The Elevation of Serum Subfatin Levels in Patients with Double Diabetes

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Abstract

Background: Hybrid diabetes (or double diabetes, DD) occur when the patient which exhibits characteristics that combine type 1 diabetes (T1DM) and type 2 diabetes (T2DM). Formerly epidemiological studies found that quarter of people with T1D also had the metabolic syndrome. Subfatin, Also called cometin, it is a small (~27kDa) cytokine secreted by protein encoded by a gene called METRNL (simeler of meteorin). is much expressed in skin in the mucosal tissues and activated macrophages. Subfatin has also been described as a hormone that effected in some diseases such as metabolic diseases (including dyslipidemia), type 2 diabetes and obesity.

Objectives: The current study objective is evaluating the subfatin in the blood serum of double diabetes patients to find predictive significance of diagnosis for this disease.

Subjects and methods: Eighty individuals were studied , divided them into two groups . Forty patients with double diabetes represented the first group (G1), and the second group (G2), which represented the control group, consisted of (40) individuals, and the range ages of the study were (18-60)years. Whole blood was used to determine HbA1c. Samples were centrifuged, and the obtained serum was used to evaluate other biochemical markers. The technique used to determine the level of subfatin in the blood was a quantitative sandwich enzyme-linked immunosorbent assay (ELISA).

Results: A significant increase shown by this study in the serum levels of subfatin in (DD) patients (n = 40) compared with control subjects (n = 40) (p value < 0.05). The ROC curves analysis for serum subfatin level when used as test for diagnosis subjects into of double diabetes cases (G1) and control group (G2), showed the AUC (area under curve) for serum of subfatin was (1.000) have interval of confidence (95%) and both lower and upper bound was (1.000).

Conclusions: serum subfatin level could be a used as a novel biomarker of double diabetes (DD) and may contribute to the early diagnosis of diabetes.

Keywords: Double diabetes, Subfatin.

INTRODUCTION

The ’double diabetes’ (DD) term, using when the patient demonstrates a mixture of T1DM and T2DM characteristics [1]. A previous studies have found that a quarter of patients with T1DM also presented with metabolic syndrome [1] in addition to the effect of hereditary factors, changes in environmental factors can be linked to the incidence increase of T1DM recorded during the past ten years, especially in children under the age of five[2]. It is very likely that the increasingly sedentary lifestyle and weight gain, both of which occur in industrialized countries, are the cause of the significant rise in the T2DM incidence in adolescents and children . Recently, there has been many number of adolescents and children with a diabetes as combination of the two forms (i.e., individuals who are obese and/or display symptoms of insulin resistance as well as positively markers of an autoimmune response to cells), Despite the lack of epidemiological evidence for such a hypothesis. According to the current classification, it is challenging to pinpoint the specific type of diabetes that these young patients have because they could be labeled as either T1DM or T2DM depending on their level of obesity and insulin resistance[3]. These individuals have overlapping T1DM and T2DM diabetes phenotypes, indicating that the present categorization of diabetes needs to be updated to include novel kind of this diabetes, often called "double diabetes" or "hybrid diabetes" [4]

Subfatin is a small (about 27 kDa) cytokine, encoded protein by a specific gene called METRNL (similer of meteorin). It is highly expressed in the skin ,mucosal tissues and the activated macrophages , subfatin or metrnl also has been described as a hormone ,In the skin-related diseases showed highly expression of METRNL in psoriasis, nodular pruritus, atopic dermatitis and actinic keratosis. Also METRNL is up regulated in the synovial membranes of human rheumatoid arthritis [5]. obesity-induced insulin resistance counteracts by metrnl which is improving adipose function, that lead inhibition of inflammation ,activation of metabolism, and adipocyte differentiation. metrnl serum levels of may be a risk factor for coronary artery disease and type 2 diabetes. Low level of subfatin with type 2 diabetes patients is plays important role in pathogenesis (the disease complications developments) which lead to increasing the insulin resistant. subfatin elevated levels have important role to prevent releasing inflammatory mediators (are chemical substances secreted by inflammatory cells and responsible of all changes occur with inflammation), subfatin elevated levels enhanced intercellular insulin signal , glucose tolerance[6].

Subjects and Methods:

In this study collection and analysis of the samples carried out in Baghdad Specialized Center for Endocrinology and Diabetes and in Najaf Specialized Center for Endocrinology and Diabetes during the period between December 2021 and March 2022, where the disease was diagnosed by specialized doctors. Eighty persons joined study whose ages ranged between (18-60) years, were divided them for two groups:

- (G1) first group that consisted of (40) patients with double diabetes, including (20) males and (20) females.
- (G2) second group, which represented the control group, consisted of (40) subjects, including (20) males and (20) females

Body mass index (BMI) has been calculated according to a specific formula which includes weight divided by the square of height [7]. Preparation of Sample is an essential task before conducting any analytical study [8].Ten milliliters of venous blood was drawn from the study cases and control samples and placed in a plain tube and left for (15 min) at room temperature. Samples were centrifuged at 4000rpm for 10 min. Serum that obtained was stored at (-20oC) unless used immediately. Whole blood was used in the determination of HbA1c. Insulin levels in patients and healthy subjects were determined by the Immunoassay for the quantitative method used in determination of human insulin[9],[10]. The electro chemiluminescence immune assay (ECLIA) is intended for use on Elecsys and cobas immunoassay analyzers and Homeostatic Model Assessment-Insulin Resistance(HOMA-IR)was calculated according as follows equation:

HOMA-IR=(Glucose× Insulin)/405

This equation is used when glucose concentration is in mg/dL [11].

Serum Subfatin Levels measurement: Enzyme linked immunosorbent assay (ELISA) Kits are used to evaluate Subfatin levels (Subfatin ELISA kit , USA) .

Statistical analysis : Using the accessible statistical tool SPSS-23, data were analyzed (Statistical Packages for the Social Sciences-Version 23). Frequency, percentage, mean, standard deviation, and simple range calculations were used to present the data . The difference between two independent means (in the quantitative data) was examined using a Student's t-test. Using the Pearson Chi-square test, the significance of the difference between the different percentages (qualitative data) was assessed (t-test). Each time the P value was equal to or less than 0.05, statistical significance was taken into account [12].

Receiver operating characteristics to specify the use of any parameter as a diagnostic or screening tool and to be able to determine the "cut-off value" for which the best sensitivity and specificity "ROC" curve technique has been applied. Data were analyzed using the statistical program available from SPSS-23 (Social Sciences Statistical Packages - Version 23). Data were represented in simple measures of frequency, mean, standard deviation, range and percentage (minimum & maximum values) [13].

Results:

Table 1 demonstrates body mass index measurements (in Kg/m2) for all patients and control subjects. It can be noticed that the mean values of BMI for double diabetes patients (G1) were (28.16±2.608 Kg/m2, while the same measurements for controls (G2) were (24.432±1.895Kg/m2) .

Table 1: Comparison of the body mass index between G1 and G2

		G1		G2		P value
		No.	%	No.	%	
BMI (Kg/m2)	Normal (18.5-24.9)	5	12.5	27	67.5	0.000 1*
	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)		

Mean ± SE of Weight (Kg)	**77.85±1.209 (57.5-92.5)**	**68.938±1.303 (54-92)**
Mean ± SE of Height (cm)	**166.35±0.961 (153-179)**	**167.85±1.277 (158-181)**

* Highly significant difference between two means independent (T-test) at a confidence interval of 0.05.

G1: Patients with double diabetes. G2: Controls.

The data presented in Table 2 showed a significant elevation in patients in G1 compared to the control group in HbA1c and FBG levels, and assessment of HbA1c used to monitor effective glycemic control as the cornerstone of diabetes care [14].

Table 2: Comparison of Glycemic Parameters Levels in G1 and G2.

parameter	Mean ± SE of G1	Mean ± SE of G2	P value
$HbA1_C$ (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***

* Highly significant difference between two means independent (T-test) at a confidence interval of 0.05.

G1: Patients with double diabetes. G2: Controls

Insulin levels and HOMA-IR (homeostasis model assessment of insulin resistance) for both groups, results showed highly increasing in insulin and HOMA-IR levels in G2 compared to G1,in other hand Table 3 presents levels of blood lipids (cholesterol, TG, HDL, LDL and VLDL) in both studied groups. The results showed a significant increase in cholesterol, triglyceride, LDL and VLDL levels in G1 compared to G2 (p value < 0.05). While decrease in HDL levels was observed when compared between the two groups (p value < 0.05).

Diabetics with a lipid abnormality, known as "diabetic dyslipidemia", often have high levels of total cholesterol [T-Chol], triglycerides [Tg], very low density lipoprotein cholesterol[VLDL] and low density lipoprotein cholesterol [LDL] [15].

Table 3: Comparison of Lipid Profile between G1 and G2.

parameter	Mean ± SE of G1	Mean ± SE of G2	P value
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***
HDL (mg/dL)	**43.325±0.449**	**48.15±1.043**	**0.043***
LDL (mg/dL)	**136.595±4.358**	**79.735±4.523**	**0.0001***
VLDL (mg/dL)	**37.48±0.900**	**19.615±0.627**	**0.0001***

* Highly significant difference between two means independent (T-test) at a confidence interval of 0.05.

G1: Patients with double diabetes. G2: Controls.

The table 4 demonstrates the measurements of serum subfatin (pg/mL) for all patients and control subjects. It can be noticed a significant elevation (p value < 0.05) of mean values of serum subfatin level for double diabetes patients (G1) were (727.129±23.525 pg/mL) , while the measurements for controls (G2) were (479.177±8.329 pg/mL) .

Table 4: Comparison of serum subfatin levels between G1 and G2.

parameter	Mean ± SE of G1	Mean ± SE of G2	P value
Serum subfatin (pg/mL)	**727.129±23.525**	**479.177±8.329**	**0.0001***

* Highly significant difference between two means independent (T-test) at a confidence interval of 0.05.

G1: Patients with double diabetes. G2: Controls.

Correlation of serum subfatin with clinical and biochemical parameters of the groups under study is summarized in Table 5, serum subfatin levels were negatively correlated with FBG, insulin, HOMA-IR and HDL in G1, additional correlated negatively with BMI, HbA1C, insulin, HOMA-IR , cholesterol, triglycerides and VLDL in G2. On the other hand, correlated positively with others in the two groups

Table 5: Correlation between serum subfatin and the clinical and biochemical parameters in G1 and G2.

parameter		G1	G2
BMI (Kg/m2)	**r**	**0.200**	**-0.102**
	P	**0.216**	**0.531**
Fasting blood sugar (mmol/l)	**r**	**-0.133**	**0.288**
	P	**0.414**	**0.071**
$HbA1_C$ (4.1-5.6%)	**r**	**0.111**	**-0.366**
	P	**0.497**	**0.020**
Insulin (µU/mL)	**r**	**-0.055**	**-0.097**
	P	**0.735**	**0.551**
HOMA-IR (µU/mL)	**r**	**-0.188**	**-0.073**
	P	**0.467**	**0.654**
Serum cholesterol (mg/dL)	**r**	**0.229**	**0.250**
	P	**0.156**	**0.120**
Serum triglycerides (mg/dL)	**r**	**0.070**	**-0.177**
	P	**0.669**	**0.275**
HDL (mg/dL)	**r**	**-0.114**	**0.190**
	P	**0.484**	**0.241**
LDL (mg/dL)	**r**	**0.227**	**0.235**
	P	**0.160**	**0.145**
VLDL (mg/dL)	**r**	**0.087**	**-0.177**
	P	**0.595**	**0.275**

r: Pearson correlation **p: P-value**

G1: Patients with double diabetes. G2: Controls.

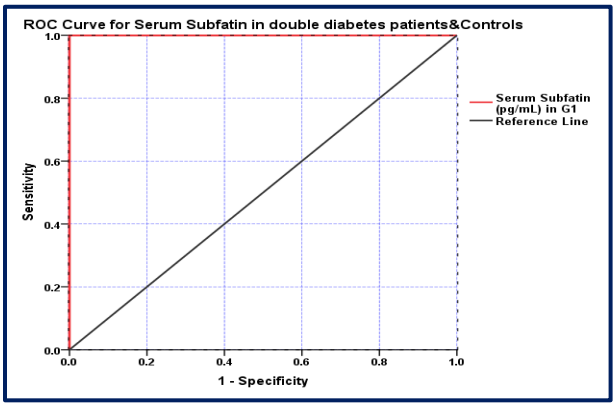
Analysis of ROC curves for serum subfatin level, when used as a subject test in double diabetic patients (G1) and control subjects (G2), showed area under curve (AUC) was (1) for subfatin level (in units pg/mL) during the interval of confidence (95%), lower and

upper limit bound was (1). As shown in Table 6 and Figure1

Table 6: Area Under the Curve for Serum subfatin in Double Diabetes patients and controls

Test Result Variable(s)	AUC Area	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Serum subfatin (pg/mL)	1.000	0.0001	0.0001*	1.000	1.000

Figure 1: Sensitivity and Specificity of Double Diabetes patients and Controls for subfatin.



Discussion:

Both BMI and waist circumference are reasonable predictors of prevalent diabetes [16], in this study the results noted a significant difference in the mean BMI between the patient group and the control group ($P < 0.05$) in disagreement with the results of a previous study that showed that the difference in The average weight, height, and BMI was not statistically significant ($p > 0.05$) between groups [17]. Overweight and body mass rising are central to the formation and increased incidence of type 1 and type 2 diabetes [18].

Attempts to halt the onset of a disorder such as DD may be particularly important given the rapidly growing problems associated with obesity. Given that consistent cell function is still present in DD at the diabetes diagnosis and that it might wane more slowly than in conventional T1D, an intervention that could prevent some putative disease-causing processes may be useful. It is said that subjects with long-term T2DM gradually resemble subjects with type 1 diabetes as their cell function declines[19], and thus an immunomodulation trial similar to that screened for T1D may be considered. While prevention of T1D remains elusive, prevention of T2D has been proved to be doable. According to the Diabetes Prevention Program [4], lifestyle modifications are important in preventing or blocking disease progression in individuals at risk for T2D. This is likely because they enhance insulin sensitivity [20]. Uncontrolled DM can result in high TC, LDL -C, high TG ,high VLDL-C, and low HDL-C values. Significant alterations in lipid and lipoprotein metabolism have been observed as complications of insulin resistance [21], early detection and assurance of optimal levels of HDL is done through healthy culture to improve diet control and avoid excessive weight gain and testing for non-alcoholic fatty liver disease (NAFLD) among patients with diabetes, Especially those with an abnormal BMI and HDL [22].In previous study founded Serum levels of Metrnl were also highest in patients with T2DM , and the serum levels of Metrnl level were higher in the prediabetes groups than in the NGT(normal glucose tolerance) subjects, Serum levels of

Metrnl level between subjects with normal weight were significantly different when compared with levels in overweight and obese subjects[23], In other side, some studies found a decreases in subfatin levels when it was studied T2D patients who suffering from highly obesity, and this may be because of delayed disease and the failure of white adipose tissue to produce the hormone, which may occur after the stage of high levels [24],[25].Results revealed a significantly elevation for levels of subfatin in G1(mean value =727.129) compared with G2(mean value =479.177) (p-value < 0.05), serum levels of subfatin were higher in patients with double diabetes compared with individuals with normal glucose tolerance (NGT). Elevated serum subfatin levels have been observed in patients with T2DM which increases the risk of complications. After ranking by sex, age, and body mass index (BMI), serum subfatin level was closely associated with glucose profile, lipid profile and insulin resistance. Multiple logistics services [26].

Conclusion:

It is difficult to diagnose double diabetes (DD) with characteristics of both T1DM and T2DM and therefore to consider the best treatment approach for these patients, the results of this study showed that the association between clinical and biochemical parameters of double diabetes (DD) allows serum subfatin to be a novel biomarker for early diagnosis of this type of diabetes .

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