SHORT COMMUNICATION   **PERIOPERATIVE MEDICINE**

Evaluation of adiponectin serum levels and their association with oxidative stress in individuals with type 2 diabetes mellitus in Iraq

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ABSTRACT

Background: Diabetes stands among the top ten contributors to worldwide mortality, with diabetics facing 2 to 3 times greater susceptibility to all-encompassing mortality. The adipose tissue has garnered growing recognition as a vigorously dynamic endocrine organ, discharging an array of biologically impactful molecules, among which is adiponectin. We investigated the influence of serum adiponectin levels and their correlation with oxidative stress in individuals with type 2 diabetes mellitus (T2DM) in the province Anbar of Iraq.

Methodology: A case-control research was carried out at Al-Ramadi Hospitals located in Anbar province, Iraq. The study encompassed a total of 84 individuals, comprising 42 patients diagnosed with T2DM, and 42 individuals enrolled as healthy controls (HCs). Venous blood samples were collected and each sample was divided into four, to obtain the serum. Serum levels of adiponectin and oxidative stress were assessed through the utilization of enzyme-linked immunosorbent assays (ELISA).

Results: The study findings revealed that there was no significant impact on the lipid profile among patients when compared to the control group, as evidenced by statistically insignificant differences (P > 0.05). However, a noteworthy increase in glucose levels was observed in patients compared to healthy individuals, demonstrating a significant difference (P < 0.05). Furthermore, the average levels of malondialdehyde (MDA) displayed a significant rise in patients in comparison to the healthy cohort (P < 0.05). In a similar vein, superoxide dismutase (SOD) levels demonstrated a notable contrast, showcasing higher values in the healthy group in contrast to the patients (P < 0.05). The adiponectin levels exhibited remarkable divergence, with notably elevated values in the T2DM group relative to the healthy group, achieving statistical significance (P < 0.05). The data indicated positive associations between serum adiponectin levels and MDA, as well as fasting serum glucose (FSG), triglycerides (TGs), and very-low-density lipoprotein (VLDL). Nevertheless, no significant correlations were identified between adiponectin and SOD, as well as the other variables.

Conclusion The findings of the study indicated that reduced adiponectin levels are associated with elevated oxidative stress among T2DM patients.

Abbreviations: FSG - fasting serum glucose; MDA - malondialdehyde; SOD - superoxide dismutase; TGs - Triglycerides; T2DM - type 2 diabetes mellitus; VLDL - Very-low-density lipoprotein

Key words: Adiponectin; SOD; Oxidative Stress; Diabetes Mellitus

1. INTRODUCTION

The term diabetes refers to a group of common endocrine disorders characterized by a continuous elevation of blood sugar levels.[1,2] This occurs either due to insufficient production of insulin by the pancreas, or because the body's cells do not properly respond to the produced insulin. Insulin, a peptide hormone, is produced by beta cells in the pancreatic islets of Langerhans and is considered the main anabolic hormone in the body.[3,4] It regulates the process of carbohydrate, fat, and protein metabolism by promoting the absorption of glucose from the blood into the liver, fat, and skeletal muscle cells.[5]

Adiponectin is a versatile hormone composed of 224 amino acids produced by white adipose tissues.[6,7] It was first identified in 1995 and is encoded by a gene located on chromosome 3q27. Adiponectin can exist in three forms with different molecular weights: low, medium and high molecular weight. Each form has distinct activities, such as glucose uptake and central obesity (waist-to-height ratio) binding.[8] Its levels increase after weight loss.[9] Adiponectin is considered one of the strongest markers of type 2 diabetes mellitus (T2DM), as its plasma levels are negatively associated with the development of insulin resistance and the occurrence of the disease. Adiponectin affects insulin sensitivity in diabetic patients through both direct and indirect mechanisms;[10] including:

1) It reduces the amount of triglycerides in adipose tissues and regulates insulin signaling. Moreover, it enhances the expression of fatty acid transporter molecules like CD36 and acyl-coenzyme oxidase, thus playing a significant role in triglyceride metabolism in skeletal muscles. The reduction in triglyceride content within muscles is likely to contribute to the improvement of insulin signaling pathway transmission.[11]

2) Adiponectin activates peroxisome proliferator-activated receptor alpha (PPAR-α), which promotes fatty acid oxidation and energy expenditure, leading to a reduction in triglyceride content in the liver and muscles. This, in turn, increases insulin sensitivity.[12]

3) Adiponectin activates the Adenosine monophosphate-activated protein kinase (AMPK) pathway, thereby stimulating beta-oxidation.[13]

The previously described oxidative stress condition contributes to the attenuation of insulin signaling, leading to an elevation in insulin resistance. Tissues sensitive to insulin, such as skeletal muscles and the liver, do not respond effectively to secreted insulin, resulting in resistance. This resistance is assumed to be the underlying cause of diabetes complications.

Furthermore, reactive oxygen species may contribute to the deterioration of pancreatic beta cells, rendering them incapable of keeping up with the increased demand for insulin secretion, consequently leading to a decrease in beta cell mass and insulin secretion.[14] Additionally, oxidative stress pathways induce oxidative damage to various large cellular molecules, promoting cell injury and impairing their function in general, ultimately affecting glucose tolerance.[15] This cellular damage ultimately leads to complications of diabetes, the most prominent of which include diabetic retinopathy (damage to blood vessels in the light-sensitive tissue of the retina, which can accumulate and result in complete loss of retinal functions), neuropathy (nerve damage), nephropathy (kidney damage), and cardiomyopathy (heart muscle damage). Additionally, oxidative stress inhibits the function of antioxidants, causing damage to various organs and disrupting their functions. Finally, it also disrupts hormonal balance.

2. METHODOLOGY

2.1. Study design:

A case-control research was carried out at Al-Ramadi Hospitals located in Anbar province, Iraq. The study encompassed a total of 84 individuals, comprising 42 patients diagnosed with T2DM, and 42 individuals enrolled as healthy controls. Between December 2022 and April 2023, blood samples were collected through venipuncture in the early morning. These samples were placed into standard gel tubes and allowed to clot at room temperature (18-25°C) for around two hours. Subsequently, centrifugation was carried out at 3000 xg for 20 min to separate the serum. The obtained serum was divided into four equal portions, each containing 500 μL. The second, third, and fourth portions were stored in Eppendorf tubes at -20°C, while the first portion was utilized to measure blood glucose levels and perform biochemical analyses. The second portion was dedicated to estimating copper, zinc, and chromium levels. The third portion was employed for measuring malondialdehyde (MDA) and superoxide dismutase (SOD), and the final portion was allocated for measuring levels of asparagine and adiponectin. Individuals using insulin or not taking antidiabetic medications were excluded from the study.

2.2. Determination of adiponectin and oxidative stress:

Adiponectin and oxidative stress markers, including SOD and MDA, were quantified utilizing a human interleukin ELISA kit sourced from Spinreact Co., Ltd. (USA). The assay procedure strictly adhered to the manufacturer's guidelines. The fasting serum glucose

Table 1: Serum levels of FSG, asprosin, MDA, and SOD in T2DM and healthy controls

Parameter	T2DM Group	Control Group	P-value
FSG (mg/dL)	190 ± 52.9	97.31 ± 20.17	< 0.0001
Adiponectin (ng/mL)	9.353 ± 3.251	4.721 ± 1.948	< 0.0001
MDA	455.2 ± 131.5	304.8 ± 67.2	< 0.0001
SOD	1.152 ± 0.4289	2.013 ± 0.4895	< 0.0001
T. Cho. (mg/dL)	162.3 ± 41.68	158.8 ± 23.79	0.6423
TGs (mg/dL)	150.9 ± 42.51	107.9 ± 29.65	< 0.0001
HDL (mg/dL)	42.6 ± 12.12	43.79 ± 8.394	0.6022
LDL (mg/dL)	91.9 ± 34.04	93.47 ± 22.25	0.8039
VLDL (mg/dL)	30.19 ± 8.502	20.6 ± 7.361	< 0.0001
Atherogenic index (AI)	2.294 ± 0.9727	2.231 ± 0.7624	0.7409
Coronary risk index (CRI)	4.02 ± 1.267	3.744 ± 0.8763	0.2494
Data presented as mean ± SD; P < 0.05 considered as significant			

(FSG) levels for both patients and controls were assessed by measuring glucose concentrations following the method outlined by Braham and Tindoer (1972).

2.3. Research ethics and approval:

Before commencing the study, all participants provided voluntary consent for the collection of blood samples. The informed consent process, was endorsed by the ethical review committee at the University of Anbar. A meticulously designed questionnaire was utilized to gather pertinent details from both the patient and control cohorts, ensuring rigorous adherence to the principles stipulated in the informed consent protocol.

2.4. Statistical analysis:

Linear regression was employed for data analysis, and the findings were presented as mean ± standard deviation. The statistical analysis was conducted using SPSS software version 23.0. Significance in statistical terms was established at a probability threshold of $P \leq 0.05$.

3. RESULTS

Comparative serum levels of FSG, asprosin, MDA, and SOD in patients known to have T2DM and the healthy controls are given in Table 1. The results demonstrated a significant and substantial elevation ($P < 0.0001$) in adiponectin levels among the T2DM group (9.353 ± 3.251) compared to the healthy group (4.721 ± 1.948), as indicated in Table 1.

Pearson correlation analyses revealed significant positive correlations between serum adiponectin

concentrations and MDA (r = 0.382, P < 0.0001), as depicted in Table 2. Additionally, there was a significant positive correlation with FSG, triglycerides (TGs), and very-low-density lipoprotein (VLDL) (r = 0.363, P < 0.0001; r = 0.575, P < 0.0001; r = 0.575, P < 0.0001, respectively), as illustrated in Table 2. Conversely, serum adiponectin levels exhibited significant negative correlations with superoxide dismutase (SOD) (r = -0.324, P < 0.003, as outlined in Table 2.

However, no significant correlations were observed between adiponectin levels and the remaining variables, as indicated in Table 2.

Table 2: Correlations between serum adiponectin concentrations and different study factors

Parameter	r (Adiponectin ng/mL)	P-value
Adiponectin (ng/mL)	1.000	0.000
MDA (ng/mL)	0.382	< 0.0001
SOD (IU/mL)	-0.324	0.003
FSG (mg/dL)	0.526	< 0.0001
T. Cho. (mg/dL)	0.178	0.105
TGs (mg/dL)	0.575	< 0.0001
HDL (mg/dL)	-0.132	0.2301
LDL (mg/dL)	0.083	0.454
VLDL (mg/dL)	0.575	< 0.0001
AI	0.187	0.088
CRI	0.299	0.006
P < 0.05 considered as significant		

4. DISCUSSION

The findings demonstrated a notable difference (P ≤ 0.05) in the levels of FSG between Control Group (97.319 ± 20.17) and T2DM Group (190 ± 52.9), as illustrated in Table 2. The increased FSG in T2DM Group was attributed to diminished insulin secretion in individuals with T2DM, resulting in reduced glucose transport out of cells for storage, thereby leading to the accumulation of larger glucose molecules.[16] The increased levels of blood glucose primarily stems from the pancreas's inadequate production of insulin or the reduced sensitivity of tissues to its effects. Individuals with diabetes encounter disruptions in their metabolic processes that result in elevated blood glucose levels. The normal action of insulin is hindered by heightened resistance, which is attributed to a decrease in the number of insulin receptors on the surfaces of target cells. Insulin, a hormone that typically aids in reducing blood glucose levels, is responsible for promoting the entry of glucose into cells for utilization and energy production.[17] The outcomes of this study are consistent with the findings of various researchers who have also investigated blood glucose levels among diabetic patients in Samarra.[18,19,20] Likewise, the heightened glucose levels observed in patients are in line with the investigation carried out by Jaeger et al., where they observed a notable rise in blood glucose levels among individuals with hypertension compared to the control group. This could be attributed to the presence of insulin resistance within cells frequently associated with hypertension. The degree of resistance tends to amplify alongside increased levels of fats and cholesterol circulating in the blood, a process largely influenced by the hormone aldosterone produced by the adrenal glands. This hormone contributes to both elevated blood pressure and insulin resistance.[21]

In the context of negative feedback pathways, glucose acts as an inhibitor of this peptide. Adiponectin levels tend to be higher in individuals with insulin resistance. Research suggests that reducing adiponectin levels may enhance insulin sensitivity. Consequently, individuals with T2DM might encounter challenges in how white adipose tissue (WAT) regulates adiponectin release, potentially leading to elevated adiponectin levels. Due to heightened adiponectin levels, the liver produces more glucose, which could result in increased insulin levels and, as a direct consequence, a deterioration of insulin resistance in the liver.[22,23]

The mean MDA level showed a markedly significant difference (P < 0.0001) between the averages of the control and patient cohorts, as elucidated in Table 2. The outcomes illustrated a noteworthy rise in MDA values among the T2DM group relative to the Control Group. The elevation in MDA levels within the T2DM group concurs with prior research findings.[21,22,24] This increase is linked to oxidative stress mechanisms primarily triggered by elevated glucose levels. The escalation in MDA levels functions as an indicator of heightened oxidative stress within the body, with its concentration corresponding to elevated blood sugar levels.[25]

The mean SOD levels demonstrated a statistically significant disparity (P ≤ 0.05) between the two groups, as depicted in Table 1. The findings showcased a notable increase in SOD levels among the healthy group in contrast to the patients. In the context of diabetes, the automatic oxidation of glucose leads to the creation of hydrogen peroxide, which suppresses the activity of SOD. Consequently, the accumulation of hydrogen peroxide might contribute to the reduced SOD activity observed in these patients. The pivotal defense mechanism that shields cells and tissues from potentially harmful reactions involving reactive oxygen species and their byproducts is the Cu/Zn-SOD enzyme. It is worth highlighting that SOD can be swiftly stimulated under specific conditions when cells or organisms confront oxidative stress. The diminished SOD activity observed in diabetes could imply a gradual waning of its efficiency and function with the progression of the disease. Furthermore, the presence of non-enzymatic glucose, which adds to hydrogen peroxide generation, further hinders Cu/Zn-SOD activity.[21] SOD stands as a pivotal antioxidant enzyme, perpetually catalyzing the transformation of the superoxide anion (O2-) into H_2O_2 and O_2. The evaluation of oxidative stress and antioxidant levels sometimes necessitates gasometric methods like blood sampling. The exploration of saliva for markers of oxidative stress and antioxidants that faithfully mirror the oxidative and reductive conditions within the body carries noteworthy clinical significance.[26]

The results presented in Table 1 indicate a non-significant difference in the means of T.Cho between Groups. Conversely, a highly significant and substantial increase < 0.0001 was observed in the means of TGs for the T2DM group compared to the healthy controls (Table 1). On the other hand, the means of HDL, LDL, AI, and CRI between the Control and T2DM groups showed insignificant and inconsequential variations. In contrast, there was a notably high and significant increase < 0.0001 in the means of very-low-density lipoprotein (VLDL) for both groups.

In this study, there was a statistically significant increase (P ≤ 0.05) in the levels of triglycerides and VLDL in the T2DM group compared to the control group. These findings align with another study,[27] which demonstrated that insulin resistance and T2DM lead to elevated levels of VLDL containing high concentrations of TG. The study described lipid abnormalities in T2DM patients,

including elevated serum TG, VLDL, and LDL.[25] These lipid disorders encompass not only quantitative but also qualitative abnormalities of fatty acids that may contribute to atherosclerosis. Quantitative abnormalities include elevated levels of total plasma cholesterol, TGs, and low-density lipoprotein cholesterol (LDL-C), as well as decreased levels of high-density lipoprotein cholesterol (HDL-C). This study's findings are consistent with our investigation.[28] In diabetes, the abnormally high level of serum lipid peroxides may result from abnormal lipid metabolism. Often, individuals with T2DM exhibit abnormal lipid profiles consisting of moderately elevated LDL-C, T.Cho, and TG. Variations in these lipid levels might stem from differences in body mass index, lifestyle, duration of diabetes, and possibly genetic variations. These study outcomes are in agreement with prior research. Dyslipidemia in T2DM plays a pivotal role in accelerating cardiovascular diseases. Insulin function diminishes when insulin resistance occurs, leading to suppressed lipid breakdown and reduced lipoprotein lipase (LPL) activity. Consequently, the production of free fatty acids (FFA) increases. Elevated hepatic FFA production enhances the production and secretion of VLDL-rich in TG, ultimately leading to decreased HDL-C levels. The metabolism of HDL-C is affected by these processes through exchange with VLDL-TG facilitated by CETP, generating TG-enriched HDL particles. These particles subsequently degrade into HDL and TG via hepatic lipase.[29]

Pearson correlation analyses revealed significant positive correlations between serum adiponectin concentrations and MDA ($r = 0.382$, $p < 0.0001$), as depicted in Table 2. Additionally, there was a significant positive correlation with FSG, TGs, and VLDL, as illustrated in Table 2. Conversely, serum adiponectin levels exhibited significant negative correlations with SOD, as outlined in Table 2. However, no significant correlations were observed between adiponectin levels and the remaining variables, as indicated in Table 2.

5. CONCLUSION

Malondialdehyde levels exhibited an increase in the type 2 diabetes group compared to the healthy group, whereas superoxide dismutase levels showed an elevation in the healthy group compared to the patients. Adiponectin levels were lower in the type 2 diabetes group compared to the healthy group. Adiponectin levels in the serum of the patients exhibited an increase and showed positive indicators. This can serve as an indicator of susceptibility to type 2 diabetes, which plays a significant role in exacerbating oxidative stress, ultimately leading to a decrease in certain antioxidants.

6. Data availability

The numerical data generated during this research is available with the authors.

9. Authors contributions

All authors actively participated in the conception, design, data analysis, statistical evaluation, drafting, editing, and review of the manuscript, making it a collaborative endeavor.

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