Evaluation of the Serum Asprosin Levels in Patients with Double Diabetes

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Abstract :

Background : Double diabetes (DD) is the term used to describe situations in which a patient exhibits characteristics that are a combination of type 1 diabetes mellitus(T1DM) and type 2 Diabetes Mellitus (T2DM) a large epidemiological study found that 25.5% of people with T1D also had the metabolic syndrome. A new protein hormone called asprosin is predominantly released by white adipose tissue. It was initially discovered in 2016 . Asprosin is important diagnoses marker for insulin resistant in diabetes patients ,additionally is very important denotation about early diagnoses of type 2 diabetes. **Objectives:** The current study aims to find predictive significance of diagnosis a double diabetes by evaluating the asprosin in the blood serum of groups under study .

Subjects and methods : Eighty individuals participated in this study and were classified into two groups. The first group(G1) consisted of (40) patients of double diabetes ,the second group (G2) which represented the control group consisted of (40) subjects ,the age range of under study groups were(18-60)years . Whole blood was used in the determination of HbA1c . Samples were centrifuged , Serum that obtained was used to Assessment the other Biochemical markers. The technique employed in the determination of serum asprosin level was the quantitative sandwich enzyme linked immune sorbent assay(ELISA).

Results: This study revealed a significant elevation in serum asprosin levels in (DD) patients(n =40) comparing to control subjects (n = 40) (p value < 0.05) . The ROC curves analysis for serum asprosin level when used as test for diagnosis subjects into of double diabetes cases (G1) When compared with control groups (G2) the area under the curve (AUC) for serum aspirin was 0.940 with a confidence interval (95% CI) and the lower band limit of the sensitivity versus specificity curve (0.867) and the upper band limit (1.000).

Conclusions: Asprosin 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 , Asprosin

1. Introduction:

The term 'double diabetes' (DD) refers to the cases where the patient demonstrates characteristics of a mixture of T1DM and T2DM [1]. Merger et al. found that a total of 25.5% of patients suffering from T1DM additionally presented with metabolic syndrome [1] . In addition to the effect of hereditary factors, changes in environmental factors can be linked to the increase in the incidence of T1DM recorded over the past ten years, especially in children under the age of five[2]. It is highly likely that an increasingly sedentary lifestyle and an increase in obesity, both of which have been happening in

lethargy, a lack of appetite, and lipodystrophy are the hallmarks of the rare genetic disorder NPS [7],[8], which has been linked to insulin resistance (Bindlish et al., 2015). Despite maintaining euglycemia, asprosin has been shown to raise mice's insulin and glucose levels [9]. Asprosin is a critical diagnostic biomarker for insulin resistance in diabetic patients [10], as well as a critical indicator of type 2 diabetes early diagnosis [11], and current research suggests a possible link between asprosin hormone levels in the blood and coronary artery disease [12].

2. Subjects and methods: This study was carried out at Specialized Center for Endocrinology and Diabetes in Baghdad and Specialized Center for Endocrinology and Diabetes in Al Najaf Al Ashraf during the period between December 2021 and March 2022. Eighty individuals with age ranged between (18-60) 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 second group (G2) which represented the control group consisted 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 **[13]**.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 (-20^{o}C) unless used immediately. Whole blood was used in the determination of HbA1c.

industrialized countries, are to blame for the significant rise in the incidence of T2DM in children and adolescents. Recently, there has been an increase in the number of kids and adolescents with a hodgepodge of the two forms of diabetes (i.e., individuals who are obese and/or show symptoms of insulin resistance and also have positive to markers of 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 this novel kind of diabetes, often known as "double diabetes" or "hybrid diabetes" [4].

Asprosin Hormone :

Asprosin is a novel protein hormone mostly secreted by white adipose tissue. The Fibrillin1 gene (Fbn1) undergoes a 3' truncating mutation on the last 50 nucleotides of exons 65 and 66, resulting in a shortened profribillin protein. This mutation was initially discovered in a study published in 2016 that supported this finding. Asprosin is produced when the profribillin protein is cleaved at the C-terminus, a reaction that is catalyzed by the enzyme Furin and yields a 140 amino acid product [5]. Several researchers proposed that the cleavage takes place between the trans-Golgi network and the cell surface, despite the fact that the specific location is unknown [6]. Asprosin was first discovered through research on infants with neonatal progeroid syndrome (NPS) [3]. Extreme

Operating Characteristics of the Receiver was using to determine of any parameter as a diagnostic or marker tool for disease and the capacity to establish the "cut-off value" which of the best sensitivity and specificity, "ROC" curve technique was applied . The analysis of data was carry-out by using the available statistical program of SPSS-23 (Statistical Packages for Social Sciences- version 23). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (lowest and highest values) [15].

4. Results: The table 1 demonstrates the measurements of the body mass index (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/m^2) .

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

3. Statistical analysis : Using the accessible statistical tool SPSS-23, data analysis was done (Statistical Packages for Social Sciences- version 23). Simple frequency, percentage, mean, standard deviation, and range calculations were used to present the data (lowest-highest values).

A significance of the difference between two independent means value in quantitative data was examined using the Students' t-test. Using the Pearson Chi-square test, the significance of the difference between various percentages (qualitative data) was evaluated (t-test). Every time the P value was equal to or less than 0.05, statistical significance was taken into account [14] .

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)		
(*)Mean, there is a significant difference between two independent means using a T-test at a confidence interval of 0.05.						

G1: Double diabetes patients. **G2: Controls.**

FBG, $HbA1_C$ assessment used for the monitoring effective glycemic control as a keystone of diabetes care [16] .

Data in table2 showed a significant elevation in patients in G1 comparing to control group in levels of $HbA1_C$ and

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***
*** Mean,there is a significant difference between two independent means using a T-test at a confidence interval of 0.05.**			

G1: Double diabetes patients. G2: Controls.

The levels of insulin and HOMA-IR (Homeostasis model assessment for insulin resistance) comparing to G2(p value < 0.05) . Diabetes patients with lipid abnormalities, often known as "diabetic dyslipidemia," frequently have high levels of total cholesterol (T-Chol), triglycerides (Tg), low levels of high density lipoprotein cholesterol (HDL-C), and an increase in tiny dense LDL particles[17]. The levels of low density lipoprotein cholesterol (LDL-C) can be slightly elevated or normal [18].

levels for G1 and G2 . Results shown significant increasing for insulin and HOMA-IR levels in G2 comparing to G1. Table 3 display levels of serum lipids(Cholesterol, TG,HDL,LDL and VLDL) in all studied groups . Results revealed a considerable elevation in the examination each of (Cholesterol , Triglyceride ,LDL and VLDL) in G1comparing to G2(p value < 0.05) . while an appreciable decrease was found in level of HDL in G1

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***
*** Mean, there is a significant difference between two independent means using a T-test at a confidence interval of 0.05.**			

G1: Double diabetes patients. G2: Controls.

be noticed a significant elevation (p value < 0.05) of mean values of serum asprosin level for double diabetes patients (G1)

The table 4 demonstrates the measurements of serum asprosin (ng/mL) for all patients and control subjects. It can

(137.466±42.327 ng/mL) .

were (170.767±37.33 ng/mL) , while the measurements for controls (G2) were

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

parameter	Mean ± SE of G1	Mean ± SE of G2	P value
Serum Asprosin(ng/mL)	170.767±5.903	137.466±6.693	0.003*
Mean, there is a significant difference between two independent means using a T-test at a confidence interval of 0.05.			

G1: Double diabetes patients. G2: Controls.

negative significant correlated with FBG ,cholesterol and LDL in G2 as shown in figure1 and 2**.**

The correlation of serum asprosin to the clinical and biochemical parameters of the under study groups is summarized in table 5 , serum asprosin levels were highly

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

parameter		G1	G2
BMI (Kg/m2)	r	0.064	0.145
	P	0.693	0.373
Fasting blood sugar (mmol/l)	r	-0.030	-0.370*
	P	0.855	0.019
HbA1C (4.1-5.6%)	r	0.014	0.215
	P	0.932	0.182
Insulin (µU/mL)	r	-0.168	-0.249
	P	0.301	0.121
HOMA-IR (µU/mL)	r	-0.158	-0.282
	P	0.331	0.078
Serum cholesterol (mg/dL)	r	0.204	-0.428**
	P	0.206	0.006
Serum triglycerides (mg/dL)	r	-0.140	0.133
	P	0.387	0.413
HDL (mg/dL)	r	-0.286	-0.137
	P	0.074	0.401
LDL (mg/dL)	r	0.257	-0.423**
	p	0.110	0.007
VLDL (mg/dL)	r	-0.094	0.133
	p	0.564	0.413
*There are significant correlation at 0.05 confidence interval, **There are highly significant correlation at 0.01 confidence interval. r: Pearson correlation p: P-value			

G1:Double diabetes patients. G2: Controls.

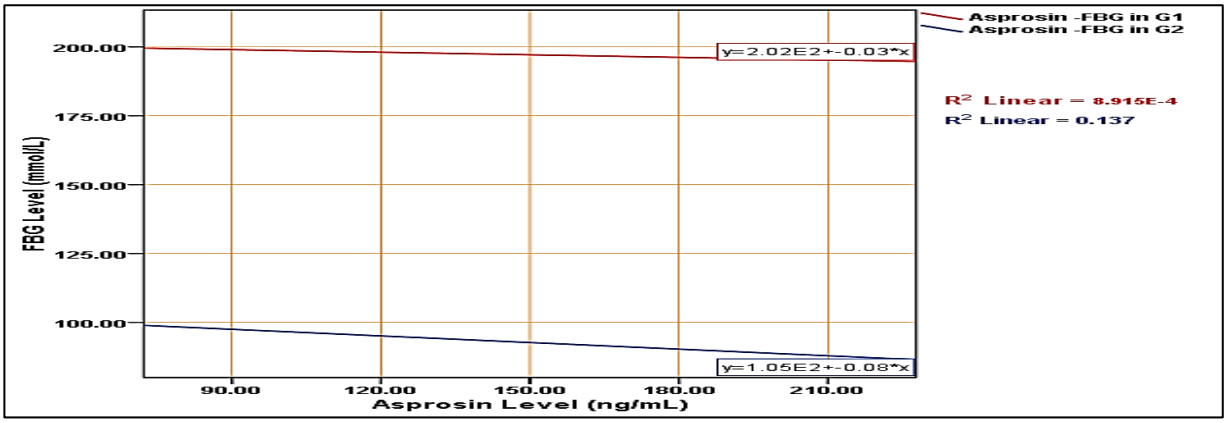

Figure 1: Correlation between serum Asprosin and FBG in G1 and G2.

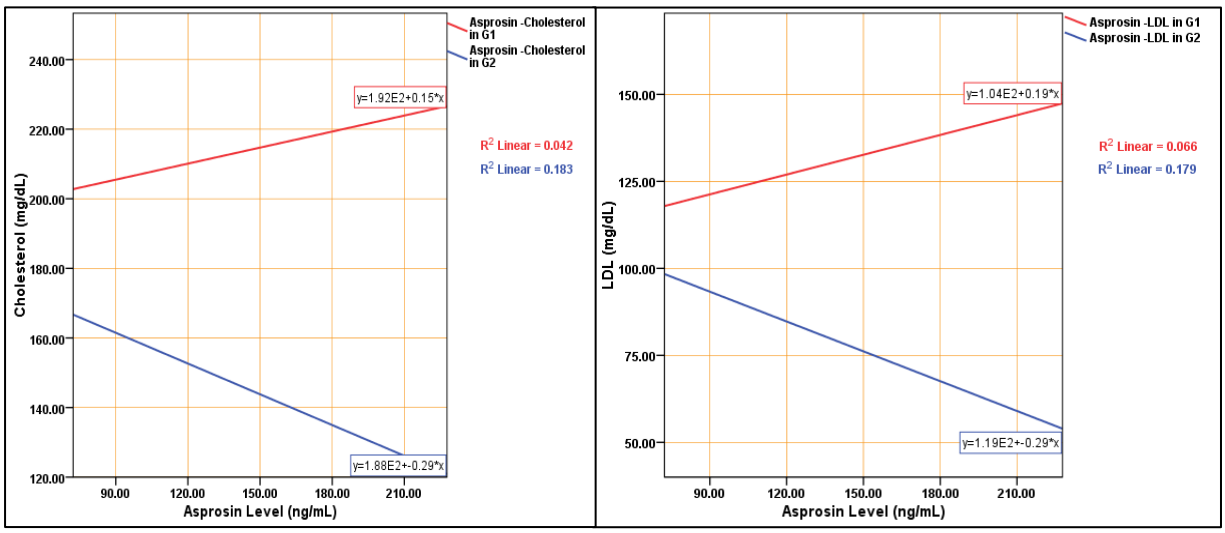

Figure 2: Correlation between serum Asprosin and Cholesterol and LDL in G1 and G2.

of serum asprosin (ng/mL) was 0.940 with confidence interval (95% CI) and lower bound (0.867)and upper bound (1.000). As shown in the Table 6 and Figure3.

The ROC curves analysis for serum asprosin level, when used as test for diagnosis subjects into of double diabetes cases (G1) and control groups (G2) indicated the area under the curve (AUC)

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

Test Result Variable(s)	AUC Area	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Serum Asprosin (ng/mL)	**0.940**	**0.037**	**0.0001***	**0.867**	**1.000**

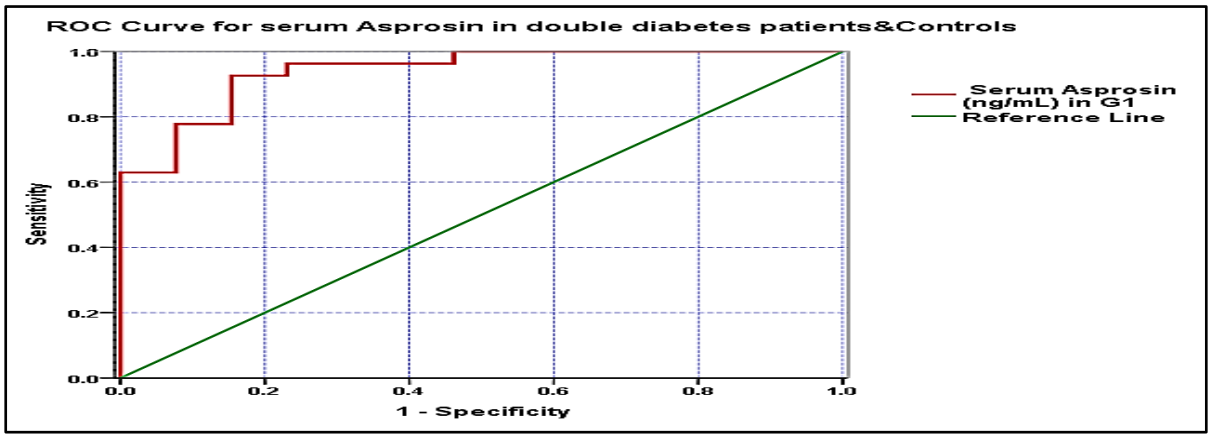

Figure 3: **Sensitivity and Specificity of Double Diabetes patients and Controls for Asprosin,.**

4. Discussion:

This study results have observed a significant difference in the mean of body mass index (BMI)between the patient groups and control group in a disagreement with the findings of a previous study that have shown that the difference in the mean of weight, height and BMI were not statistically significant ($p>0.05$) between the groups [19].

Following adult T1D patients for an average of 18 years, the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study found that the prevalence of overweight increased from 29 to 42% and that of obesity increased seven-fold from 3 to 23% [20].

HbA1c testing is utilized as a cornerstone of diabetes therapy due to the considerable increase in HbA1c and FBG levels in patient group G1 when compared to control group [20]. Attempts to stop the onset of a disorder like DD may be particularly significant given the fast growing issues linked with obesity. Given that consistent β-cell functionality is still exists in DD at the time of its diagnosis and that it perhaps diminish more slowly than in traditional T1D, an interjection that can block some of the presumed disease-causing processes may be beneficial. People with long-term T2DM are said to gradually resemble people with type 1 diabetes mellitus as their β-cell function declines[21].A trial of immunomodulation comparable to one being examined for T1D may therefore be considered for DD. While T1D prevention is still a long way off, T2D prevention has been proved to be doable. According to the Diabetes Prevention Program [4],]. lifestyle modifications are important in preventing or retarding the development of disease in individuals at dangers for T2D. This is likely because they enhance insulin sensitivity. Uncontrolled DM can result in high TC, LDL -C, high TG,high VLDL-C, and low HDL-C values. Significant alterations in lipid metabolism and lipoproteins are observed as a result of insulin resistance [22]. Health education to improve dietary control and avoid excessive weight gain and testing for non-alcoholic fatty liver disease (NAFLD) among diabetic patients, especially those with abnormal BMI and HDL,are

recommended for early detection and to ensure optimal levels of HDL [23]

Results revealed a significant elevation in asprosin levels in the patients group (G1) comparing to the control group G2 (p value < 0.05) , Asprosin is linked to other pathologies that are related to the metabolic syndrome, such as diabetic retinopathy, polycystic ovary syndrome, and anorexia nervosa, in addition to the characteristics of the metabolic syndrome, such as glucose and lipid metabolism, insulin resistance, obesity, and inflammation. A small number of pathways have already been identified, but much more study is required to fully comprehend asprosin's therapeutic potential in the metabolic syndrome [24].

5. Conclusion:

The new diabetes phenotype (DD) with features of both T1DM and T2DM is difficult to diagnose and consequently to consider the best therapeutic approach to these patients .The results of this study showed that the correlation between and the clinical and biochemical parameters of double diabetes (DD) allow serum asprosin to be a novel biomarker for the early diagnosis for this type of diabetes .

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