DOI: https://doi.org/10.31351/vol33iss1pp104-112

Association of Apelin, Chemerin and Omentin Levels with Oxidative Stress Markers in Non-Diabetic and Induced Diabetic Rats

Hawzheen K.Othman*,1 ,Almas M. Mahmud2 and Ismail M. Maulood3

[1] Department of Biology, College of Science, Salahaddin University, Erbil, Iraq.
[2] Department of Biology, College of Science, Salahaddin University, Erbil, Iraq.
[3] Department of Biology, College of Science, Salahaddin University, Erbil, Iraq.

Abstract

Oxidative stress significantly impacts the etiology of type one diabetes mellitus. However, the associations of adipokines with oxidative stress biomarkers have not yet been investigated. Therefore, the present study aimed to investigate the estimation of serum apelin, chemerin, and omentin levels in induced type one diabetes mellitus and their correlations with oxidative stress markers. The study included sixty male albino rats divided into two groups. The first group (n = 30) was diabetic (diabetes induced with an intraperitoneal injection of 40mg/kg streptozotocin). The non-diabetic control group was the second (n: 30). The enzyme-linked immunosorbent test was used to quantify the serum levels of apelin, chemerin, omentin and asymmetric dimethylarginine. Furthermore, blood glucose, lipid profile triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, liver enzymes (alanine aminotransferase and aspartate transaminase), malondialdehyde and nitric oxide parameters were evaluated. Rats with induced type one diabetes mellitus had significantly higher serum levels of apelin, chemerin, omentin, malondialdehyde, asymmetric dimethylarginine, and nitric oxide compared to non-diabetics. According to the coefficient of correlation analysis, the results revealed a positive association between apelin and low-density lipoprotein, alanine aminotransferase, and malondialdehyde and a negative correlation between apelin and aspartate aminotransferase: alanine aminotransferase. In addition, no correlation was found between apelin and serum levels of glucose, cholesterol, high-density lipoprotein, low-density lipoprotein and high-density lipoprotein ratio, triglyceride, triglyceride and high-density lipoprotein ratio, very low-density lipoprotein, aspartate aminotransferase, creatinine, uric acid, albumin, nitric oxide, and asymmetric dimethylarginine. Low-density lipoprotein, alanine aminotransferase, and malondialdehyde. Serum chemerin level correlated positively with Low-density lipoprotein: high-density lipoprotein and asymmetric dimethylarginine. Also, the plasma level of omentin showed a significant and positive correlation with both triglyceride and high-density lipoprotein ratio and malondialdehyde values. In conclusion, the results of this study introduced apelin and chemerin as potential biomarkers for the early detection of diabetes. Elevated serum apelin levels in induced type one diabetes mellitus could provide compensatory action for low insulin levels. While, apelin, chemerin and omentin's positive correlations with malondialdehyde levels suggest the potential roles of these adipokines in diabetes-induced complications caused by oxidative stress.

Keywords: Induced type one diabetes mellitus, Apelin, Chemerin, Omentin, Oxidative stress.

Introduction

Diabetes mellitus, a complex, long-term metabolic and hormonal disorder have traditionally been characterized by hyperglycemia, which could be caused by insufficient insulin synthesis, problems in insulin action, or both [(1)]. A substantial body of evidence suggests that oxidative stress and reactive oxygen species (ROS) are key factors in the development and progression of diabetes. Oxidative stress occurs when the natural dynamic homeostasis is overwhelmed by an excessive buildup of free radicals([2]). It has been shown that diabetes results in the systemic formation of ROS in many organs, as well as the depletion of antioxidant capacity, all of which contribute to organ damage and the onset of complications[(2)].

Nitric oxide (NO), a vital regulator of metabolic activities and blood vessels, is generated from L-arginine by the action of NO synthase, which is suppressed by endogenous asymmetric dimethylarginine (ADMA), an amino acid naturally present in plasma and many organs. In diabetic individuals higher blood concentrations of ADMA have recently been demonstrated, which may be associated with the accelerated development of diabetes-related complications[(3)] Apelin, a regulating adipokine and endogenous ligand for G protein-coupled receptors was demonstrated to be highly expressed in both human and animal tissues, including the adipocytes, and nervous and vascular systems [(4)]. The apelin-(apelin–angiotensin receptor-like 1 (APJ) system has recently received greater attention due to its possible involvement in many physiological processes, including homeostasis, body regulation, cell proliferation, and

*Corresponding author E-mail: hawzheen.othman@su.edu.krd
Received: 27/11 /2022
Accepted: 5/4 /2023

energy consumption. The real link between oxidative stress and apelin and it is receptor apelin–angiotensin receptor-like 1 has been reported in several investigations. A review study [5] showed that apelin could counteract peroxidation in cardiac tissue and the synthesis of ROS. However, a different finding observed that apelin can enhance the ROS production in cells of vascular smooth muscle [6]

Chemerin was initially found as a new retinoic acid-responsive gene in psoriatic skin lesions, suggesting an immunomodulating role. It was then recognized as one of the adipokines exhibiting paracrine, autocrine and even endocrine effects *in vivo* [7]. According to Helfer and Wu chemerin improves insulin-stimulated glucose absorption in adipocytes and simultaneously enhances insulin signalling. Chemerin has also been stated to cause insulin resistance in muscles *via* activating IRS-1 and Akt signaling [8]. In addition to controlling inflammatory and metabolic processes, chemerin may affect vascular function. An *in-vitro* investigation showed that chemerin reduces NO-dependent cGMP signaling, lowering vascular relaxation in the rat aorta, and this finding was attributed to enhanced free radical production following chemerin-induced endothelial NO synthase uncoupling [9]. Another novel adipokine was identified from omental adipocytes known as omentin-1 or intelectin-1[10]. Moreover, Eimal Latif et al revealed that omentin is expressed selectively in mesothelial cells, visceral fats, vascular cells, small intestine, colon, and goblet cells and is abundantly present in plasma[11], As Habi attributed these widespread expressions of omentin in such critical areas to the roles of this peptide to a variety of physiological functions like cardiovascular function, insulin action and inflammatory response [1] . Omentin-1 has also been demonstrated to have the capacity to modify insulin sensitivity [1] and promote the relaxation of vascular smooth muscle cell[s][12]. A recent study examining the link between omentin-1 concentrations and diabetes found conflicting results[13]. In a study by[12] and his coworkers found that omentin-1 concentrations were reduced in diabetic patients. However, another study discovered that diabetic patients had higher levels of omentin-1 than non-diabetics patients [13]. In addition, recent clinical investigations have found a negative correlation between plasma levels of omentin and oxidative stress, which is considered a possible signal for lowering oxidative stress-related complications in diabetes [11].

Due to the limited and inconsistent results from the previous studies, the current study was created to evaluate changes in serum apelin, chemerin and omentin levels and how they correlate with oxidative stress markers in both non-diabetic and induced-diabetic rats.

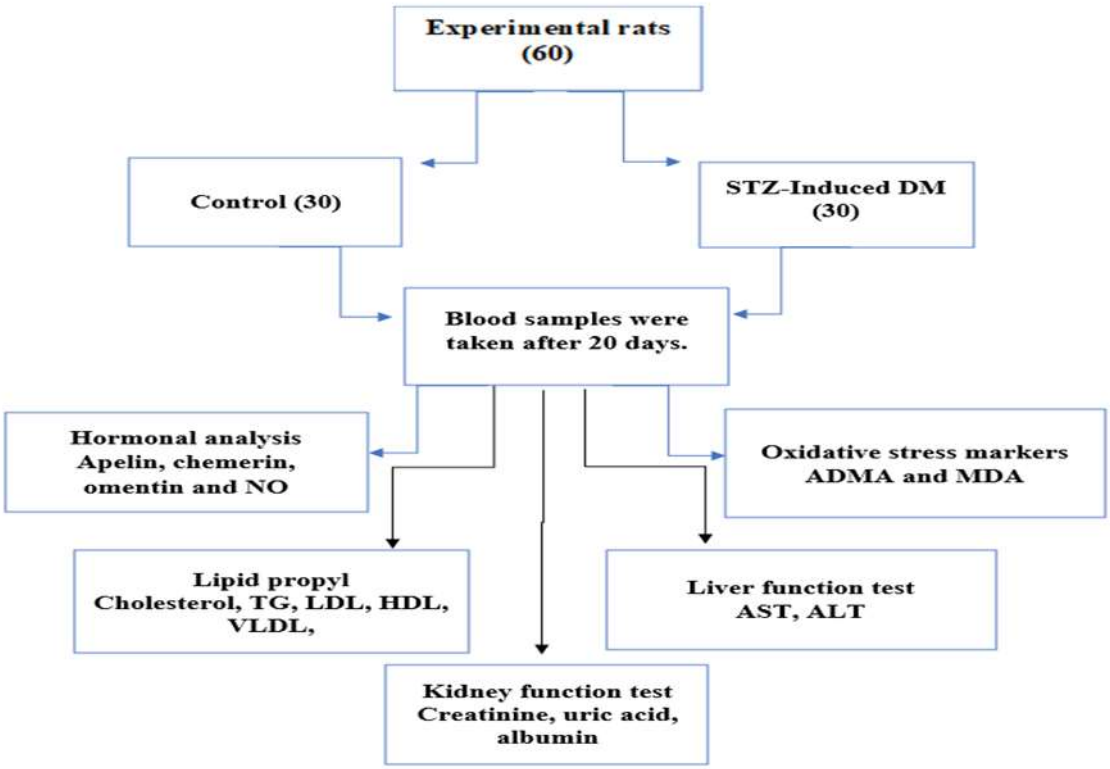
Materials and methods

Animals

Sixty inbred male albino rats weighing about 140–160 grams were used in the experiments. Rats were housed in sterilized plastic cages bedded with soft wood chips and subjected to standard animal house conditions (temperature 232 °C, relative humidity 5510 %,, and 12-hour light/dark cycles), with access to regular rodent chow including the following ingredients (wheat 66.6%, soya 25.6 %, corn oil 4.4 %,, limestone 1.5 %,, salt 0.63 %,, methionine 0.158 %,, Lysine 0.24 %,, choline chloride and tap water *ad libitum*. From August 2019 to June 2022, the study was conducted in the Biology Department /College of science/ Salahaddin University-Erbil.

Studied groups and induction of type one diabetes mellitus

The animals were divided equally into two groups:
Group 1 (control): This group consisted of 30 male albino rats and served as a control group.
Group 2 (induced type one diabetic group): This group consisted of 30 male albino rats in which STZ (40 mg/kg b.w, [14]Sigma-Aldrich, St. Louis, MO, USA) dissolved in citrate buffer was injected intraperitoneally once to experimentally induced diabetes (0.01 M, pH 4.5). To avoid a fatal drop in blood sugar caused by a significant release of insulin after STZ injection, rats treated with STZ were given access to a 5% glucose solution for the first 24 hours (Merck KGG, A Darmstadt, Germany). (Okoduwa et al., 2017). Glucose levels were monitored by tail prick before induction of diabetes and every three days after using a porTable glucose meter. (Accu-check Roche Diagnostics GmbH, Mannheim, Germany). Type one diabetic rats were identified when their blood glucose level exceeded 16.70 mmol/l, and rats were then kept for about 20 days to elicit endothelial dysfunction..



Figureure1. flow chart demonstrates the experimental design.

Sampling of blood

Blood samples were obtained from rat hearts after being anesthetized with a mixture of ketamine and xylazine (90 mg/kg, i.p. and 10 mg/kg, i.p., respectively). The serum was then separated by allowing the blood to clot then centrifuged at 3000 rpm for 10 minutes and kept at - 20 °C until used.

Hormonal analysis

Circulating serum apelin, chemerin, omentin and ADMA were measured each individually with distinct and fixed procedure protocol respectively according to the commercial kit type rat apelin ELISA kit, SunLong Biotech Co.,LTD , rat chemerin ELISA kit, SunLong Biotech Co.,LTD, rat omentin ELISA kit, SunLong Biotech Co.,LTD and rat omentin ELISA kit were used by semi-automated human Enzyme-Linked Immunosorbent Assay (ELISA), NAMRU3/China. Nitric oxide (NO) concentrations are indirectly determined *via* Griess's reaction depending on the measurement of nitrite concentration. Nitrate was reduced to nitrite in the presence of cadmium, then converted to nitric acid, concentrations were determined by spectrophotometric analysis at 543 nm and the products expressed as µmoles (UNICO spectrophotometer SN SQU10111012002) (15). Buege and Aust's method was depended to measure serum malondialdehyde (MDA) (UNICO spectrophotometer SN SQU10111012002), this method was based on the reaction between lipid oxidation products with thiobarbituric acid leading to a colored product [(16)].

Biochemical analysis

Serum glucose, lipid profile tests (triglyceride (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL)), liver function profile tests (alanine aminotransferase (ALT) and aspartate transaminase (AST,)), Creatinine, uric acid, and albumin were determined by a fully automated biochem ical analyzer (Cobas e 411, HITACHI, USA).

Results

Rats with induced type one diabetes mellitus and non-DM had significantly different serum apelin concentrations, as shown in (Figure 2). According to the Mann-Whitney test, apelin levels were substantially elevated in induced type one diabetes mellitus rats compared to non-DM rats. Additionally, apelin was identified as a potential biomarker for TIDM using the ROC curve with AUC values (0.70) and($p<0.014$).

In induced type one diabetes mellitus, there was no correlation between serum apelin and glucose, cholesterol, HDL, LDL: HDL TG, TG: HDL, VLDL, AST, creatinine, uric acid, albumin, NO, and ADMA levels. LDL, ALT, and MDA. While, serum apelin levels were inversely correlated with AST: ALT and positively with LDL, ALT, and MDA (Table 1 and Figure 4)

Table 1. Correlation coefficient of apelin, chemerin and omentin as dependent variables with metabolic parameters of both non-diabetic and induced diabetic rats

Parameters	Apelin (n=30)		Chemerin(n=30)		Omentin (n=30)	
	r	P	r	P	r	P
Glucose	0.212	0.144	0.117	0.396	0.239	0.095
Cholesterol	0.187	0.199	-0.101	0.463	-0.001	0.996
LDL	0.52	0.004	0.129	0.348	0.096	0.507
HDL	0.117	0.423	-0.224	0.100	-0.140	0.333
LDL: HDL	0.186	0.200	0.245	0.071	0.242	0.091
TG	0.120	0.410	-0.020	0.887	0.141	0.330
TG: HDL	0.022	0.879	0.098	0.477	0.286	0.044
VLDL	0.120	0410	-0.020	0.887	0.141	0.330
AST	-0.072	0.621	-0.012	0.931	0.190	0.187
ALT	0.41	0.004	0.028	0.839	0.238	0.100
AST: ALT	-0.305	0.039	-0.066	0.637	-0.096	0.511
Creatinine	0.028	0.849	0.134	0.330	0.108	0.457
Uric acid	0.132	0.373	0.060	0.661	-0.076	0.599
Albumin	-0.077	0.599	-0.085	0.538	-0.092	0.526
NO	-0.063	0.672	-0.140	0.314	-0.139	0.342
MDA	0.374	0.010	0.195	0.157	0.294	0.040
ADMA	0.032	0.831	0.290	0.035	0.110	0.458
Apelin			-0.238	0.112	-0.096	0.547
Chemerin	-0.238	0.112			0.309	0.031
Omentin	-0.096	0.547	0.309	0.031		

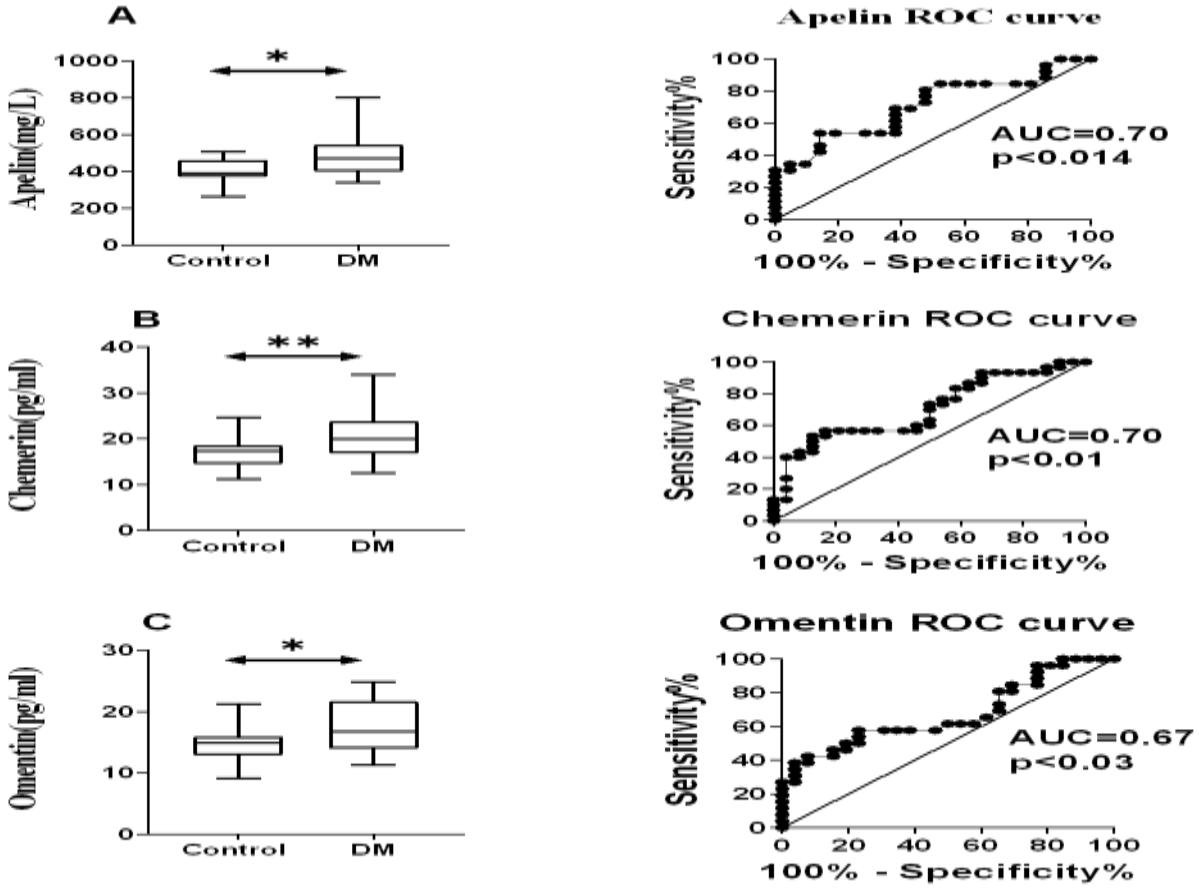


Figure 2. Evaluation of apelin, chemerin and omentin levels in control and induced TIDM and ROC curve analysis. The ROC curves in (A) apelin and (B) chemerin as biomarker of diabetes.

As shown in (Figure 2), rats with T1DM reported significantly increased blood chemerin levels than non-DM rats, and chemerin appears to be a viable biomarker for T1DM based on ROC curve data (0.70), ($p<0.01$). Based on Spearman correlation coefficient (r) analysis, the data reveals that there was a positive association between the chemerin and LDL: HDL and ADMA in the studied rat groups. Furthermore, this study found no significant correlation between serum chemerin levels and the other parameters studied (Table 1 and Figure 5).

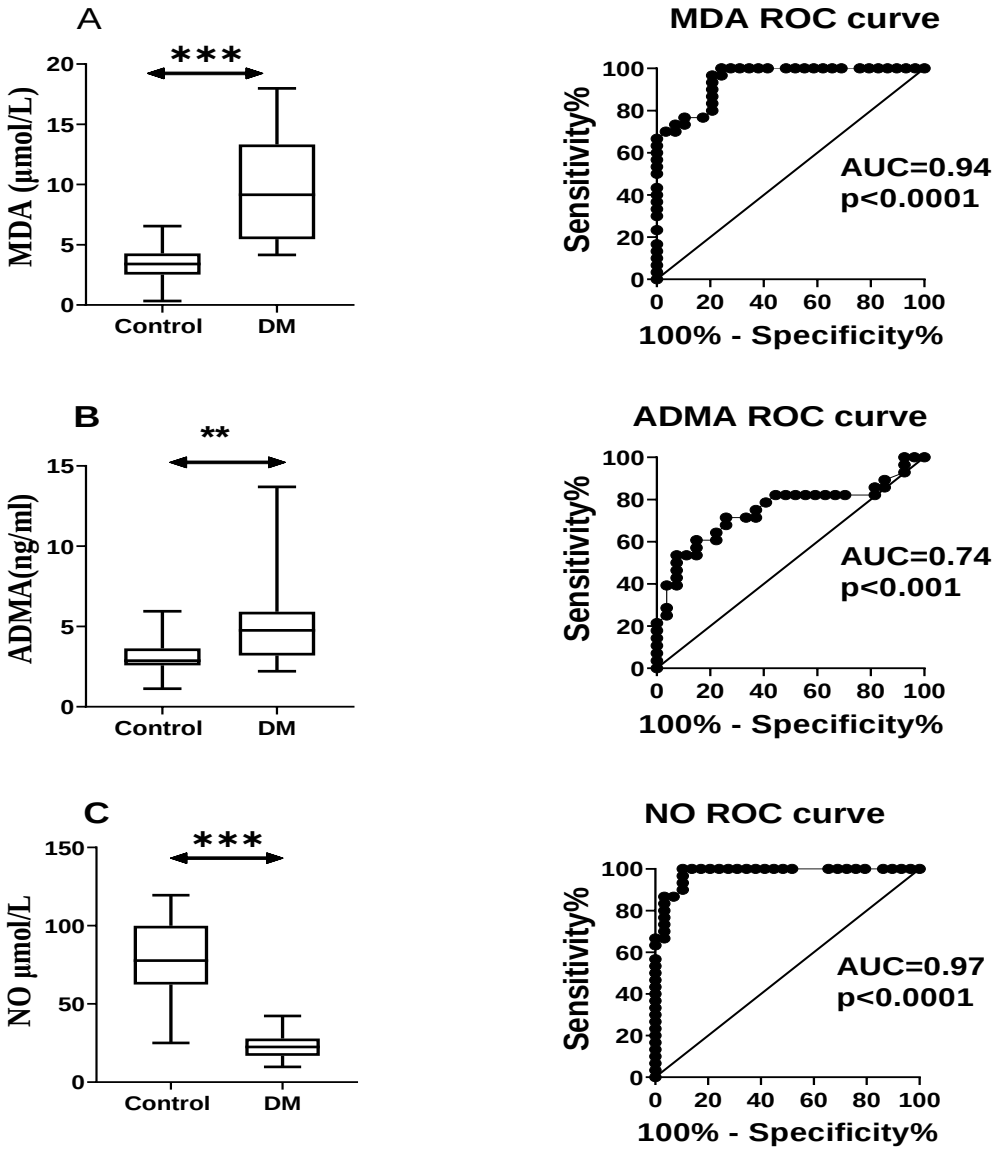


Figure 3. Evaluation of MDA, ADMA and NO levels in control and induced type one diabetes mellitus and the ROC curves in (A) MDA, (B) ADMA, and (C) NO represent a good biomarker of diabetes.

Animals with induced type one diabetes mellitus demonstrated significantly increase serum omentin levels than non-diabetic rats. This kind of adipokine, according to rock curve data analysis (AUC 0.67) ($p> 0.03$), did not become a reliable biomarker for diabetes (Figure2). Moreover, serum omentin levels associated positively with TG: HDL and MDA. No association was identified between serum omentin levels and plasma glucose, cholesterol, LDL, HDL, LDL: HDL, TG, TG: HDL, VLDL, AST, ALT, creatinine, uric acid, albumin, NO, and ADMA (Table 1 and Figure 5).

In induced type, one diabetes mellitus serum MDA, NO, and ADMA levels were significantly higher than those for the non-diabetic group (Figure 3). Data from the ROC curve analysis, such as the AUC and P values for apelin, chemerin, and omentin, show that all of the adipokines represent as good biomarkers for diabetes.

.

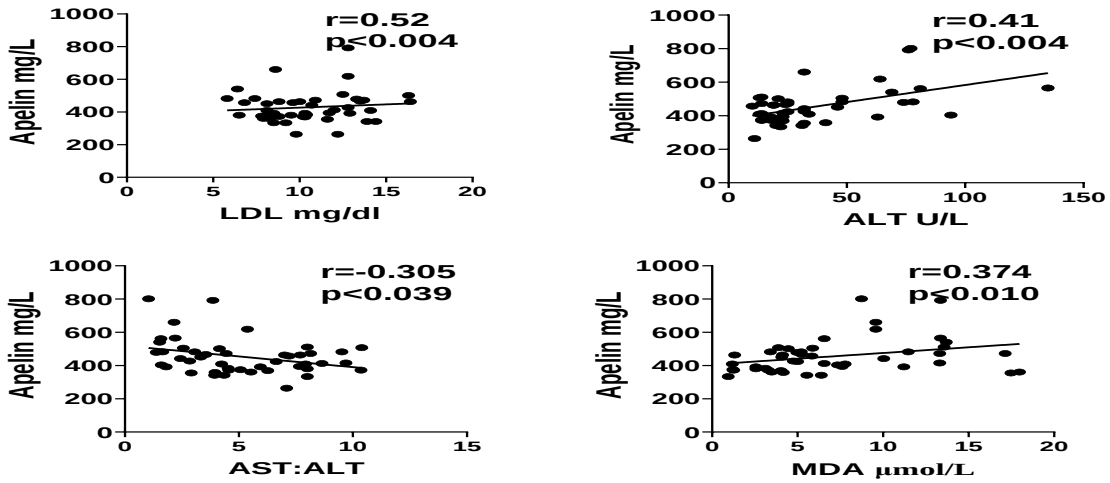


Figure 4. Correlation between serum levels of apelin and LDL, ALT, AST: ALT and MDA

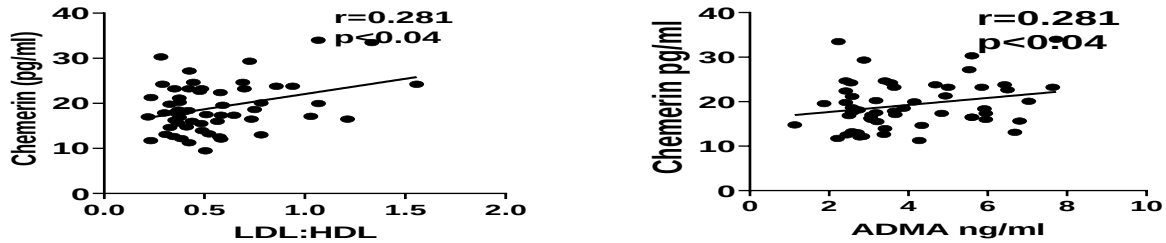


Figure5A. Correlation between serum levels of chemerin and LDL: HDL and ADMA

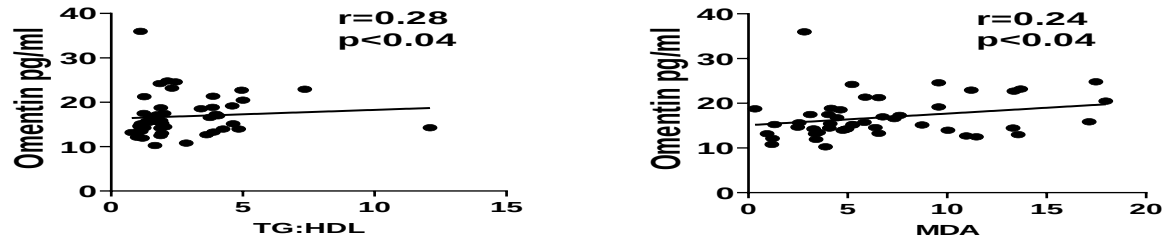


Figure 5B. Correlation between serum levels of omentin with TG: HDL and MDA.

Discussion

Apart from the overwhelming evidence of oxidative stress's harmful effects and its connection to experimental diabetes, many clinical studies are being conducted in an attempt to find new biological markers for the earlier detection of diabetes and its link to oxidative stress, as well as for controlling disease's progression and reducing side effects. Because of their vital role in human metabolic processes, adipocytes have recently attracted a lot of interest. Adipose tissue, which acts as an endocrine gland, produces and releases many adipokines, including apelin, chemerin, and omentin.The exploration of a potential adipokine linked to diabetes and oxidative stress may offer clinicians new opportunities to diagnose diabetes earlier and successfully manage its complications.

The findings from this study provide evidence that diabetic rats' serum apelin levels were higher than those of the non-diabetic control rats. This finding was in line with that of Marana Habchi and his coworkers. This research reported higher serum apelin levels in both categories of diabetics, and type 1 DM patients experiencing a greater increase than type 2 DM patients [17]. The majority of human investigations have demonstrated that apelin's effect on glucose metabolism is simultaneous to insulin because it enhances tissue glucose absorption and transport[18]. It was recorded that an injection of apelin efficiently increases serum insulin levels while lowering blood sugar levels[19]. In addition to this, chronic apelin administration greatly increases insulin levels and pancreatic islet mass through the increase of PERK-IRE1a-CHOP

signaling and the inactivation of AKT, ERK, and AMPK pathways[20]. Thus, our stud confirmed the notion that elevated apelin levels in T1DM are the result of a compensatory mechanism meant to counteract low insulin levels.

In the present investigation, it was found that T1DM rats had considerably higher levels of MDA than non-diabetics and apelin positively correlated with this elevation. According to research conducted in both clinical and experimental contexts, free radicals are created by the breakdown of glucose, nonenzymatic protein glycation, and oxidative protein degradation in diabetes, elevated amounts of free radicals could cause lipid peroxidation, and MDA levels are frequently evaluated as a recognized indicator of lipid peroxidation [21].

According to the available literature, apelin activation of APJ improves oxidative stress in human adipocytes, nervous tissues and cardiovascular system [5, 22]. However, the hypothesis that apelin suppresses ROS contradicts the conclusion of our findings because we found that apelin has a positive association with a free radical marker such as MDA. There is evidence that apelin stimulates the production of Nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4). [23]. Apelin has also been demonstrated to promote the production of ROS in endothelia [24] and to trigger the production of ROS during the proliferation of vascular smooth muscle cells via a ROS-ERK-dependent pathway [25].

In our study, we observed a decrease in nitric oxide levels in induced diabetes. This reduction may be attributed to several factors, including increased oxidative stress, impaired endothelial function, and alterations in the insulin signaling pathway, all of which can negatively impact nitric oxide production and bioavailability. Understanding the underlying mechanisms of this phenomenon is crucial for developing targeted interventions to prevent or mitigate vascular complications associated with diabetes [26-28].

Data obtained from our study reveal that apelin positively correlated with LDL levels. It has been demonstrated that apelin prevents lipolysis and makes lipid vacuoles more resistant to lipases, increasing their stability. Therefore, this positive correlation may be a protective mechanism for increased apelin levels against lipidemia produced by oxidative stress brought on by diabetes.

As regards to liver function, our results showed a significant positive association of apelin with serum ALT, which is consistent with Wang and his coworkers' findings that apelin has a role in increasing liver fibrosis through apelin-mediated profibrotic gene production through the ERK signaling pathway [29]. The direct hepatotoxic action of excess free radicals within hepatocytes by apelin may be the reason for the positive connection between rising levels of ALT and apelin. The idea that oxidative stress is the typical mechanism for the etiology of liver damage has received support from experimental and clinical studies [30].

In our investigation, it was discovered that induced type one diabetes mellitus patients had considerably higher serum chemerin levels (Sun et al and Elsehmaway et al) both reported similar results[31] [32]. In contrast to their findings, we could not discover any evidence of a positive link with glycemic variables, indicating that the raised levels of chemerin in diabetic rats were likely caused by other factors rather than increased glucose levels. This finding was further supported by the fact that both diabetics with high and low HbA1C had equal chemerin levels [33].

Chemerin levels and the LDL: HDL ratio, a risk factor for stenosis, were shown to positively correlate in the current study. This result suggested chemerin as a promoting factor for atherosclerosis [34]. Our results support earlier research' conclusions that there is a favorable correlation between plasma chemerin levels and total LDL cholesterol levels [35]. Some researchers have positively linked aortic chemerin expression and atherosclerosis in aortic VSMC and foam cells and they demonstrated the accumulation of chemerin in the atherosclerotic lesion of the aorta [36].

Additionally, we observed a significant and noTable positive relationship between level of chemerin and ADMA for the first time ever in the literature. ADMA has been considered a new risk biomarker of cardiovascular disease. It is believed that elevated circulatory levels of ADMA have been observed to be positively related to different pathological circumstances including atherosclerosis, hypercholesterolemia, chronic heart failure, hypertension, DM and chronic renal failure [37]. Along with earlier study, our findings indicating that chemerin can be regarded as an early prognostic indicator for future complications brought on by diabetes [32].

Serum level of omentin experienced a significant elevation in induced type one diabetes mellitus. Contradictory findings were found in other researches on the relationship between circulating omentin-1 levels and DM. For instance, clinical trials found that diabetic individuals had reduced omentin-1 concentrations[12] . However, according to previous research, DM had greater omentin-1 levels than controls [13]. During a course of a year-long follow-up experiment, the findings showed that the upregulation of omentin levels was associated with greater HbA1c levels in diabetic patients (38). Also, omentin expression and/or release in human adipocytes was inhibited by insulin and enhanced by fibroblast growth factor 21 and dexamethasone (39). A clinical study found that an increase in blood omentin levels was associated with a decrease in high-sensitivity C-reactive protein levels, regardless of changes in glucose or HOMA-R [38]. Additionally,

a recent study further demonstrated that omentin levels rise to combat the acute period following the development of cardiovascular disease [13]. The lack of an inverse relationship with MDA and TG: HDL in our study leads us to conclude that the increase in serum omentin levels is due to vascular damage or inflammation caused by hyperglycemia in rats with T1DM.

Conclusion

Elevated circulating serum apelin and chemerin levels in induced type one diabetes mellitus rats may be an indicator for the progression of diabetes, and hence it is beneficial to measure serum apelin in the diabetic individuals to identify disease problems early.

Acknowledgement

I appreciate Almas MR. Mahmud and Ismail M. Maulood's participation in examining early drafts of the full work critically.

Conflict of Interest

I do not have any conflict of interest or any other relevant connection or shared interest.

Funding

This research was self-funded by the author

Ethics Statements

All experiments involving rats were conducted in compliance with the guidelines set forth by the Institutional Animal Care and Use Committee at Salahaddin university

Author Contribution

The authors contributed to all aspects of the study, including conceiving and designing the study, conducting experiments, analyzing data, and writing the manuscript.

References

1. As Habi A, Sadeghi M, Arab A, Hajianfar H. The association between omentin and diabetes: a systematic review and meta-analysis of observational studies. Diabetes Metab Syndr Obes. 2019;12:1277-86.
2. Pasupuleti VR, Arigela CS, Gan SH, Salam SKN, Krishnan KT, Rahman NA, et al. A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. Oxid Med Cell Longev. 2020;2020:8878172.
3. Gawrys J, Gajecki D, Szahidewicz-Krupska E, Doroszko A. Intraplatelet L-Arginine-Nitric Oxide Metabolic Pathway: From Discovery to Clinical Implications in Prevention and Treatment of Cardiovascular Disorders. Oxid Med Cell Longev. 2020;2020:1015908.
4. Lv S, Feng Y, Jiang Q, Lv X, Yang Y, Cirillo G. Relationship between Apelin/APJ Signaling, Oxidative Stress, and Diseases. Oxidative Medicine and Cellular Longevity. 2021;2021:1-7.
5. Zhou Q, Cao J, Chen L. Apelin/APJ system: A novel therapeutic target for oxidative stress-related inflammatory diseases (Review). Int J Mol Med. 2016;37(5):1159-69.
6. Lu T, Lee HC. Coronary Large Conductance Ca(2+)-Activated K(+) Channel Dysfunction in Diabetes Mellitus. Front Physiol. 2021;12:750618.
7. Helfer G, Wu QF. Chemerin: a multifaceted adipokine involved in metabolic disorders. J Endocrinol. 2018;238(2):R79-r94.
8. Neves KB, Nguyen Dinh Cat A, Alves-Lopes R, Harvey KY, Costa RMD, Lobato NS, et al. Chemerin receptor blockade improves vascular function in diabetic obese mice via redox-sensitive and Akt-dependent pathways. Am J Physiol Heart Circ Physiol. 2018;315(6):H1851-H60.
9. Zhou Y, Zhang B, Hao C, Huang X, Li X, Huang Y, et al. Omentin-A Novel Adipokine in Respiratory Diseases. Int J Mol Sci. 2017;19(1).
10. Liu F, Fang S, Liu X, Li J, Wang X, Cui J, et al. Omentin-1 protects against high glucose-induced endothelial dysfunction via the AMPK/PPARδ signaling pathway. Biochem Pharmacol. 2020;174:113830.
11. Eimal Latif AH, Anwar S, Gautham KS, Kadurei F, Ojo RO, Hafizyar F, et al. Association of Plasma Omentin-1 Levels With Diabetes and Its Complications. Cureus. 2021;13(9):e18203.
12. Abdelraouf Korany M, Sonbol A, Mohamed Elgouhary S. Omentin-1 and diabetic retinopathy in type 2 diabetic patients. Alexandria journal of medicine. 2018;54(4):323-6.
13. Hayashi M, Morioka T, Hatamori M, Kakutani Y, Yamazaki Y, Kurajoh M, et al. Plasma omentin levels are associated with vascular endothelial function in patients with type 2 diabetes at elevated cardiovascular risk. Diabetes Research and Clinical Practice. 2019;148:160-8.
14. Yanni AE, Antoniou N, Kostomitsopoulos N. Improvements needed in reporting the methodology for STZ-induced diabetes in rats. Am J Physiol Endocrinol Metab. 2021;320(5):E898-E9.
15. Ratajczak-Wrona W, Jablonska E, Antonowicz B, Dziemianczyk D, Grabowska SZ. Levels of biological markers of nitric oxide in serum of patients with squamous cell carcinoma of the oral cavity. International Journal of Oral Science. 2013;5(3):141-5.
16. Ali IMM, Yenzeel JH, Al-ansari HMS. Evaluation of Oxidative Stress and LeptinLevel in Samples of Iraqi Obese Women. Iraqi Journal of Science. 2020:1565-70.
17. Habchi M, Duvillard L, Cottet V, Brindisi MC, Bouillet B, Beacco M, et al. Circulating apelin is increased in patients with type 1 or type 2 diabetes and is associated with better glycaemic

control. Clin Endocrinol (Oxf). 2014;81(5):696-701.
18. Li M, Fang H, Hu J. Apelin-13 ameliorates metabolic and cardiovascular disorders in a rat model of type 2 diabetes with a high-fat diet. Mol Med Rep. 2018;18(6):5784-90.
19. Li C, Cheng H, Adhikari BK, Wang S, Yang N, Liu W, et al. The Role of Apelin-APJ System in Diabetes and Obesity. Front Endocrinol (Lausanne). 2022;13:820002.
20. Huang Q, Liu X, Cao C, Lei J, Han D, Chen G, et al. Apelin-13 induces autophagy in hepatoma HepG2 cells through ERK1/2 signaling pathway-dependent upregulation of Beclin1. Oncol Lett. 2016;11(2):1051-6.
21. Shahvali S, Shahesmaeili A, Sanjari M, Karami-Mohajeri S. The correlation between blood oxidative stress and sialic acid content in diabetic patients with nephropathy, hypertension, and hyperlipidemia. Diabetol Int. 2020;11(1):19-26.
22. Yamazaki S, Sekiguchi A, Uchiyama A, Fujiwara C, Inoue Y, Yokoyama Y, et al. Apelin/APJ signaling suppresses the pressure ulcer formation in cutaneous ischemia-reperfusion injury mouse model. Sci Rep. 2020;10(1):1349.
23. Bi Y, Lei X, Chai N, Linghu E. NOX4: a potential therapeutic target for pancreatic cancer and its mechanism. Journal of translational medicine. 2021;19(1):1-8.
24. Liu M, Li H, Zhou Q, Zhao H, Lv D, Cao J, et al. ROS-Autophagy pathway mediates monocytes-human umbilical vein endothelial cells adhesion induced by apelin-13. J Cell Physiol. 2018;233(10):6839-50.
25. Chen L, Zhou T, White T, O'Brien A, Chakraborty S, Liangpunsakul S, et al. The Apelin-Apelin Receptor Axis Triggers Cholangiocyte Proliferation and Liver Fibrosis During Mouse Models of Cholestasis. Hepatology. 2021;73(6):2411-28.
26. Sokolovska J, Dekante A, Baumane L, Pahirko L, Valeinis J, Dislere K, et al. Nitric oxide metabolism is impaired by type 1 diabetes and diabetic nephropathy. Biomed Rep. 2020;12(5):251-8.
27. Meza CA, La Favor JD, Kim DH, Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? Int J Mol Sci. 2019;20(15).
28. Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation. Antioxidants & Redox Signaling. 2021;35(15):1291-307.
29. Wang Y, Song J, Bian H, Bo J, Lv S, Pan W, et al. Apelin promotes hepatic fibrosis through ERK signaling in LX-2 cells. Mol Cell Biochem. 2019;460(1-2):205-15.
30. Arroyave-Ospina JC, Wu Z, Geng Y, Moshage H. Role of Oxidative Stress in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: Implications for Prevention and Therapy. Antioxidants. 2021;10(2):174.
31. Sun J, Ren J, Zuo C, Deng D, Pan F, Chen R, et al. Circulating apelin, chemerin and omentin levels in patients with gestational diabetes mellitus: a systematic review and meta-analysis. Lipids Health Dis. 2020;19(1):26.
32. Elsehmawy A, El-Toukhy SE, Seliem NMA, Moustafa RS, Mohammed DS. Apelin and chemerin as promising adipokines in children with type 1 diabetes mellitus. Diabetes Metab Syndr Obes. 2019;12:383-9.
33. Akgul Balaban Y, Yilmaz N, Kalayci M, Unal M, Turhan T. IRISIN AND CHEMERIN LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS. Acta Endocrinol (Buchar). 2019;15(4):442-6.
34. Lőrincz H, Katkó M, Harangi M, Somodi S, Gaál K, Fülöp P, et al. Strong correlations between circulating chemerin levels and lipoprotein subfractions in nondiabetic obese and nonobese subjects. Clin Endocrinol (Oxf). 2014;81(3):370-7.
35. Szpakowicz A, Szpakowicz M, Lapinska M, Paniczko M, Lawicki S, Raczkowski A, et al. Serum Chemerin Concentration Is Associated with Proinflammatory Status in Chronic Coronary Syndrome. Biomolecules. 2021;11(8).
36. Spiroglou SG, Kostopoulos CG, Varakis JN, Papadaki HH. Adipokines in periaortic and epicardial adipose tissue: differential expression and relation to atherosclerosis. J Atheroscler Thromb. 2010;17(2):115-30.
37. Gac P, Poreba M, Jurdziak M, Trzmielewska E, Goclawska K, Derkacz A, et al. Cardiovascular risk factors and the concentration of asymmetric dimethylarginine. Adv Clin Exp Med. 2020;29(1):63-70.
38. Tan BK, Adya R, Farhatullah S, Chen J, Lehnert H, Randeva HS. Metformin treatment may increase omentin-1 levels in women with polycystic ovary syndrome. Diabetes. 2010;59(12):3023-31.
39. Watanabe T, Watanabe-Kominato K, Takahashi Y, Kojima M, Watanabe R. Adipose Tissue-Derived Omentin-1 Function and Regulation. Compr Physiol. 2017;7(3):765-81.