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Evaluation of Mid-regional pro-adrenomedullin (MR-proADM) and same biochemical parameters in diabetes mellitus type 2 patients

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Abstract
Background: The pro-adrenomeduline central region (MR-proADM) and biochemical parameters perform many biological activities throughout the human body. Thus, an increase in their level in plasma and blood is directly associated with an increased risk of developing type 2 diabetes mellitus (T_2 DM). However, the potential association between MR-proADM and biochemical parameters in patients with T_2 DM is still not well understood and studies in this area are limited. The present study aimed to assessment the relationship between MR-proADM and biochemical parameters and its association with T2DM in the population of Kirkuk, Iraq.
Methods: The current cross-sectional study included a total of 90 participants (55 with T_2 DM and 45 healthy without T2DM) and all of them underwent several important routine health examinations. The patients' ages ranged from 19 to 34 years. The samples were collected for the measurement of MR-proADM and biochemical parameters by biochemical and clinical measurement methods specified in this study. The association between MR-proADM and biochemical parameters in T_2 DM was evaluated by statistical analysis methods represented by SPSS version 20, and the associations for these variables were found by Pearson's correlation.
Results: The mean value of glucose, Alkaline phosphatase (ALP) and MR-proADM for participants with T2DM was significantly higher (P = 0.007), (P = 0.020), and (P =0.005) compared to the control group. While the mean value of total serum bilirubin of the participants with T_2 DM was low and significantly uncorrelated (P = 0.457) respectively compared to the control group. The mean value of calcium and vitamin D of participants with T_2 DM was low but significantly associated (P = 0.031) and (P = 0.029) respectively compared to control group. Regarding MR-proADM values and biochemical parameters in T_2 DM, found that MR-proADM was positively correlated with blood glucose levels and ALP in T_2 DM patients and negatively correlated with vitamin D. While there was no correlation between MR-proADM and serum bilirubin and calcium in T_2 DM patients.
Conclusions: Elevated levels of blood glucose, ALP and MR-proADM were observed in patients with T2DM, and it was found that there is a correlation between MR-proADM and biochemical parameters of T_2 DM. This study recommend testing and screening for MR-proADM and biochemical parameters on a larger scale as it has been shown to be associated with type 2 diabetes.

Keywords: Mid-regional pro-adrenomedullin, biochemical parameters, type 2 diabetes mellitus, liver enzyme

Introduction
Adrenomedullin (ADM) is a group of amino acid with peptide (52 amino acids) that are vasoactive in vascular [1-3], are considered a potent vasodilator, hypotensive in the circulatory system, and have metabolic and immune-modulating effects [3]. ADM is secreted from different types of cells in organs and tissues, including cardiomyocytes cells, monocytes, and fibroblasts, and mainly from vascular endothelial smooth muscle cells [1, 2], and it performs many physiological functions [1]. In addition, ADM is produced as a result of a lack of ischemia or hypoxia [4] and the process of its secretion in the tissues helps in the continuation of the blood supply to individual organs [3]. Also, ADM levels rise in the plasma of patients with diseases such as hypertension, diabetes mellitus, and arterial stifness [1, 5]. Furthermore, a multifunctional regulatory peptide regulated by hypoxia and inflammation is pro-Adrenomedullin, which produces a peptide known as the Mid-regional pro-Adrenomedullin (MR-proADM) [3, 6, 7].

MR-proADM is the most stable of ADM and represents active peptide levels in patients' plasma and blood samples [6, 8]. Moreover, MR-proADM is considered a biomarker of endothelial function, and its increased level is a sign of organ dysfunction weakness and failure or an indicator of organ dysfunction [7, 9, 10].

Diabetes mellitus is known as a non-communicable and chronic disease that affects humans as a result of a deficiency in insulin production, and it is known as type 1 diabetes mellitus (T1DM) or insulin weak in its function and is known as type 2 diabetes (T2DM) [11]. In addition to that, individuals suffer from diabetes for a genetic or acquired reason [11], the World Health Organization (WHO) stated that it is considered one of the five major chronic diseases and one of the most important health problems affecting people all over the world, especially T_2 DM [11, 12]. One of the symptoms of diabetes mellitus in patients is hyperglycemia and the possibility of hypertension and cardiovascular disease [11-13]. Since there are strong associations between elevated levels of MR-proADM in the plasma of blood samples and risks of cardiovascular events such as congestive heart failure (CHF) and kidney disease in patients with T_2 DM [2, 14]. Therefore, the need to evaluate the role of MR-proADM and the biochemical parameters of T_2 DM has recently been highlighted. In addition, MR-proADM performs many biological activities throughout the human body [15], and therefore its increased level in patients with T_2 DM may be a reaction and response to cellular strain and adaptation to any type of cellular aggression associated with diseases related to increase the secretion of MR-proADM [14]. Recent studies have indicated that higher levels of circulating MR-proADM in plasma are directly associated with an increased risk of Heart Failure (HF), a major health problem experienced by most of patients with T_2 DM [2, 16, 17].

Moreover, a study reported that there is an association between MR-proADM levels and biochemical parameters through clinical outcomes for patients with T2DM and kidney disease [15]. While currently, no studies are showing the relationship between MR-proADM levels in plasma and the risk of developing liver disease in patients with from T_2 DM. In addition, most deaths from T_2 DM were due to liver disease, which plays an important role in glucose homeostasis [18, 19]. Therefore, it was important to determine the relationship between MR-proADM levels and elevated liver enzymes in patients with T_2 DM, which are associated with elevated biochemical parameters indicated by clinical results such as liver enzyme (ALP) alkaline phosphatase [18, 20]. In general, these enzymes are elevated in individuals with T_2 DM [20], so they were focused on in this study, in addition to some biochemical parameters. Accordingly, the present study aimed to evaluate MR-proADM levels and biochemical parameters among T_2 DM diabetic patients in Kirkuk, Iraq.

Materials and Methods

Study design and Participants

The current study is a prospective, cross-sectional, and observational study. The total participants were 200 eligible individuals all of whom underwent medical examinations and of the 200 participants, 110 were excluded because their laboratory data were incomplete and they did not meet the inclusion criteria. The study included 90 randomly selected participants who all underwent several important routine health check-ups. This study included 55 patients with confirmed T2DM (26 males and 29 females) and 35 healthy individuals free of diabetes, which is known as the control group. Where the ages of male ranged from 19 to 34 years, and the ages of female ranged from 19 to 34 years. Also, the control group included participants who m*et al*l inclusion criteria. The patients' ages ranged from 19 to 34 years. The current study was conducted during the period starting from August 1, 2021 to January 8, 2022, at Kirkuk general Hospital.

Inclusion and Exclusion Criteria

Inclusion Criteria

Cases were adults within the aforementioned age groups without Diabetic complications.

Exclusion Criteria

Diabetics complications: Heart disease, myocardial infraction, unstable angina, stable angina, heart failure, Diabetic neuropathy, problems with feet, oral health, Diabetic retinopathy, hearing, and Gestational diabetes.

Biochemical and Clinical measurements

Blood samples were collected and drawn from all participants after 15 minutes of rest and a ten-hour fast. Blood samples were kept at 10 °C until centrifugation. All blood samples were stirred and centrifuged at 3,600 g for 10 minutes at 28 °C after collection. Plasma was withdrawn and separated from blood samples immediately for MR-proADM assessment, then stored at −80 °C until MR-proADM measurement. Plasma MR-proADM was measured and determined by an enzyme-linked immunosorbent assay (ELISA, Elabscience company, USA) technique kit (). Plasma concentrations of MR-proADM were measured and analyzed using (BIO-TEK INSTRUMENTS, INC, USA). Biochemical parameters that included blood glucose, direct bilirubin, indirect bilirubin, total bilirubin, vitamin D, calcium (Ca+), and liver enzyme (ALP) were measured by biometrics. Blood glucose (BG) levels, or blood glucose were assessed by the Trinder reagent test using the GOD-PAP method (BIOLABO company, France). Direct bilirubin mg /dl and total serum bilirubin mg /dl were determined by the sulfanilic acid method (BIOLABO Company, France) while indirect bilirubin mg /dl was measured through the following relationship: Conc of total bilirubin – Conc of direct bilirubin= mg /dl. ALP enzyme activity in plasma was measured by the Community of Clinical Chemistry (DEA) method (BIOLABO Company, German). The Ca+ concentration was determined using the Arsenazo III method (BIOLABO company, German), and Serum vitamin D was determined by an enzyme-linked immunosorbent assay (ELISA) (technique Inc. kit, USA) and this analysis was performed using (BIO-TEK INSTRUMENTS, INC, USA).

Statistical analysis

SPSS version 20 was used to perform the statistical analyses. The value of $p \leq 0.05$ was taken and determined to indicate statistical significance. Quantitative data are expressed as means ± standard error (SE), which is used to used also to refer the standard deviation ± (SD) of the samples. The use of Pearson's correlation was used to find the correlations between patients' biochemical parameters and MR-proADM.

Results

In the current study, a total of 90 participants were enrolled. Among them were 55 (61%) participants with T_2 DM and 40 (38%) without diabetes (control). The ages of the participants with T_2 DM were recorded and the minimum and maximum ages were 19 and 34 years. Males constituted 26 (47%) of the T_2 DM participants and females 29 (52%).

The mean value of glucose 289.5 mg/dl for participants with T2DM was significantly higher (P = 0.007) high compared to participants (control) whose mean glucose value was 99.6 mg/dl as shown in Table 1. It is also clear from the obtained results that the mean values of direct bilirubin, indirect bilirubin, and total serum bilirubin were 0.056 mg/dl, 0.490 mg/dl, and 0.546 mg/dl for participants with T2DM, respectively, while the mean values were 0.166 mg/dl, 0.558 mg/dl, and 0.724 mg/dl for the participants (control), respectively. Moreover, the level of direct bilirubin, indirect bilirubin, and total serum bilirubin of participants with T_2 DM and their levels for participants (control) were not significantly correlated (P = 0.681), (P = 0.488), and (P = 0.457), respectively (Table 1). The results of the liver enzyme ALP indicated that the levels of ALP were elevated in participants with T_2 DM, where the mean values were 225.4 U/L, compared to participants in the control group, where the mean values of ALP were 112.4 U/L. Based on these results, the mean values of ALP participants with T2DM were found to be significantly higher than in the control group (P = 0.020), as shown in Table 1. Regarding calcium (Ca^+) mg/dl, the results showed that the mean value of calcium 7 for participants with T2DM was significantly low (P = 0.031) compared to participants (control) whose mean value of Ca^+ was 9.6. Finally, it was noted through the results that the mean value of vitamin D (Vit D) was 16.294 ng/ml for participants with T2DM and it was 25.1 ng/ml for control group. Thus the mean values of Vit D in participants with T_2 DM are significantly low compared to the control group (P = 0.029) (Table 1). In addition, the results related to biochemical parameters were further illustrated by Figure 1, which shows the comparison of these parameters between participants with T2DM and healthy participants (control group).

From Table 2 we find that the mean value of MR-proADM is 210.8 pg/ml for participants with T2DM was significantly high (P = 0.005) compared to participants (control), where the mean value of MR-proADM was 90.8 pg/ml. Moreover, the results related to MR-proADM was further illustrated by Figure 2, which shows the comparison of MR-proADM between participants with T2DM and healthy participants (control group). It was evident that the MR-proADM levels of the patients were higher than the control group.

Table 1: Comparing between biochemical parameters in T2DM patients and healthy participants (control), (N = 90) in Kirkuk, Iraq, 2022.

Parameters	Control Mean± SE (N=40)	Patients Mean± SE (N=50)	P-Value
Glucose mg/dl	99.600±2.760	289.510±3.640**	0.007
Direct bilirubin mg/dl	0.166±0.035	0.056±0.027	0.681
Indirect bilirubin mg/dl	0.558±0.149	0.490±0.176	0.488
ALP U/I	112.400±3.668	225.417±2.157*	0.020
Ca^+ mg/dl	9.618±0.597*	7.044±0.181	0.031
Total serum bilirubin mg/dl	0.724±0.184	0.546±0.157	0.457
Vitamin D ng/ml	25.076±0.248*	16.294±0.290	0.029

(ALP) alkaline phosphatase (Ca+) Calcium.

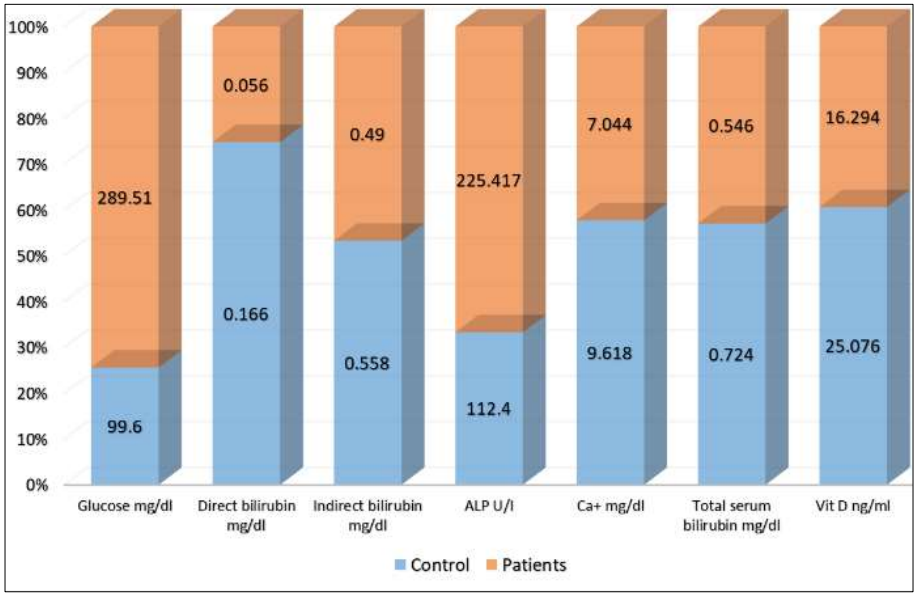

Fig 1: Comparison (means) of biochemical parameters among T2DM patients participants and healthy participants (control).

Table 2: Comparing between MR-proADM levels in T2DM patients and healthy participants (control).

Parameters	Control Mean ±SE (N=40)	Patients Mean ±SE (N=50)	P-Value
MR-proADM pg/ml	90.801±2.071	210.802±1.547**	0.005

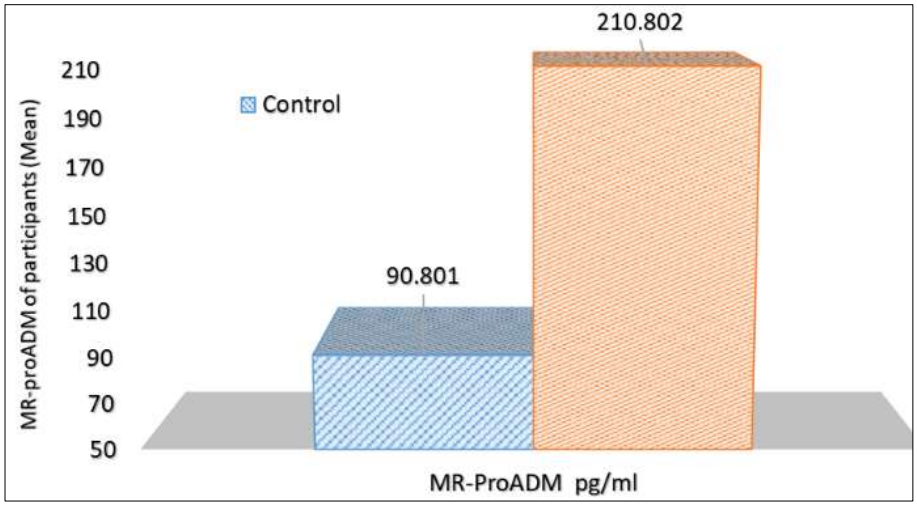

Fig 2: Comparing of MR-proADM levels between control and patients.

The results showed that the mean values of MR-proADM were 215.6 pg/ml and 204.1 pg/ml for male and female participants with T2DM, respectively. However, the MR-proADM level of participants with gender was not significantly associated (P = 0.374) (Table 3). While Figure 3 shows that the levels of MR-proADM through the mean values were slightly higher for males with type 2 diabetes than for females with the same disease.

Table 3: Comparing MR-proADM levels between male and female.

Parameters	Male Mean ±SE (N=23)	Female Mean ±SE (N=27)	P-Value
MR-proADM pg/ml	215.575±3.140	204.120±2.113	0.374

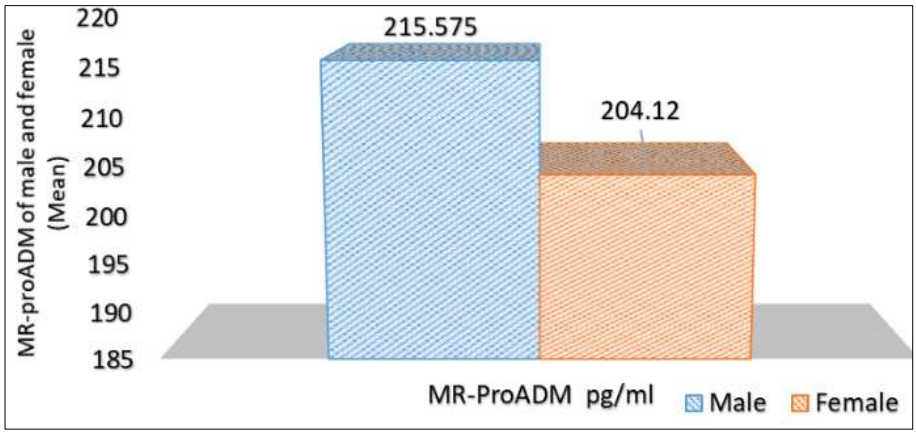

Fig 3: Comparing MR-proADM levels between male and female.

The results of the correlation between biochemical parameters and MR-proADM for patients with T2DM are shown in Table 4. There was no significant correlation between MR-proADM and direct bilirubin, indirect bilirubin, total serum bilirubin and calcium (Ca^{+}) (P > 0.05) in patients with T2DM, but there was a significant positive correlation between MR-proADM and glucose (P = 0.01) in T2DM patients. Also, the results showed that there was a significant positive correlation between MR-proADM and liver enzymes ALP (P = 0.05) in T2DM patients, while MR-proADM was negatively correlated with Vit D (P = 0.05) in T2DM patients.

Table 4: Correlation between biochemical parameters and MR-proADM in T2DM patients.

Correlation Patients Parameters	Glucose	Direct bilirubin	Indirect bilirubin	ALP	Ca^{+}	Total serum bilirubin	Vit D
MR-proADM	0.991**	0.210	0.371	0.921*	-0.738	0.548	-0.998*
P-Value	0.01	0.314	0.251	0.05	0.175	0.081	0.05

*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level, Positive number = Direct proportion, and Negative number =inverse proportion.

Discussion

In the current study, the blood glucose level was significantly higher in participants with T_2 DM compared to healthy participants, this because individuals with T_2 DM had higher levels of plasma glucose concentration [21]. Also, the reason for the higher glucose levels in patients with T_2 DM than normal is that they have insulin resistance and thus result in the abnormality in plasma glucose balance [19]. Currently, many studies have been conducted that are concerned with the level of bilirubin in the blood and its association with T_2 DM. Through the results of our study, we found that the levels of direct bilirubin, indirect

bilirubin, and total serum bilirubin in the blood of patients with T_2 DM were low compared to healthy participants. This result is consistent with the results of many recent studies that focused on the relationship between bilirubin levels in the blood and the risk of developing T_2 DM, where they found that individuals with T_2 DM have bilirubin levels low and it was inversely associated with T2DM [22-24].

The results of the current study revealed that there were significantly elevated levels of the liver enzyme ALP in participants with T_2 MD. This occurs in T_2 MD patients as a result of several reasons, including irregular blood glucose levels and the production of glycation, which leads to oxidative stress, which in turn causes a change in liver enzyme levels from the normal level [19, 25]. In addition, glucose metabolism in T_2 DM patients increases the risk of Liver damage and consequently high its enzymes in the blood [26]. Moreover, the results in this study indicated that there is a significant association between elevated levels of ALP enzyme and T_2 DM. These findings are in agreement with the results of a study conducted by Shibabaw *et al*. where they found that liver enzymes were elevated in patients with T_2 DM compared to the control group [19]. Also, the study by Forlani *et al*. reported that levels of the liver enzyme were elevated and their prevalence rate was high in T_2 DM [27].

Calcium and vitamin D are among the most important elements needed by the body, especially the bones, but at specific levels. Many studies have mentioned that there is a link between blood calcium levels, vitamin D, and T_2 DM, as those studies confirmed that individuals with type 2 diabetes suffer from a deficiency in vitamin D and calcium [28]. This is what was reached through the results related to Ca and Vit D. We found that the patients with T_2 DM had a decrease in the level of Ca and Vit D, while the healthy control group had higher levels for these two parameters. This is because calcium and vitamin D affect the level of glucose balance in the blood as a result of their association with insulin secretion by pancreatic cells [28, 29]. In addition, this result showed that there is an association between blood Ca and Vit D levels and type 2 diabetes, and this result is consistent with what was reported by some previous studies [28-30].

In our study, we aimed to evaluate MR-proADM and understand the pathophysiological effect of it on individuals with T_2 DM. An increase in the concentration of MR-proADM has been associated with several pathological conditions in obese individuals, including insulin resistance, dyslipidemia, oxidative stress, and low-grade infammation [31]. Furthermore, these cases were directly related to patients with T_2 DM. Thus this association could explain why the concentration of MR-proADM is increased in patients with T_2 DM. Our results indicate that the levels of MR-proADM were significantly higher for those with T2DM than the control group and that there is a close association between high MR-proADM concentration and T2DM. There are not many studies evaluating MR-proADM levels and its association with T_2 DM. In view of this, the results we obtained were important and consistent with the results of the study conducted by Suthahar *et al*. where their results indicated that there is a significantly positive association between MR-proADM and type 2 diabetes [32]. In addition to that, another study conducted by Sujana *et al*. indicated that there is an association between the higher concentration of MR-proADM and type 2 diabetes, and the association was positive between them, especially in obese patients [31]. Regarding to the gender and its association with MR-proADM levels in patients with T_2 DM, the result that showed the relationship of gender (males and females) with type 2 diabetes with different levels of MR-proADM that there is a difference, but not significantly in the levels of MR-proADM between males and females, and no significant association between MR-proADM and gender. There is a lack of studies that have examined the relationship between these biomarkers variables and gender, but there is one study in China reported that biomarkers such as MR-proADM possessed gender-related differences, and MR-proADM levels were higher in males than in females [33].

Furthermore, the results of our study regarding the correlation between biochemical parameters and MR-proADM for patients with T_2 DM indicated that MR-proADM was correlated with some parameters and there was no association with the rest. Where we not found correlation between MR-proADM and serum bilirubin and calcium in patients with T_2 DM, and we not found previous studies addressing the correlation between MR-proADM and these biochemical parameters to predict them. Thus, our current study it will be evidence of a lack of association between MR-proADM levels and serum bilirubin and calcium levels of patients with T_2 DM. Also we found that MR-proADM levels were positively correlated with blood glucose levels in T_2 DM patients. This is explained by the fact that the high concentration of MR-proADM in plasma leads to an increase in insulin resistance. Thus this indicates the correlation between MR-proADM and glucose in patients with T_2 DM, in addition to that elevated levels of MR-proADM also cause variations in glucose metabolism [34, 35, 36]. A recent study examined the correlation between liver enzymes and some biochemical parameters in T2DM [20]. However, in this study, we evaluated the correlation between MR-proADM and liver enzymes in patients with T_2 DM. The results showed that MR-proADM was positively correlated with ALP and negatively with vitamin D. These results represent one of the strengths of this study, as when looking at previous and recent studies, no study was obtained that reported the results of the correlation between MR-proADM and liver enzymes on the one hand, and between it and vitamin D in patients with T_2 DM before. However, the true relationship between MR-proADM and biochemical parameters in T_2 DM requires more future research to predict it more clearly and accurately.

Conclusion

In conclusion, the results of the current study showed that the levels of blood glucose, liver enzyme (ALP) and MR-proADM were significantly higher in T_2 DM patients than in control group participants and significantly associated with T2DM. While the levels of direct bilirubin, indirect bilirubin and total bilirubin in T_2 DM patients were low. While calcium and vitamin D levels were lower in T T_2 DM patients compared to control group but significantly associated with T_2 DM. In addition, there was no significant association between gender and MR-proADM levels in patients with T_2 DM. Regarding the MR-proADM and biochemical parameters values in T_2 DM, we found that MR-proADM was positively correlated with blood glucose levels and liver enzymes in T_2 DM patients and negatively correlated with vitamin D. While there was no correlation

between MR-proADM and serum bilirubin and calcium in T2DM patients T_2 DM. After the relationship between MR-proADM and biochemical parameters in T_2 DM has been evaluated, we recommend that tests and examinations of MR-proADM and biochemical parameters be conducted more broadly as it has been proven to be associated with type 2 diabetes. This may justify future studies to predict the effect and role of these parameters and MR-proADM and thus reducing the risk of diabetes.

References

1. Koyama T, Kuriyama N, Suzuki Y, Saito S, Tanaka R, Iwao M, *et al*. Mid-regional pro-adrenomedullin is a novel biomarker for arterial stiffness as the criterion for vascular failure in a cross-sectional study. Scientific Reports. 2021;11(1):305.
2. Fraty M, Velho G, Gand E, Fumeron F, Ragot S, Sosner P. & Surdiagene Study Group. Prognostic value of plasma MR-proADM vs NT-proBNP for heart failure in people with type 2 diabetes: The surdiagene prospective study. Diabetologia. 2018;61:2643-2653.
3. Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Müller B. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. Critical care. 2005;9;1-9.
4. Cormier-Regard S, Nguyen SV, Claycomb WC. Adrenomedullin gene expression is developmentally regulated and induced by hypoxia in rat ventricular cardiac myocytes. Journal of Biological Chemistry. 1998;273(28):17787-17792.
5. Levy D, Hwang SJ, Kayalar A, Benjamin EJ, Vasan RS, Parise H, *et al*. Associations of plasma natriuretic peptide, adrenomedullin, and homocysteine levels with alterations in arterial stiffness: The Framingham Heart Study. Circulation. 2007;115(24):3079-3085.
6. Montrucchio G, Sales G, Balzani E, Lombardo D, Giaccone A, Cantù G, *et al*. Effectiveness of mid-regional pro-adrenomedullin, compared to other biomarkers (Including lymphocyte subpopulations and immunoglobulins), as a prognostic biomarker in COVID-19 critically ill patients: New evidence from a 15-month observational prospective study. Frontiers in Medicine, 2023, 10.
7. Indirli R, Bandera A, Valenti L, Ceriotti F, Di Modugno A, Tettamanti M, *et al*. Prognostic value of copeptin and mid-regional proadrenomedullin in COVID-19-hospitalized patients. European Journal of Clinical Investigation. 2022;52(5):e13753.
8. Lundberg OH, Bergenzaun L, Rydén J, Rosenqvist M, Melander O, Chew MS. Adrenomedullin and endothelin-1 are associated with myocardial injury and death in septic shock patients. Critical Care. 2016;20:1-11.
9. Fialek B, De Roquetaillade C, Pruc M, Navolokina A, Chirico F, Ladny JR, *et al*. Systematic review with meta-analysis of mid-regional pro-adrenomedullin (MR-proADM) as a prognostic marker in Covid-19-hospitalized patients. Annals of Medicine. 2023;55(1):379-387.
10. Li P, Wang C, Pang S. The diagnostic accuracy of mid-regional pro-adrenomedullin for sepsis: A systematic review and meta-analysis. Minerva Anestesiologica. 2021;87(10):1117-1127.
11. Ferdi NEH, Khalida ABLA, Chenchouni H. Biochemical profile of an adult diabetic population from Algeria in relation with anthropometric parameters, age and gender. Iranian journal of public health. 2018;47(8):1119.
12. Rahbar S, Naimi SS, Soltani AR, Rahimi A, Akbarzadeh Baghban A, Rashedi V, *et al*. Improvement in biochemical parameters in patients with type 2 diabetes after twenty-four sessions of aerobic exercise: A randomized controlled trial. Iranian Red Crescent Medical Journal, 2017, 19(7).
13. Jayan A, Dubey RK, Padmavati P, Jha AC, Gautam N. Association of lipid profile with fasting and post prandial glucose level in type 2 diabetic patients. Journal of Universal College of Medical Sciences. 2015;3(1):2-5.
14. Velho G, Ragot S, Mohammedi K, Gand E, Fraty M, Fumeron F, *et al*. Plasma adrenomedullin and allelic variation in the ADM gene and kidney disease in people with type 2 diabetes. Diabetes. 2015;64(9):3262-3272.
15. Montrucchio G, Sales G, Rumbolo F, Palmesino F, Fanelli V, Urbino R, *et al*. Effectiveness of mid-regional pro-adrenomedullin (MR-proADM) as prognostic marker in COVID-19 critically ill patients: An observational prospective study. PLoS One. 2021;16(2):e0246771.
16. Holmager P, Schou M, Egstrup M, Gustafsson I, Goetze JP, Gustafsson F, *et al*. The influence of diabetes mellitus on mid regional pro adrenomedullin concentrations and prognostic value in heart failure outpatients. Journal of Cardiac Failure. 2015;21(3):250-257.
17. Giglio RV, Stoian AP, Haluzik M, Pafili K, Patti AM, Rizvi AA, *et al*. Novel molecular markers of cardiovascular disease risk in type 2 diabetes mellitus. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2021;1867(8):166148.
18. Yashim N, Obazee DY, Ogbe OP, Ajobiewe HF, Ajobiewe JO, Dah TY, *et al*. 7, Ogunkoya OF. Evaluation of Biochemical Parameters among Diabetes Patients and Healthy Participants in a Nigerian Tertiary Hospital. Sch J App Med Sci. 2022;10:1785-1789.
19. Shibabaw T, Dessie G, Molla MD, Zerihun MF, Ayelign B. Assessment of liver marker enzymes and its association with type 2 diabetes mellitus in Northwest Ethiopia. BMC research notes. 2019;12:1-5.
20. Kashinakunti SV, Rangappa M, Kallaganada GS. Correlation between liver enzymes and lipid profile in type II diabetes mellitus-A case-control study. J. Biotechnol. Biochem. 2017;3(5):01-05.
21. Al-Adwi ME, Al-Haswsa ZM, Alhmmadi KM, Eissa YA, Hamdan A, Bawadi H, *et al*. Effects of different diets on glycemic control among patients with type 2 diabetes: A literature review. Nutrition and health. 2023;29(2):215-221.
22. Yang M, Ni C, Chang B, Jiang Z, Zhu Y, Tang Y, *et al*. Association between serum total bilirubin levels and the risk of type 2 diabetes mellitus. Diabetes research and clinical practice. 2019;152:23-28.
23. Abbasi A, Deetman PE, Corpeleijn E, Gansevoort RT, Gans RO, Hillege HL, *et al*. Bilirubin as a potential causal factor in type 2 diabetes risk: A Mendelian randomization study. Diabetes. 2015;64(4):1459-1469.

24. Jung CH, Lee MJ, Kang YM, Hwang JY, Jang JE, Leem J, *et al*. Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: A 4 year retrospective longitudinal study. Metabolism. 2014;63(1):87-93.
25. Bigagli E, Lodovici M. Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. Oxidative medicine and cellular longevity; c2019.
26. Dinarvand N, Cheraghian B, Rahimi Z, Salehipour Bavarsad S, Bavarsad A, Mohammadtaghvaei N. Examining dyslipidaemia, metabolic syndrome and liver enzyme levels in patients with prediabetes and type 2 diabetes in population from Hoveyzeh cohort study: A case–control study in Iran. Endocrinology, Diabetes & Metabolism, 2023, e401.
27. Forlani G, Di Bonito P, Mannucci E, Capaldo B, Genovese S, Orrasch M, *et al*. Prevalence of elevated liver enzymes in Type 2 diabetes mellitus and its association with the metabolic syndrome. Journal of endocrinological investigation. 2008;31:146-152.
28. Muñoz-Garach A, García-Fontana B, Muñoz-Torres M. Vitamin D status, calcium intake and risk of developing type 2 diabetes: An unresolved issue. Nutrients. 2019;11(3):642.
29. Maddaloni E, Cavallari I, Napoli N, Conte C. Vitamin D and diabetes mellitus. Vitamin D in Clinical Medicine. 2018;50:161-176.
30. Kim KN, Oh SY, Hong YC. Associations of serum calcium levels and dietary calcium intake with incident type 2 diabetes over 10 years: The Korean Genome and Epidemiology Study (KoGES). Diabetology & metabolic syndrome. 2018;10:1-7.
31. Sujana C, Salomaa V, Kee F, Seissler J, Jousilahti P, Neville C, *et al*. Associations of the vasoactive peptides CT-proET-1 and MR-proADM with incident type 2 diabetes: results from the BiomarCaRE Consortium. Cardiovascular Diabetology. 2022;21(1):1-10.
32. Suthahar N, Meijers WC, Brouwers FP, Heerspink HJ, Gansevoort RT, van der Harst P, *et al*. Heart failure and inflammation-related biomarkers as predictors of new-onset diabetes in the general population. International journal of cardiology. 2018;250:188-194.
33. Chen Z, Zhu Y, Zhang L. Study of three novel biomarkers, MR-proADM, midkine, and stromelysin2, and peripheral atherosclerosis in a Chinese Han population: A case-control study. European Journal of Inflammation. 2020;18:2058739220960558.
34. Sujana C, Seissler J, Jordan J, Rathmann W, Koenig W, Roden M, *et al*. Associations of cardiac stress biomarkers with incident type 2 diabetes and changes in glucose metabolism: KORA F4/FF4 study. Cardiovascular diabetology. 2020;19(1):1-12.
35. Jasim SN, Ahmed AM, Saleh SS. Estimation of trace elements (Selenium, iron) and their biological effect in serum levels of breast cancer patients. Med J Babylon. 2020;17:89-92.
36. Khalaf Al-Hadidi EE, Al-Obaidi WML. Assessment of asprosin level and some of physiological variables in patients with cardiovascular diseases in Kirkuk city, Iraq. Biomedicine. 2022;42(5):973-977.
37. Jasim SN, Ahmed AM, Saleh SS. Estimation of trace elements (selenium, iron) and their biological effect in serum levels of breast cancer patients. Med J Babylon. 2020;17:89-92.
38. Saleh SS, AL-Salihi SS, Mohammed IA. Biological activity Study for some heterocyclic compounds and their impact on the gram positive and negative bacteria. Energy Procedia. 2019;157:296-306.