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*Research Article*

# Assessment of Serum Metalloendopeptidase level in Patients with Double Diabetes

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**ABSTRACT**

***Background:*** Double diabetes is the term used to describe situations in which a patient exhibits characteristics that are a combination of type 1 and type 2 Diabetes Mellitus. Metalloendopeptidase or Neprilysin is membrane-bound metallopeptidase. It has a wide range of physiological function and a variety of substrates. It has a significant impact on the proteolytic functions of the kidney, cardiovascular health, immunological response, cell proliferation, and fetal development. It also has a preventative effect on the onset of type 2 diabetes, obesity, and cancer.

***Objective:*** The study aims to assess the level of MEP in patients with double diabetes and determine its predictive value in the diagnosis of double diabetes.

***Subjects and Methods:*** Eighty participants were divided into two groups for this study. 40 patients with double diabetes made up the first group (G1), whereas 40 age- and gender-matched apparently healthy subjects made up the second group (G2), which served as the control group. ELISA was used to measure the serum's metalloendopeptidase level. For the measurement of HbA1c, whole blood was used. The measurement of insulin, blood glucose, and lipid profile were performed using serum. The HOMA test measured insulin resistance.

***Results:*** This study revealed a significant elevation in serum metalloendopeptidase levels in patients with DD ($p$ value $< 0.05$). The ROC curves analysis for serum metalloendopeptidase level showed the area under the curve (AUC) of serum metalloendopeptidase (pg/mL) was 0.992.

***Conclusions:*** Serum metalloendopeptidase level could be used as a novel biomarker in patients with double diabetes.

## Introduction

Double diabetes (DD) is a distinct subgroup of type 1 diabetes (T1DM) that has been identified as being associated with a worse metabolic phenotype and an elevated risk of macro- and microvascular complications. This type had clinical features of insulin resistance (IR). The major goal of detecting double diabetes is to promptly adopt the best therapeutic strategies to lower the elevated risk of chronic complications and other detrimental metabolic features connected with this illness (1). Highlighting DD is crucial because the prevalence of T1DM is rising by 3-5% annually across the world (2). The three main categories of proposed diagnostic criteria include Insulin resistance, obesity/metabolic syndrome, and family history of type 2 diabetes mellitus (T2DM). Perhaps the most accurate indicator

of double diabetes is the predicted glucose disposal rate. Yet, clinical professionals believe that 4% of all T1D patients may also develop T2DM (3). Worldwide, there has been an upsurge in obesity over the past 20 years. 2.8% and 37.1%, of patients with T1D were overweight which was linked to poor diabetes management, unstable blood glucose levels, and higher insulin dosage (4). Similar rates were observed by Merger et al. for T1D patients, who also had higher rates of microvascular and macrovascular-associated comorbidities, such as coronary heart disease and stroke, both of which were unrelated to glucose management. 7% of 200 adolescent diabetics in a different research were identified as having DD(5).

A membrane-bound, widely expressed endopeptidase called Metalloendopeptidase (MEP) or Neprilysin (NEP) works by cleaving regulatory peptides that are located on the N-terminal side of hydrophobic residues. It has an impact on the immune, cardiovascular, and neurological systems. MEP has been shown to target a variety of small peptides including amyloidβ, insulin B-chain, and several neuropeptides (6-8) Disrupted glucose homeostasis may be caused by NEP. Previous research showed that in high-fat-fed obese mice, higher plasma NEP levels are positively correlated with insulin resistance and decreased beta-cell function. Moreover, there are links between NEP deficit and/or inhibition and improved glucose tolerance, protection against weakened-cell function, and higher insulin sensitivity. As a possible target for therapeutic strategies in the control of diabetes, MEP has gained in popularity (9,10). The study aims to assess the level of MEP in patients with double diabetes and determine its predictive value in the diagnosis of double diabetes.

## Subjects and Methods

The study design is a case-control study. It was carried out at the specialized center for Endocrinology and Diabetes in Baghdad and the specialized center for Endocrinology and Diabetes in Al Najaf Al Ashraf during the period between December 2021 and December 2022.

The present study was conducted according to the guidelines of the Declaration of Helsinki of 1975, revised in 2013, and approved by the scientific and ethical committee in College of Education for Pure Science (Ibn Al-Haitham)- the University of Baghdad. informed consent was obtained from each participant.

Eighty individuals with age ranged between (18-40) years were enrolled in this study and they were divided into two groups:

- The first group(G1) consisted of (40) patients with double diabetes, (20) of them were males, and (20) were females. The inclusion criteria were patients diagnosed with type 1 diabetes on insulin therapy since diagnosis, followed by the endocrinology and diabetes centers by at least 12 months then the patients develop features of insulin resistance and/or metabolic syndrome.
- The second group (G2) represented an age and gender-matched apparently healthy control group consisting of (40) subjects, (20) of them were males and 20 were females.

Body mass index (BMI) has been calculated according to a specific formula which includes weight divided by the square of height.

Ten milliliters of venous blood were drawn from the participants and the samples and placed in a plain tube and left for (15 min) at room temperature. Samples were centrifuged at 4000rpm for 10 min. A serum that was obtained was stored at (-20oC) unless used immediately. Whole blood was used in the determination of HbA1c. Serum was used in the determination of Fasting plasma glucose (FPG), total cholesterol, HDL-cholesterol, and triglycerides by spectrophotometer. LDL-cholesterol was calculated by Friedwald's equation (11).

Enzyme-linked immunosorbent assay (ELISA) Kits are used to evaluate MEP levels (Metalloendopeptidase ELISA kit, USA). Insulin was also measured by ELISA.

Insulin resistance was calculated by the following equation (12): HOMA-IR: fasting Glucose(mg/dl) x fasting Insulin(μU/mL) / 405.

## Statistical Analysis:

Data analysis was done using simple frequency, percentage, mean, and standard deviation using the easily accessible statistical application SPSS-23. The students' t-test was used to determine whether the difference between the two independent means (in quantitative data) was significant. The significance of the difference between different percentages (qualitative data) was assessed using the Pearson Chi-square test (t-test). Statistical significance was considered whenever the P value was equal to or less than 0.05. The Receiver Operating Characteristic "ROC" curve approach was used to create the "cut-off value" of the best sensitivity and specificity for diagnosing disease and to assess the usage of any parameter as a diagnostic or screening tool for disease.

## Results

Table 1 demonstrates the body mass index (Kg/m2) for all patients and control subjects. It can be noticed that the mean values of BMI for the patients with double diabetes (G1) were (28.16±2.608 Kg/m2).

**Table1**: distribution of the body mass index of the participants

| | | G1 | | G2 | | P value |
|---|---|---|---|---|---|---|
| | | No. | % | No. | % | |
| BMI (Kg/m2) | Normal (18.5-24.9) | 5 | 12.5 | 27 | 67.5 | 0.0001* |
| | Overweight (25-29.9) | 28 | 70 | 13 | 32.5 | |
| | Obese I (30-34.9) | 7 | 17.5 | - | - | |
| | Obese II (=>35) | - | - | - | - | |
| | Mean ± SE of BMI (Kg/m2) | 28.16±0.299 (23.34 - 33.73) | | 24.432±0.413 (19.13-29.38) | | |

*Significant difference between two independent means T-test at 0.05 level.

G1: Double diabetes patients. G2: Controls

Data in table 2 showed a significant elevation in the levels of insulin, FBG and HbA1c in G1.

**Table2**: Comparison of biochemical Parameters Levels in G1 and G2

| Parameter | Mean ± SE of G1 | Mean ± SE of G2 | P value |
|---|---|---|---|
| HbA1C (4.1-5.6%) | 8.48 ± 0.204 | 5.342 ± 0.081 | 0.0001* |
| Fasting blood sugar(mmol/L) | 196.475±6.149 | 93.7±1.449 | 0.0001* |
| Insulin (µIU/mL) | 15.855±0.724 | 5.117±0.482 | 0.0001* |
| HOMA-IR | 7.747±0.452 | 1.189±0.115 | 0.0001* |
| Serum cholesterol (mg/dL) | 217.9±4.435 | 147.5±4.601 | 0.0001* |
| Serum triglycerides (mg/dL) | 184.025±3.683 | 98.07±3.136 | 0.0001* |
| S.HDL level (mg/dL) | 43.325±0.449 | 48.15±1.043 | 0.043* |
| S.LDL level (mg/dL) | 136.595±4.358 | 79.735±4.523 | 0.0001* |
| S.VLDL level (mg/dL) | 37.48±0.900 | 19.615±0.627 | 0.0001* |

*Significant difference between two independent means T-test at 0.05 level.

G1: Patients with Double diabetes G2: Controls.

Additionally, it display levels of serum lipids (total cholesterol, TG, HDL, LDL and VLDL) in all studied groups. Results revealed a significant elevation in levels of (Cholesterol, TG, LDL and VLDL) in G1 compared to G2(p value < 0.05). While a significant decrease was found in HDL

Table 3 demonstrates the measurements of serum MEP for the participants. It can be noticed a significant elevation (p value < 0.05) of the mean values of serum MEP level in double diabetes patients (G1).

**Table3**: Comparison of serum metalloendopeptidase levels between G1 and G2

| Parameter | Mean ± SE of G1 | Mean ± SE of G2 | P value |
|---|---|---|---|
| Serum metalloendopeptidase (pg/mL) | 163.6±27.11 | **123.44±21.24** | **0.0001*** |

*Significant difference between two independent means T-test at 0.05 level

G1: Double diabetes patients. G2: Controls.

The correlation of serum MEP to the studied parameters is summarized in table 4, the most important finding is the significant correlation between serum MEP and HOMA-IR in G1.

Analysis of ROC curves for serum MEP level, when used as a subject test showed that the area under curve (AUC) was (0.992) for MEP level (pg/mL)as shown in Table 5 and Figure1.

## Discussion

Double Diabetes describes a condition in which TIDM is superimposed with obesity and or insulin resistance (13). Overweight/obesity is an extra burden on the health of people with T1DM. In this study, 70 % of patients with double diabetes were overweight and 17.5% were obese. A Previous study showed the prevalence of overweight was (53.3 %) and obesity (25.2 %) (1,14). It is obvious that one key factor contributing to insulin resistance and putting a subject with type 1 diabetes into the DD category is excessive weight gain. The accelerator hypothesis (15) proposes that individuals with type 1 diabetes are more likely to develop insulin resistance if they put on a lot of weight. According to the accelerator hypothesis, excessive weight growth and the emergence of insulin resistance are to blame for the rise in double diabetes incidence . The B-cells are put under stress by insulin resistance, which forces them to produce more insulin. Interventions to prevent weight gain and/or to encourage weight loss may help lower the risk of diabetic complications in this population. Given the increasingly expanding issues related to obesity, efforts to prevent the progress of T1DM to DD may be especially crucial. According to the Diabetes Prevention Program lifestyle modifications are important in preventing or blocking disease progression in individuals at risk. This is likely because they enhance insulin sensitivity (16-19).

**Table4:** Correlation between serum metalloendopeptidase and the studied parameters in G1 and G2

| Parameter | | G1 | G2 |
|---|---|---|---|
| BMI (Kg/m2) | r | 0.161 | 0.063 |
| | P | 0.322 | 0.701 |
| Fasting blood glucose (mg/dL) | r | 0.203 | -0.049 |
| | P | 0.208 | 0.672 |
| HbA1C (%) | r | 0.283 | 0.075 |
| | P | 0.076 | 0.646 |
| Insulin (µU/mL) | r | 0.296 | 0.320* |
| | P | 0.064 | 0.044 |
| HOMA-IR ( µU/mL) | r | 0.398* | -0.305 |
| | P | 0.011 | 0.055 |
| Serum cholesterol (mg/dL) | r | 0.016 | 0.422** |
| | P | 0.923 | 0.007 |
| Serum triglycerides (mg/dL) | r | -0.152 | 0.007 |
| | P | 0.348 | 0.965 |
| Serum HDL (mg/dL) | r | -0.194 | -0.221 |
| | P | 0.231 | 0.172 |
| Serum LDL (mg/dL) | r | 0.068 | -0.380* |
| | P | 0.675 | 0.016 |
| Serum VLDL (mg/dL) | r | -0.156 | 0.007 |
| | P | 0.337 | 0.965 |

*Significant correlation at 0.05 level, **Highly significant correlation at 0.01 level.

R: Pearson correlation p: P-value

G1: Double diabetes patients. G2: Controls.

**Table5:** Area Under the Curve for Serum metalloendopeptidase in Double Diabetes

| Test Result Variable(s) | AUC Area | Std. Error | P value | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Serum metalloendopeptidase (pg/mL) | 0.992 | 0.009 | 0.0001* | 0.974 | 1.000 |

The result of the present study showed that patients with double diabetes had considerably higher serum levels of MEP than did the control group, with an excellent area under the curve. A previous study found that diabetic patients have elevated serum MEP levels, which raises the risk of complications, and that the serum MEP level was closely related to glucose profile, lipid profile, and insulin resistance after adjusting for sex, age, and BMI. Another study revealed that elevated levels of soluble MME in the blood are linked to higher BMI and insulin resistance as estimated by HOMA-IR [20]. Moreover, pharmacological MME inhibitor therapy in rats has been linked to improved insulin sensitivity (21) .

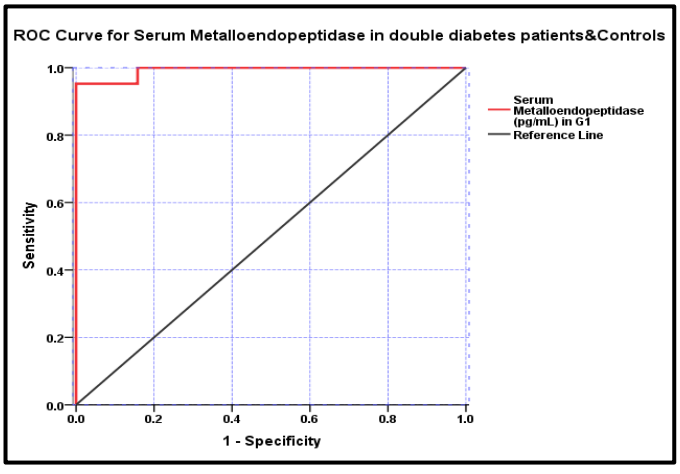


**Figure1:** Receiver Operating Curve for serum metalloendopeptidase in double diabetes

MEP is thought to have a particular cellular function in regulating insulin signaling at the insulin receptor level as well as a somewhat specific systemic effect in regulating the body's general sensitivity to insulin by degrading a variety of small peptide hormones. Understanding each of these hierarchical roles in detail will be necessary for developing effective treatments for adipose-associated illnesses mediated by MEP. Therapeutic treatments for adipose-associated illnesses mediated by MEP will depend greatly on how each of these hierarchical roles is changed (17,22).

## Conclusion

Serum metalloendopeptidase level is elevated in patients with double diabetes and can be suggested as a novel biomarker in this disease.

**Competing Interests**

Authors have declared that no competing interests exist.

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