found higher in patients with type 2 diabetic. Another study demonstrated fasting glucose in galectin-3 knockout mice were reported higher than normal mice [11]. Thus, galectin-3 can be a marker showing the condition of inflammatory diseases such as atherosclerosis and especially complications in diabetic patients. Moreover, galectin-3 deficiency led to the rapid development of diabetic nephropathy [10].

Galectin-3 might have an up-regulatory effect on advanced glycated human serum albumin-induced expression of endothelial cell specific molecule-1 or endocan [24]. In fact, the expression of endothelial cell-specificmolecule-1 gene is under the regulation of inflammatory cytokines, which contribute diabetes-induced endothelial dysfunction [25]. There are evidence that galectin-3 may directly activate the peroxisome proliferator-activated receptors (PPARγ) on adipocytes and leads to adipocyte differentiation in vitro and in vivo [26]. Thus, galectin-3 may consider a biomarker of vascular injury at early stage development of diabetes mellitus.

Galectin-3, is a scavenger receptor of AGEs [5], play a role in many biological effects both inside and outside the cell by interacting with various molecules [9]. Both our findings and other studies reported that galectin-3 levels increased in patients with type 2 diabetic. One of the reasons why levels of galectin-3 in patients with type 2 diabetic were higher than according to healthy individuals is also the AGEs consisting of the presence of hypoglycemia for a long time. In this way, it can be interpreted that galectin-3 has effects on the function of AGEs/ALEs and plays mediately a role in diabetic complications. However, it is not known whether galectin-3 causes the development or reduction of diabetic complications via AGEs/ALEs. In some studies reported that galectin-3 in circulation or on the cell surface protection from the effects of AGEs/ALEs and some studies have reported that galectin-3 knockout mice develop accelerated diabetic complication [10,26].

Few studies have been reported that high CRP level association with prediabetes and diabetes [21,22]. We found that CRP levels in the only dysregulated type 2 diabetic groups between all groups were statistically higher than the other groups. We can say that the reason for the CRP levels were significantly higher in dysregulated diabetics is due to acute inflammations that occur during diabetes. Chronic inflammation is known as a major risk factor for diabetes-related mortality [27]. CRP level has been reported to be a useful biomarker in early estimating chronic processes in diabetic patients. These studies demonstrated that high CRP levels have been shown to be associated with increased cardiovascular risk in type 2 diabetics [28,29]. The finding recommends that CRP levels are important in the progression of diabetic patients with chronic inflammation. But galectin-3 may be a more useful marker than CRP in the pathogenesis of inflammation-mediated diabetes. Moreover, Qi-hui et al. (2013) reported that there was a positive correlation between serum galectin-3 and CRP and that serum galectin-3 was better than CRP in the predictor of vascular complications [30].

Increasing levels of ADMA that is an endogenous inhibitor of nitric

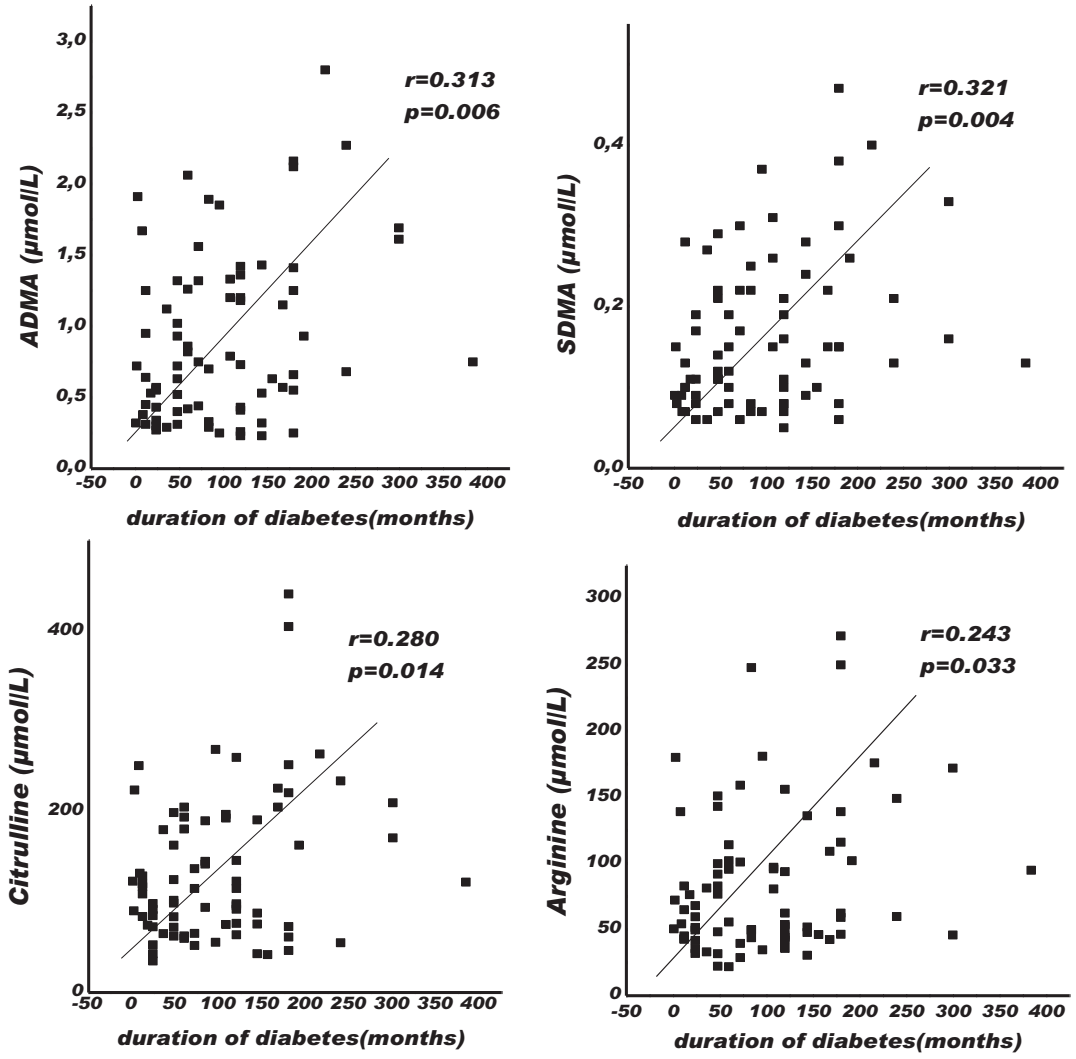

Fig. 7. Positive correlation of duration of diabetes with ADMA, SDMA, citrulline and arginine in the subjects were calculated by simple regression analysis.

oxide synthase leads to deterioration of endothelium-dependent vasodilatation by reducing NO synthesis [31]. In studies until today, ADMA has been shown to be a risk marker for endothelial dysfunction and cardiovascular diseases. In addition, ADMA has been associated with kidney failure, hypertension, hyperthyroidism, diabetes and many other diseases [32]. According to our findings, ADMA levels were significantly higher in dysregulated diabetics than in the other groups. But, we found that serum ADMA levels were not significantly higher in the prediabetic stage and regulated diabetics than in the control group. These data show that ADMA may be a biomarker for cardiovascular risk in diabetics. Similarly, Wang et al. have also emphasized that ADMA is important to predict cardiovascular disease risk as a predictor of endothelial dysfunction [33].

There was no correlation between ADMA and galectin-3, but we found that only L-NMMA from the compounds of methylated arginine had a positive correlation with galectin-3. It should be investigated the relationship between L-NMMA and galectin-3 among the compounds of methylated arginine. On the other hand, serum ADMA levels were no significant difference with hs-CRP and neopterin. Galectin-3 levels are a positive correlation with neopterin.

Galectin-3 may be an indicator for the early detection of prediabetes and diabetes. Additionally, the findings of this study suggest that galectin-3 plays an important role in the progression of prediabetes to diabetes. We propose that galectin-3 contributes to the development of prediabetes and diabetes due to inflammation, insulin resistance, and additional unidentified mechanisms, which likely include beta cell dysfunction. Assessment of the levels of these biomarkers will be helpful in not only early diagnosis but also prognosis of type 2 diabetes mellitus. Thus, becoming a useful prognostic marker in clinical practice.

5. Conclusions

The present study shows the levels of hs-CRP, ADMA, neopterin and galectin-3 biomarkers in different stages of diabetes. Thus, diagnosis and treatment of individuals in the early stages of diabetes can be provided. Because serum galectin-3 levels in diabetics were found higher than healthy subjects and positively correlated with both hs-CRP and LNMMA, it may be a strong predictor of cardiovascular disease and inflammation in type 2 diabetic patients. Property, because AGEs are key role in diabetic complications, the finding of drugs that block the interaction of galectin-3 with and AGEs will be effective in reducing the complications in diabetic individuals. Moreover, the investigation of the levels of these biomarkers in both serum and urine of diabetic groups with nephropathy that grouped by albuminuria will be important especially in terms of showing whether kidney damage can be detected early. As a result, we believe that this study will shed light on this type of research in diabetes.

Funding

All sources of financial and material support were provided by the Scientific Research Projects Coordination Unit, Selcuk University, Konya, Turkey.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

References

[1] International Diabetes Federation, Diabetes Atlas, 8th edition, (2017), pp. 32–34.
[2] J.L. Chiang, M.S. Kirkman, R.M.B. Laffel, A.L. Peters, Type 1 diabetes through the lifespan: a position statement of the American Diabetes Association, Diabetes Care (2014) 2034–2054.
[3] G. Sypniewska, Diabetes epidemics-classification and prevalence, Biochemia Medica 24 (Suppl1) (2014) 1–78.
[4] International Diabetes Federation, Diabetes Atlas, 7th edition, (2015), pp. 12–18.
[5] G. Pugliese, C. Iacobini, C.M. Pesce, S. Menini, Galectin-3: an emerging all-out player in metabolic disorders and their complications, Glycobiology 25 (2) (2015) 136–150.
[6] L. Wan, F.T. Liu, Galectin-3 and inflammation glycobiol, Insights 6 (2016) 1–9.
[7] D. Stajica, D. Selakovic, N. Jovicicc, J. Joksimovic, N. Arsenijevicd, M.L. Lukicd, G. Rosi, The role of galectin-3 in modulation of anxiety state level in mice, Brain, Behavior, and Immunity journal 78 (2019) (2019) 177–187.
[8] S.Y. Li, P.J. Davidson, N.Y. Lin, et al., Transport of galectin-3 between the nucleus and cytoplasm. II. Identification of the signal for nuclear export, Glycobiology 16 (2006) 612–622.
[9] J. Dumic, S. Dabelic, M. Flögel, Galectin-3: an open-ended story, Biochim. Biophys. Acta 1760 (2006) 616–635.
[10] G. Pugliese, C. Iacobini, C. Ricci, C.B. Fantauzzi, S. Menini, Galectin-3 in diabetic patients, Clin. Chem. Lab. Med. 52 (10) (2014) 1413–1423.
[11] J. Pang, D.H. Rhodes, M. Pini, R.T. Akasheh, K.J. Castellanos, R.J. Cabay, D. Cooper, M. Perretti, G. Fantuzzi, Increased adiposity, dysregulated glucose metabolism and systemic inflammation in galectin-3 KO mice, PLoS One 8 (2013) e57915.
[12] A.E. Karlsen, Z.M. Størling, T. Sparre, M.R. Larsen, A. Mahmood, J. Størling, P. Roepstorff, K. Wrzesinski, P.M. Larsen, S. Fey, K. Nielsen, P. Heding, C. Ricordi, J. Johannesen, O.P. Kristiansen, U.B. Christensen, I. Kockum, H. Luthman, J. Nerup, F. Pociot, Immune-mediated beta-cell destruction in vitro and in vivo—a pivotal role for galectin-3, Biochem. Biophys. Res. Commun. 344 (2006) 406–415.
[13] American Diabetes Association, Classification and diagnosis of diabetes. Sec. 2. In standards of medical care in diabetesd-2015, Diabetes Care 38 (Suppl. 1) (2015) S8–S16 2015.
[14] M. Alrashed, M. Abougoush, E.O. Akgul, M.K. Erbil, Detection method of serum and urine neopterin levels by high performance liquid chromatography and clinical applications, Gulhane MJ 44 (2002) 273–277.
[15] I. Mehmetoğlu, S. Gökce, S. Kurban, R. Gökce, M.N. Atalar, M. Celik, Publication investigation of the relationships ofobesity with melatonin and dehydroepiandrosterone levels, Nobel Medicus 13 (2) (2017) 70–75.
[16] David M. Nathan, Judith Kuenen, Rikke Borg Hui Zheng, David Schoenfeld, J. Robert, Heine, for the A1c-derived average glucose (ADAG) study group, Diabetes Care 31 (8) (2008) 1473–1478.
[17] I.M. Di Gangi, L. Chiandetti, A. Gucciardi, V. Moret, M. Naturale, G. Gi¬ordano, Simultaneous quantitative determination of N(G),N(G)-dimethyl-L-arginine or asymmetric dimethylarginine and related pathway's metabolites in biological fluids by ultrahigh-performance liquid chromatography/electrospray ionization-tandem mass specterometry, Anal. Chim. Acta 677 (2010) 140–148.
[18] A. Berezin, The rationality to use of galectin-3 as target in biomarker-guided therapy of type 2 diabetes mellitus, Endocrinol Metab Syndr 5 (2016) 1.
[19] H. Yilmaz, M. Cakmak, O. Inan, T. Darcin, A. Akcay, Increased levels of galectin-3 were associated with prediabetes and diabetes: new risk factor? J. Endocrinol. Investig. 38 (2015) 527–533.
[20] J. Weigert, M. Neumeier, J. Wanninger, S. Bauer, S. Farkas, M.N. Scherer, et al., Serum galectin-3 is elevated in obesity and negatively correlates with glycosylated hemoglobin in type 2 diabetes, J. Clin. Endocrinol. Metab. 95 (3) (2010) 1404–1411.
[21] D.P. Misra, S. Das, P.K. Sahu, Prevalence of inflammatory markers (high-sensitivity C-reactive protein, nuclear factor-κB and adiponectin) in Indian patients with type 2 diabetes mellitus with and without macrovascular complications, Metab. Syndr. Relat. Disord. 10 (2012) 209–213.
[22] S.B. Asegaonkar, A. Marathe, M.L. Tekade, et al., High-sensitivity C-reactive protein: a novel cardiovascular risk predictor in type 2 diabetics with normal lipid profile, J. Diabetes Complicat. 25 (2011) 368–370.
[23] N. Pejnovic, J. Pantic, I. Jovanovic, G. Radosavljevic, M. Milovanovic, I. Nikolic, N.S. Zdravkovic, A.L. Djukic, N.N. Arsenijevic, M.L. Lukic, Galectin-3 deficiency accelerates high-fat diet induced obesity and amplifies inflammation in adipose tissue and pancreatic islets, Diabetes 62 (2013) 1932–1944.
[24] N.C. Henderson, T. Sethi, The regulation of inflammation by galectin-3, Immunol. Rev. 230 (1) (2009) 160–171.
[25] J. ten Oever, E.J. Giamarellos-Bourboulis, F.L. van de Veerdonk, F.F. Stelma, A. Simon, M. Janssen, M. Johnson, A. Pachot, B.J. Kullberg, L.A. Joosten, M.G. Netea, Circulating galectin-3 in infections and non-infectious inflammatory diseases, Eur. J. Clin. Microbiol. Infect. Dis. 32 (12) (2013) 1605–1610.
[26] R. Lakshminarayan, C. Wunder, U. Becken, M.T. Howes, C. Benzing, S. Arumugam, S. Sales, N. Ariotti, V. Chambon, C. Lamaze, et al., Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrinindependent carriers, Nat. Cell Biol. 16 (2014) 595–606.
[27] G.S. Hotamisligil, Inflammation and metabolic disorders, Nature 444 (2006) 860–867.
[28] A. Pfützner, T. Schöndorf, M. Hanefeld, T. Forst, High-sensitivity C-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with pioglitazone, J. Diabetes Sci. Technol. 4 (2010) 706–716.
[29] E.Y. Choi, R.T. Yan, V.R. Fernandes, et al., High-sensitivity C-reactive protein as an independent predictor of progressive myocardial functional deterioration: the multiethnic study of atherosclerosis, Am. Heart J. 164 (2012) 251–258.
[30] J.I.N. Qi-hui, L.O.U. Yu-feng, L.I. Tian-lang, C.H.E.N. Huai-hong, L.I.U. Qiang, H.E. Xiao-jun, Serum galectin-3: a risk factor for vascular complications in type 2 diabetes mellitus, Chin. Med. J. 126 (11) (2013) 2109–2115.
[31] P. Vallance, A. Leone, A. Calver, J. Collier, S. Moncada, Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis, J. Cardiovasc. Pharmacol. 20 (Suppl. 12) (1992) 60–62.
[32] J.P. Cooke, Does ADMA cause endothelial dysfunction? Arterioscler. Thromb. Vasc. Biol. 20 (2000) 2032–2037.
[33] J. Wang, A.S. Sim, X.L. Wang, et al., Relations between plasma asymmetric dimethylarginine (ADMA) and risk factors for coronary disease, Atherosclerosis 184 (2) (2006) 383–388.