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Research Article

The Association between Serum Galectin-3 and Biochemical Parameters with Cardiovascular Diseases among Type 2 Diabetes Patients in Gaza Strip, Palestine: A Case-Control Study

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Abstract

Background: Galectin-3 considered as β-galactoside binding lectin expressed in human tissues and has a vital numerous biological activities. This study was conducted to assess Galectin-3 level among type 2 diabetes mellitus patients (T2DM), who have CVDs, and to evaluate its association with other classical biochemical parameters.

Methods: A case-control study recruited 180 participants of both genders, aged between 45-65 years. The participants were assigned into three groups: T2DM patients, T2DM patients with CVDs, and control group; sixty participants in each group, matching for age. Fasting blood samples were collected from all participants and used for laboratory analysis. ELISA was used for measurement of Galectin-3 level. Additional information regarding demographic and medical history variables was obtained with an interview-based questionnaire. All statistical analysis was performed using SPSS version 23.

Results: There were a significant difference in serum Galectin-3 level in T2DM with CVDs group in compared with T2DM and control group (P value < 0.01). Furthermore, a positive significant correlations were found between serum Galectin-3 and BMI, smoking, FBS, HbA1c, urea, creatinine, uric acid, cholesterol, triglyceride, LDL-C, LDH, CK, CK-MB, urinary Alb/Cr ratio, Ccr and NT-BNP in the studied group ($p<0.05$); whereas there was a negative correlation between Galectin-3 and HDL-C, but no significant correlation was found between Galectin-3 and age.

Conclusion: Galectin-3 involved in cardiac processing pathway, and closely related to the severity of diabetes in T2DM with and without cardiovascular complications, thereforGalectin-3 may be helpful in the diagnosis and prognosis of CVDs in T2D Mpatients.

Keywords: Biomarkers; Cardiovascular Diseases; Diabetes Mellitus; Galectin-3; Palestine

Abbreviations

DM: Diabetes Mellitus; CVDs: Cardiovascular Diseases; T2DM: Type 2 Diabetes Mellitus; CCU: Coronary Care Unit; HbA1c: Glycated Hemoglobin; FBG: Fasting Blood Glucose; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; LDH: Lactate Dehydrogenate; CK: Creatine Kinase; CKMB: Creatine Kinase Muscle Brain; NT-BNP: N. Terminal Brain Natriuretic Peptide; Alb/Cr ratio: Albumin/creatinine ratio; Ccr: Creatinine clearance rate

Introduction

The prevalence of Diabetes Mellitus (DM) and Cardiovascular Diseases (CVDs) are steadily increasing everywhere, most markedly in the world's low and middle-income countries [1]. Globally, the World Health Organization estimates that, 422 million adults were living with DM in 2014, and projects that DM will be the seventh leading cause of death in 2030 [2]. Most of DM deaths occur in low and middle-income countries [3]. Due to sophisticated laboratory tests that are usually required to distinguish between type 1 diabetes and type 2 diabetes (T2DM), separate global estimates of diabetes prevalence for type 1 diabetes and T2DM do not exist [3]. In fact, the majority of people with diabetes are affected by T2DM [1]. In Palestine, the prevalence rate of DM was 10.5% in the West Bank and 11.8% in the Gaza Strip among the registered Palestinian refugees [3]. Abu Rmeileh et al. [4] estimated the prevalence of DM in Palestine at 20.8% and 23.4% in 2020 and 2030, respectively. When DM is uncontrolled, it has dire consequences for health and well-being [1]. In addition, diabetes and its complications impact harshly on the finances of individuals and their families and to health systems and national economies through direct medical costs and loss of work and wages [1]. Complications can arise as the disease progresses. Long term complications such as coronary heart disease which can lead to a heart attack, cerebrovascular disease which can lead to stroke,

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retinopathy which can lead to blindness, nephropathy which can lead to kidney failure and the need for dialysis, and neuropathy which increases the chance of foot ulcers, infection and the eventual need for limb amputation [3]. In Palestine, cardiac diseases were reported to be the number one cause of death [5].

Physicians have used additional tools to aid clinical assessment, which were helpful in making an accurate diagnosis, and effectively prognosticate, treat and better identify high-risk subjects. Biomarkers are one of such tool that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention there for biomarkers should fulfill to be useful clinically [6]. Galectin-3 considered as β-galactoside binding lectin expressed in human tissues and have a vital numerous biological activities [7]. Galectin-3 as a novel biomarker that may aid in the diagnostic and prognostic evaluation of acute and chronic cardiac heart diseases. Our study was conducted to determine the association between serum Galectin-3 and other biochemical parameters with CVDs among patients with T2DM in Gaza Strip, Palestine.

Materials and Methods

Study Participants

This case-control study recruited 180 participants of both genders, aged between 45-65 years. The study was conducted in the year 2019, among a representative sample of Palestinian participants, at the central laboratory of Al-Remal Clinic, in Gaza Strip, Palestine. The participants were assigned into three groups: control group (Group 1), T2DM patients (Group 2), and T2DM patients with CVDs (Group 3), and; sixty participants in each group. T2DM patients without CVDs were recruited from the outpatient clinic at the diabetic department of Al-Remal Clinic; while T2DM with CVDs were recruited from the Coronary Care Unit (CCU) at Al-Shifa Medical Complex, in Gaza Strip, Palestine. Additionally, the control group (healthy subjects) was equal number of an age matched and in-residence place. Pregnant, lactating women and patients with other types of serious illness such as cancer or end stage kidney disease were excluded from the study.

Assessment of Anthropometric Measurements

Height was measured in all participants (Participants bare footed and head upright) with a measuring rod attached to the balanced beam scale; the height was reported to the nearest 0.5 cm. Weight (kg) was measured using standard scale (Seca); the scale was placed on a hard-floor surface; patients were asked to remove their heavy outer garments and weight was measured and recorded to the nearest 0.1 kg. Furthermore, the Body Mass Index (BMI) was calculated by dividing weight in kilograms by the square of height in meters [8].

Assessment of Blood Pressure

Blood pressure was measured from the left arm (mmHg) by mercury sphygmomanometer. Three readings on different days, while the participant was seated after relaxing for at least fifteen minutes in a quiet environment, empty bladder. The average of three measurements was recorded [9].

Assessment of Other Variables

Additional information regarding demographic and medical history variables was obtained with an interview-based questionnaire.

Biochemical Analysis

After 12 hours fasting, venous blood samples were collected from all participants by well-trained and experienced nurses. Venous blood (7.0 ml) was drawn into vacationer tubes and was used for blood chemistry analysis including: glycated hemoglobin (HbA1c) (%), Fasting Blood Glucose (FBG) (mg/dl), urea (mg/dl), creatinine (mg/dl), uric acid (mg/dl), cholesterol (mg/dl), triglyceride (mg/dl), High And Low Density Lipoprotein Cholesterol [HDL-C and LDL-C (mg/dl)], Lactate Dehydrogenate (LDH) (U/L), Creatine Kinase (CK) (U/L), and creatine kinase muscle brain (CKMB)(U/L). Two ml of the blood was left with anticoagulant (EDTA) in the first tube for HbA1c and five ml in the second plain tube and was allowed to clot used for biochemical tests which done by chemistry analyzer (Erba XL 200 manufactured by Erba Diagnostic Mannheim, Germany), Galectin-3 and N. Terminal Brain Natriuretic Peptide (NT-BNP) (pg/ml) by Eliza kit (R & D company U.S.A) [10]. Furthermore, urinary albumin (mg/dl), creatinine (mg/dl), albumin/creatinine ratio (Alb/Cr ratio) (mg/g), and Creatinine Clearance Rate (Ccr) (ml/min) were also determined. All laboratory tests were analyzed in the central laboratory of Al-Remal Clinic, in Gaza Strip, Palestine.

Statistical Analysis

All statistical analysis was performed using SPSS version 23. Descriptive statistics and ANOVA test were used to find the difference between the study groups. In addition, the associations between Galectin-3 with other biochemical parameters were performed using Pearson correlations test. P value less than 0.05 was considered as statistically significant.

Ethical Consideration

The study protocol was approved by the Palestinian Health Research Council (Helsinki Ethical Committee of Research PHRC/HC/287/17). In addition, written informed consent was also obtained from each participant.

Result and Discussion

Characteristics of the Study Population by the Study Groups

A total of 180 participants of both genders, aged between 45-65 years were included in this study. The participants were assigned into three groups: control group (Group 1), T2DM patients (Group 2), and T2DM patients with CVDs (Group 3), and; sixty participants in each group. Table 1 shows the characteristics of the study population by the study groups. The result revealed that, 35 (58.3%) males and 25 (41.7%) females were included in the control group (Group 1), 38 (63.3%) males and 22 (36.7%) females were included in the T2DM patients (Group 2), while 23 (38.3%) males and 37 (61.7%) females were included in the T2DM patients with CVDs (Group 3). In addition, the mean of age (years) for the three groups were 53.8±6.5, 53.9±5.6 and 55.6±6.9 respectively. No significant difference according to age, as the sample of our study matched for age. Furthermore, the BMI (kg/m^2) for the three groups were 26.9±3.2, 32.2±6.6 and 32.4±6.9 respectively. Table 1 also shows that, 3.3%, 11.7% and 28.3% of the study participants are current smoker in the three groups respectively. The mean of systolic blood pressure (mmHg) for the three groups were 114.8±5.7, 128.8±15.7, and 126.3±8.7 respectively. And the mean of diastolic blood pressure (mmHg) for the three groups were

Table 1: Characteristics of the study population by the study groups.

Variables	Control group (Group 1) n=60	T2DM patients (Group 2) n=60	T2DM patients with CVDs (Group 3) n=60	P Value
Gender				
Male, No. (%)	35.0 (58.3)	38.0 (63.3)[2]	23.0 (38.3)[2]	0.014*
Female, No. (%)	25.0 (41.7)	22.0 (36.7)	37.0 (61.7)	
Age (years)				
Mean ± SD	53.8±6.5	53.9±5.6	55.6±6.9	0.197NS
Rang	(4565)	(4565)	(4565)	
BMI (kg/m²)				
Mean ± SD	26.9±3.2[1&3]	32.2±6.6[1]	32.4±6.9[3]	0.001**
Rang	(2233.4)	(1944.9)	(2357.8)	
Smoking status				
Non-smoker, No. (%)	55.0 (91.7)[1&2]	34.0 (56.7)[1]	34.0 (56.7)[2]	0.001**
Past-smoker, No. (%)	3.0 (5)	19.0 (31.7)	9.0 (15)	
Current-smoker, No. (%)	2.0 (3.3)	7.0 (11.7)	17.0 (28.3)	
Systolic blood pressure (mmHg)				
Mean±SD	114.8±5.7[1&2]	128.8±15.7[1]	126.3±8.7[2]	0.001**
Rang	(100120)	(105180)	(100150)	
Diastolic blood pressure (mmHg)				
Mean±SD	74.3±6.1[1&2]	84.3±11.4[1]	81.7±6.2[2]	0.001**
Range	(6080)	(65130)	(7095)	

Similar number between two groups means significant difference, p*-significant, p**-highly significant, NS: non significant.

Table 2: Level of HbA1c, serum glucose, urea, creatinine and uric acid in the studied groups.

Parameters	Control group (Group 1) n=60	T2DM patients (Group 2) n=60	T2DM patients with CVDs (Group 3) n=60	F	P Value
HbA1c (%)					
Mean±SD	5.8±0.4[1&2]	7.8±1.2[1]	25.7±10.5	98.911	0.001**
Range	4.86.3	6.0-10.3	6.0-11.3		
FBG (mg/dl)					
Mean±SD	91.7±11.8[1&2]	173.6±88.3[1]	186.8±64.7[2]	39.453	0.001**
Range	64115	70-469	91-393		
Urea (mg/dl)					
Mean±SD	28±8.4[1&2]	36±16[1]	42.9±23.4[2]	11.798	0.001**
Range	15-47	15-81	21-142		
Creatinine (mg/dl)					
Mean±SD	0.7±0.2[1&2]	0.9±0.6[1]	1.2±0.5[2]	14.265	0.001**
Range	0.41	0.53.2	0.74.2		
Uric acid (mg/dl)					
Mean±SD	4.0±1.2[3]	4.8±1.7	5.2±1.5[3]	9.933	0.001**
Range	2.1-8.1	2.1-8.1	2.5-8.7		

Similar number between two groups means significant difference, p*-significant, p**-highly significant, NS: non-significant.

74.3±6.1, 84.3±11.4, and 81.7±6.2 respectively. ANOVA test revealed that, there was a significant difference in the gender between the three groups (P<0.05), Post-hoc statistical analysis showed that, there was no significant difference between group one with group two and group three; while there was a significant difference between group two and group three. As the studied groups matched for age they were given reliable comparable results, similar results were found by the previous studies, which did not found significant difference in gender and age [11,12]. In contrast, these results were not agree with the previous studies that indicated presence of significant relation between gender and age [13,14].

With regard to BMI, in our study the ANOVA tests how that, there was a highly significant difference in the studied groups according to BMI (P<0.01). Post-hoc elucidated that, there was a highly significant difference between group two and group three (P<0.01) compared with group one, whereas no significant difference

Table 3: Serum lipid profiles in the studied groups.

Parameters	Control group (Group 1) n=60	T2DM patients (Group 2) n=60	T2DM patients with CVDs (Group 3) n=60	F	P Value
Cholesterol (mg/dl)					
Mean±SD	178.3±20.2[1&2]	219.7±39.1	224.8±51.8[2]	25.401	0.001**
Range	123 200	146-336	119-350		
Triglyceride (mg/dl)					
Mean±SD	140.2±79[1&2]	218.3±114.7[1]	226.5±103.7[2]	13.539	0.001**
Range	46411	60-614	100-564		
HDL-C (mg/dl)					
Mean±SD	46.2±7.8[1&2]	42±4.9[1]	38.8±3.9[2]	24.657	0.001**
Range	31-67	32-52	2846		
LDL-C (mg/dl)					
Mean±SD	127.8±29.8[3]	134.1±42	148.8±28.7[3]	14.265	0.003**
Range	70.2-223.6	59-237	102-242		

Similar number between two groups means significant difference, p*- significant, p**-highly significant, NS: non-significant.

Table 4: Levels of cardiac enzyme activities in the studied groups.

Enzymes	Control group (Group 1) n=60	T2DM patients (Group 2) n=60	T2DM patients with CVDs (Group 3) n=60	F	P Value
LDH (U/L)					
Mean±SD	328.8±62.4[2]	363.5±69.6[3]	467.4±212.6[2&3]	5.576	0.001**
Range	210476	240592	2611146		
CK (U/L)					
Mean±SD	101.5±39[1&2]	124±63.6[1]	208.8±312.5[2]	7.879	0.001**
Range	36175	47290	571695		
CKMB (U/L)					
Mean±SD	13.1±4.3[1&2]	20.8±10.2[1]	34.4±50.4[2]	17.368	0.001**
Range	420	961	10280		

Similar number between two groups means significant difference, p*- significant, p**-highly significant, NS: non-significant. LDH: Lactate dehydrogenate; CK: Creatine kinase; CKMB: Creatine kinase muscle brain.

between group two and group three. Regarding smoking status, in the present study, most of the study participants were non-smoker, but we found a small proportion of participants in the three groups were current smoker, and ANOVA test defined that, there was a significant difference among the studied groups (P<0.001), and Post-hoc has been appointed that, there was a significant difference between group one and group three (P<0.001), and no significant difference between group two and group three. These results of BMI and smoking among the studied groups coincide with the findings of the previous studies [15]. Conversely, other studies found [14] that, there was significantly difference between above variables in the three studied groups. Rebholz et al. [16] show a significant difference between BMI and smoking. The reason for these conflicting results may be due to the variation in the sample size from one study to other. Regarding, systolic and diastolic blood pressure, ANOVA test clarified that, there was a significant difference between three studied groups (P<0.001), and the Post-hoc explained that, there was a significant difference in group two and group three in compared with group one, while there was no significant difference between group two and group three in systolic and diastolic blood pressure. These results were coincide with [17] findings.

Level of HbA1c, Serum Glucose, Urea, Creatinine and Uric Acid in the Studied Groups

As shown in Table 2, the mean of HbA1c levels (%) among three studied groups were 5.8±0.4, 7.8±1.2, and 8.2±1.2 respectively; and the mean of glucose levels (mg/dl) were 91.7±11.8, 173.6±88.3, and 186.8±64.7 respectively. While the means of serum urea levels (mg/dl) were 28±8.4, 36±16, and 42.9±23.4 respectively; and the mean of serum creatinine levels (mg/dl) were 0.7±0.22, 0.9±0.6, and 1.2±0.5 respectively. On the other hand, the mean of serum uric acid levels (mg/dl) were 4.0±1.2, 4.8±1.7 and 5.2±1.5 respectively for the three studied groups. ANOVA test showed statistically significant differences (P<0.01) of the means for both HbA1c and serum glucose among three studied groups. The outcome of Post-hoc test showed highly significant differences (P<0.01) in the HbA1c levels in group two and group three compared to the control group, while there was no significant difference between group two compared with group three. In addition, serum glucose concentration showed significant differences in group two and group three (P<0.01) compared to the control group; and no significant difference between group two and three in glucose levels. The results were agreement with the previous studies [18,19], whom indicated that HbA1c and glucose levels significantly predicted CVDs and mortality, even below the threshold commonly accepted for the diagnosis of DM and independent of age and classic risk factors. Therefore, the findings of the present study suggested that HbA1c might be superior to fating blood glucose levels as a risk factor for CVDs, this result agree with Jin QH et al. [20] who defined that, HbA1c significantly predicts future heart failure

Table 5: Urinary parameters in the studied groups.

Variables	Control group (Group 1) n=60	T2DM patients (Group 2) n=60	T2DM patients with CVDs (Group 3) n=60	F	P Value
Albumin (mg/dl)					
Mean±SD	4.6±1.8[1&2]	35.9±56[1]	38.8±51[2]	11.267	0.001**
Range	1.3 8.3	2.1 233	2 265		
Creatinine (mg/dl)					
Mean±SD	195.2±54.9[2]	169.6±75.5	150.5±108.8[2]	4.414	0.013**
Range	45327	60397	40612		
Alb / Creat ratio (mg/g)					
Mean±SD	24.1±7.9[1]	264.7±886[1]	309.5±320.3	10.971	0.001**
Range	8.240.2	12.24827.3	11.51675		
CCr (ml/min)					
Mean±SD	127±23.0[1&2]	98.1±17.6[1]	74.3±13.7[2]	121.74	0.001**
Range	90.0163.1	72.6124	50.096.3		

Similar number between two groups means significant difference, p*-significant, p**-highly significant, NS: Non-Significant. Alb/Cr ratio: Albumin/Creatinine ratio; Ccr: Creatinine clearance rate.

parameters in patients with T2DM.

As indicated in our data, serum urea concentrations were significantly higher in T2DM with and without CVDs, compared to control group. ANOVA test showed that, there was a significant difference between the three studied groups, and Post-hoc test showed that there was a significant difference between group two (P<0.05), with group one and highly significant between group three (P<0.01) compared to group one; at the same time there was no significant difference between group two and group three (P>0.05). These findings concise with Abdelsalam et al. [21] who showed highly urea levels accompanied with cardio-diabetic complication, and agree with Pietrement et al. study [22]. The increase in serum urea observed here may be due to impairment in filtration in kidney and may be as a result of impaired hepatic function and due to a disturbance in protein metabolism. These outcomes explained that increase in urea level and decrease kidneys function is higher incidence of CVDs in patients with T2DM. As for creatinine considered a waste product that normally filtered from the blood and excreted in the urine. In this work, serum creatinine levels showed a statistically significant (P<0.01) among the three studied groups by ANOVA test. In addition, Post-hoc test showed highly significant (P<0.01) difference in serum creatinine levels in group three compared to the control group, as well as in group two compared to the group three (P <0.05), and there was no significant difference between group one and group two. These results were in concordant with the previous studies [23,24] which reported that, high creatinine levels observed in diabetic patients might be due to impaired function of the nephrons. In line with these observations, Bostom et al. [25] showed an independent influence of elevated creatinine on CVDs morbidity and mortality. High serum creatinine concentration within the normal range is a marker for increased risk of cerebrovascular diseases in both normotensive and hypertensive subjects [26]. These findings support the evidence indicating that impairment of renal function may be a factor for increased risk of CVDs.

On the other hand, means levels of serum uric acid gradually increased in the studied groups. The differences in the three studies groups in the mean of uric acid levels were statistically significant (P<0.05). Post-hoc test showed significant differences only in serum uric acid between group one and group three (P<0.05). In contrast, no significant difference in serum uric acid levels were found between group one and group two, also group two and three. The present study showed significantly higher serumuric acid level in diabetics with CVDs compared to control group (P<0.05). These findings agreement with the majority of studies that found a significant interrelationship between hyperuricemia and cardiovascular outcomes [27,28]. Therefore, it has been debate that hyperuricemia could be a compensatory mechanism to void oxidative damage related to atherosclerosis and aging in humans [29]. Results of this study give further credit to this protective concept of uric acid with spread its effects to the CVDs, suggesting that increased uric acid concentration provided a more efficient antioxidant capacity that decreases the clinical repercussion of brain ischemia. In the current study, the increase in serum uric acid level in group two was non-significant (P>0.05) compared to the control group. This results agreement with that of Seghieri et al. [30] who found that serum uric acid levels were similar in both diabetic and non-diabetic patients. We conclude that, uric acid levels provided an evidence for causal relationship between uric acid and diabetic macrovascular diseases this conclusion agree with the study [31]. As we noticed from research results, HbA1c, fasting blood glucose, urea, creatinine and uric acid were positively association with diabetic CVDs complication development.

Serum Lipid Profiles in the Studied Groups

Lipid profiles including total cholesterol, triglyceride, high and low density lipoprotein cholesterol (HDL-C and LDL-C) for the three studied groups are presented in Table 3. The mean levels of total cholesterol (mg/dl) for the studied groups were 178.3±20.2, 219.7±39.1 and 224.8±51.8 respectively; and mean levels for triglyceride (mg/dl) were 140.2±79, 218.3±114.7 and 226.5±103.7 respectively. The mean levels for LDL-C (mg/dl) were 82.8±37.8, 134.1±42 and 148.8±28.7 respectively. In contrast, the mean levels of HDL-C (mg/dl) showed gradual decrease with values of 46.2±7.8, 42±4.9 and 38.8±3.9 in the three studied groups respectively. The study results comparison of means differences among studied groups were found to be highly statistically significant (P <0.01) for total cholesterol, triglyceride and HDL-C. The outcome of Post-hoc test for total cholesterol, triglyceride

Table 6: Correlation between serum Galectin-3 with studied parameters.

Variables	Serum Galectin-3 level	
	Pearson correlation (r)	p-value
Age (years)	0.013	0.861^{NS}
BMI (kg/m^2)	0.407	0.000**
FBG (mg/dl)	0.441	0.000**
HbA1c (%)	0.544	0.000**
Urea (mg/dl)	0.334	0.000**
Creatinine (mg/dl)	0.428	0.000**
Uric acid (mg/dl)	0.297	0.000**
Cholesterol (mg/dl)	0.333	0.000**
TG (mg/dl)	0.335	0.000**
HDL-C (mg/dl)	-0.397	0.000**
LDL-C (mg/dl)	0.328	0.000**
LDH (IU/L)	0.482	0.000**
CK (IU/L)	0.406	0.000**
CK-MB (IU/L)	0.409	0.000**
Alb/Cr (mg/g)	0.191	0.010**
Ccr (ml/min)	0.248	0.001**
NT-BNP (pg/ml)	0.456	0.000**

Strong positive correlation (0.75-0.99), moderate positive correlation (0.5-0.75), weak correlation (0.25-0.49), "minus" negative correlation, "0" no correlation. *p - value significant at p≤0.05, **p - value significant at p≤0.01.

and HDL-C showed highly significant differences (p <0.01) between group two and group three compared to the control group, and there were no significant differences (P>0.05) in group two compared to the group three for cholesterol, triglyceride and HDL-C. The levels of lipids were found to be significantly increased in studied groups, and value of HDL levels was significantly decreased in studied groups when compare to control. The difference in LDL-C was significant between group three (P <0.05) compared to the control group, but there was no significant difference between group two and group three. These results indicated that lipid profiles might be used as strongly predictive of development CVDs in patients with T2DM.

This study revealed that increase in total cholesterol, triglyceride and LDL-C in diabetic patients with and without CVDs. These results agreement with the previous studies [32,33] which reported that cholesterol, triglyceride and LDL-C are elevated in diabetic patients. Raised levels of cholesterol, triglyceride, LDL-C, and low level of HDL-C were reported to be major risk factors for CVDs and DM according to previous studies [32,33]. These findings of elevating in serum concentrations of LDL-C, and decrease concentrations of HDL-C, and to some extent high total triglyceride concentrations strongly predictive of cardiovascular risk this result agree with [34]. As indicated in the present study, there was a significant decrease (P<0.05) in HDL-C levels in diabetics with and without CVDs compared to the control group. The abnormal high concentrations of serum lipids in diabetics is mainly, due to an increase in the mobilization of free fatty acids from fat depots, since insulin inhibits the hormone sensitive lipase. Excess fatty acids in serum of diabetics converted into triglyceride, phospholipids and cholesterol in liver. These three substances with proteins may be discharged into blood in the form of lipoproteins; this agrees with who hypothesized that increased triglyceride caused elevated glucose levels by triggering insulin resistance [35]. Watt et al. [36] indicate that, accumulation of lipids such as triglyceride resulted in skeletal muscle insulin resistance. On the other hand, Kabir et al. [37] concluded that dyslipidemia might effect on the levels of glucose in the blood. The relationship between triglyceride and glucose was very important in managing DM and in the prevention of CVDs [33]. Furthermore, Al Qahtani et al. [38] noted that, low levels of HDL-C contributed to significant mortality risk among patients with heart failure. Alsheikh-Ali et al. [39] whom found that dyslipidemia were the most prevalent CVDs risk factor burden in Africa and the Middle East. High serum concentrations of LDL-C, low concentrations of HDL-C and to some extent high total cholesterol and triglycerides concentrations were associated with increased CVDs risk especially with T2DM patients and associated with morbidity and mortality. These findings revealed to important clinical implications because patients with T2DM have an increased risk of developing CVDs. Identifying novel risk factors for CVDs may help in the development of strategies for the prevention and treatment of CVDs in T2DM patients. Some conventional risks biomarkers (fasting blood glucose, HbA1c, creatinine, uric acid, cholesterol, triglyceride, HDL-C, LDL-C, and albumin/creatinine ratio were independently associated with CVDs in T2DM [40].

Levels of Cardiac Enzyme Activities in the Studied Groups

Table 4 shows serum cardiac enzyme activities in the three studied groups, including Lactate Dehydrogenate (LDH), Creatine Kinase (CK) and Creatine Kinase Muscle Brain (CKMB). The mean of LDH levels (U/L) among the studied groups were 328.8±62.4, 363.5±69.6 and 467.4±212.6 respectively. On the other hand, the mean of CK and CKMB were gradual increased with values of 101.5±39, 124±63.6 and 208.8±312.5 (U/L) for CK respectively; and 13.1±4.3, 20.8±10.2 and 34.4±50.4 (U/L) for CKMB among three studied groups, respectively. Cardiac enzyme activities increase gradually among three studied groups as shown in table 4. This increase was clinically and statistically significant for group three compare with group two and group one. ANOVA test showed significant difference in the levels of serum LDH among three groups (P<0.001), Post-hoc test revealed significant difference (P<0.05) between group two and three, and highly significant (P<0.01) between group one and three. In contrast, no significant differences (P>0.05) were found between group one and group two. Also, ANOVA test showed, highly significant difference (P<0.01) in the means levels of serum CK and CK-MB among three groups and Post-hoc test for CK showed, highly significant difference (P<0.01) between group one with group two and three. While in CKMB there was highly significant difference (P >0.01) between group one and three also a moderate significant between group two and group three (P<0.05). DM is an independent risk factor for CVDs. Cardiac enzymes have been demonstrated to be important prognostic determinants to identify high-risk patients [41]. The present study showed that, the increase in serum LDH, CK and CKMB activities in the T2DM with CVDs group was significant (P<0.05) compared to the T2DM without CVDs and control groups. Similar results were previously reported [41]. The significant elevation of LDH, CK and CKMB may be explained by their specificity to CVDs diagnostic. Also Kaneda et al. [42] noted that value of serum CKMB, LDH in study group show statistically significant when compared to control group.

Urinary Parameters in the Studied Groups

The mean levels of urinary albumin; creatinine, Albumin/Creatinine ratio (Alb/Cr ratio) and Creatinine Clearance Rate (Ccr) in the three studied groups are shown in the table 5. Urinary albumin mean (mg/dl) was 4.6±1.8, 35.9±56 and 38.8±51 in group one, two and three respectively. On the other hand, mean of urinary creatinine levels (mg/dl) was 195.2±54.9, 169.6±75.5 and 150.5±108.8 respectively. On the other hand, the mean of Alb/Cr ratio (mg/dl) was 24.1±7.9, 364.3±886 and 309.5±320.3 respectively; and creatinine clearance rate (ml/min) were 127±23.0, 98.1±17.6 and 74.3±13.7 in the three studied groups respectively. The study show a significant difference in urinary albumin between group one ($P<0.01$) in compared with group two and three; while there was no significant difference ($P>0.05$) between group two and group three. About for creatinine in urine, there was a significant difference between group one and three ($P<0.05$), and no significant difference between group two with group one and group three. The increase in the urinary albumin and the decrease in creatinine levels were statistically significant ($P<0.05$) in the three studied groups. The increase in the urinary Alb/Cr ratio and in the Ccr were statistically significant ($P<0.01$) in group two compared to the control group. Also, urinary Ccr were statistically significantly ($P<0.05$) in group two and group three compared to group one.

The increase presence of albumin in the urine has marked clinical significance as an early indicator of underlying renal pathology, and considered to be independent cardiovascular risk marker subsequently morbidity and mortality [43]. In the present study Alb/Cr ratio in spot morning urine samples was used to measure albuminuria. Elevation of urinary albumin level and Alb/Cr ratio was significant in diabetics with and without CVDs compared to the control group. In addition, the increase in the Alb/Cr ratio was not significant in group two compared to group three. This result is in coincide with Muhaisen et al. [44] and Carter et al. [45] studies whose noticed that, there was a continuous association between levels of albuminuria with the risk for CVDs and, thus, albuminuria or clinical proteinuria is associated with a higher risk for CVDs morbidity and mortality. The presence of albuminuria is a clear manifestation of obvious nephropathy and is associated with impairment of kidney function. On the other hand, the decrease in urinary creatinine in group two and in group three were significant ($P<0.05$) compared to the control group, this result was in harmony with that of Sinkeler et al.[46] who reported that lower creatinine excretion rate was strongly associated with T2DM and nephropathy. Results of this study revealed that the decrease in the Ccr was significant ($P<0.05$) in group two and group three compared to the control group. This result was in concordant with the findings of the previous studies [23,24]. It is noticed from these results that T2DM patients with CVDs are more susceptible to kidneys diseases than T2DM without CVDs and control.

Correlation between Serum Galectin-3 with Studied Parameters

Correlation between serum Galectin-3 levels with different parameters in the studied groups is shown in Table 6. Pearson correlation coefficient test showed, statistically strong positive correlation between serum Galectin-3 with BMI, smoking, FBS, HbA1c, urea, creatinine, uric acid, cholesterol, triglyceride, LDL-C, LDH, CK, CKMB, Alb/Cr ratio, Ccr and NT-BNP in the studied groups ($P<0.01$), while there was a significant but moderate positive correlation between serum Galectin-3 and urinary Alb/Cr ratio ($P<0.05$); whereas there was negative correlation between Galectin-3 and HDL-C; but no correlation was found between Galectin-3 and age. In contrast, there was a significant strong positive correlation between serum NT-BNP and BMI, smoking, FBG, HbA1c, urea, CKMB ($P <0.01$). Weak correlation found with LDH and Ccr ($P<0.05$). However, negative correlation within HDL-C. At the same time, we noted no correlation between NT-BNP with creatinine, uric acid, LDL-C, CK and Ccr. The results of the study was consonance with Tan et al. [47] that revealed the association between serum Galectin-3 and serum creatinine as well as incident microalbuminuria in individuals with T2DM and lead to the development and progression of diabetic nephropathy there for serum Galectin-3 is a perfect biomarker mediator of the development and progression of diabetic nephropathy. Also some of the previous studies [11,12] agree in some variables with current study that show positive correlation with renal function, BMI and NT-BNP levels, in contrast disagreement in the Galectin-3 level relation with age, where clarified that there were negative correlation with Galectin-3 level. Fashanu et al. [15] showed that, there was significantly ($P< 0.05$) and positive correlation between Galectin-3level with cholesterol, HDL-C and disagree with Falcone et al. [48] study that explained that, there was no significant difference between Galectin-3 level, total cholesterol, HDL-C and LDL-C. Another study of Felker et al. [11] show that the strongest association of Galectin-3 with elevated glucose, blood urea, and creatinine. On the other hand, this study showed, there was strong positive correlation between Galectin-3 and NT-BNP in the three studied groups. This result is agreed with that of Lok et al. [49]. This explanation the high Galectin-3 level with NT-BNP levels may be responsible for processing the increased risk in T2DM with and without CVDs patient. Clearly, we can conclude that serum Galectin-3 levels affected by BMI, age and most of biochemical studied parameters among studied groups, but NT-BNP levels less affect with study parameters.

The main strength of our study was its being the first study, which describes the association between serum Galectin-3 and biochemical parameters with CVDs among T2DM patients in Gaza Strip, Palestine. Our results, clearly demonstrated, a significant difference in serum Galectin-3 level in T2DM with CVDs group in compared with T2DM and control group (P value < 0.01). Furthermore, a positive significant correlations were found between serum Galectin-3 and BMI, smoking, FBS, HbA1c, urea, creatinine, uric acid, cholesterol, triglyceride, LDL-C, LDH, CK, CKMB, urinary Alb/Cr ratio, Ccr and NT-BNP in the studied groups ($p<0.05$); whereas there was a negative correlation between Galectin-3 and HDL-C, but no significant correlation was found between Galectin-3 and age.

Conclusion

Finally, we conclude that Galectin-3 involved in cardiac processing pathway, and closely related to the severity of diabetes in T2DM with and without cardiovascular complications, therefore Galectin-3 may be helpful in the diagnosis and prognosis of CVDs in T2DM patients.

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