Association of Protein Z with Type 1 Diabetes

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Abstract

Background Diabetes mellitus type 1 (T1DM) is a chronic immune system disease characterized by the loss or injury of ß-cells in the Langerhans isle, resulting in insulin deficiency and hyperglycemia. Several parameters may change in diabetes mellitus type 1 the current study focused on determining the novel marker Z-Protein level, and investigate the relationship between Diabetes Mellitus Type 1 and other factors. **Method** One hundred twenty type 1 patients with ages ranging from 4 to 17 years were recruited from three distinct locations in Iraq (central child hospital, Alkindi center for diabetes and endocrinology, and Children's Education Hospital) from 20 December 2021 to 25 March 2022. Patients were placed into three groups: group one (67) T1DM with insulin therapy; group two (20) newly diagnosed T1DM; and group three (33) healthy patients as a control. FBG, Cholesterol, Triglyceride, HDL, LDL, VLDL, HbA1c, GOT, GPT, Total Oxidant Status, Total Antioxidant Status, Oxidative Stress Index, and Z protein were measured. **Result** Serum levels of Z protein were significantly high (p-value = 0.00). There was a significant difference in the newly compared with healthy groups in Z protein and there was a significant difference in the treatment compared with healthy groups. Serum Z protein was weak positively correlated with FBG, cholesterol, HDL, TAS, and medium positively correlated with TG, VLDL in the patient groups. According to ROC analysis, the cutoff value for Z protein was (94.8) in newly and treatment group but the Sensitivity was 100 in newly and 89 in treatment.**Conclusion** Levels of Z protein are elevated in DM patients. Z protein was significantly correlate with treatment and newly groups.

Keywords: Z protein, antioxidant, Type 1 diabetes

1. Introduction

Diabetes mellitus (DM) was one of the leading causes of mortality globally in the previous century, becoming increasingly pressing in the last few decades with the exponential development of obesity (1). Diabetes Type 1 is an autoimmune disease characterized by pancreatic beta cell damage and absolute insulin deficiency. Affected people have weakened glucose metabolism and are more likely to develop chronic hyperglycemia problems as well as severe hypoglycemia and ketoacidosis (2). In childhood or adolescence, type 1 diabetes commonly presents as T1DM (3). Hyperglycemia can enhance the generation of reactive oxygen species (ROS), and thereby cause vascular dysfunction by up-regulating chronic inflammatory biomarkers. In contrast, increased oxidative stress and inflammation can lead to insulin resistance and reduced insulin production. (4). Resulting in a lifetime need for exogenous insulin and the regulation of blood glucose levels by food and activity. With a ketoacidosis, there is a lifetime risk of both severe hypoglycemia and severe hyperglycemia., controlling blood glucose levels following a T1D diagnosis is challenging. Due to lifetime exposure to incomplete glucose control, people with T1D are at an elevated risk of micro-and macrovascular injuries, which may decrease the risk of long-term diabetes-related complications (5).

Protein Z (PZ) is a vitamin K-dependent factor (factors VII, IX, and X, protein C, and protein S) containing 396 amino acids and having a molecular mass of 50 KDa (6). Protein Z is synthesized from liver tissue and functions as a cofactor in the inactivation of factor Xa in the physiologic suppression of coagulation. (7). Researchers found a serum protein Z-dependent protease inhibitor (ZPI) that suppresses activated factor X has been found (FXa) in a mechanism that involves protein Z (PZ), calcium ions, and cephalin are all present on phospholipid surfaces (8). The human PROZ gene is situated on chromosome 13q34, close to the F8 (FVII) and TSTA3 (FX) genes, and is structured into nine exons, one of which is an alternate exon. PZ functions as a cofactor in the inhibition of FXa and FXIa (6). Disruption of the body's vascular system can occur due to the impaired metabolism of carbohydrates and lipids that result from hyperglycemia to form numerous complications of diabetes (9). According to the American Diabetes Association (ADA), that people with T1D aim for an HbA1c of 7.0 percent (53 mmol/mol) (7.5 percent in children with T1D) (5). HbA1c is the most often utilized measure for the diagnosis of diabetes, coupled with fasting plasma glucose (FPG). FPG findings may change depending on food influences and fasting length. The oral glucose tolerance test is used to diagnose IFG. The IGT test is difficult for patients to complete in a clinical environment since it is time-consuming and involves numerous blood samples (10). In this research, the authors aim to investigate the relation of serum Z protein measurement with T1DM.

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2. Materials and methods

Patients

The current research was conducted in Iraq to determine Z levels in type 1 diabetic patients, this research included 120 individuals, varying in age from four to seventeen years. 90 patients with DM1 were collected from (Central Child Hospital, Alkindi center for diabetes and endocrinology and Child Protection Teaching Hospital) between (20 December 2021 to 25 March 2022). They were classified into three groups: group 1 contained 67 people (33 girls and 34 boys) receiving insulin injection treatment; and group 2 included (20) (9 girls and 11 boys) newly diagnosed. In addition, 33 people (14 girls and 19 boys) were used as a control group.

Blood sample collection

The blood sample was taken following an overnight fast. 5 ml of blood was taken from every patient and control through venipuncture using a 10 ml syringe. 2 ml of blood was placed into a tube containing ethylene diamine tetraetic acid (EDTA), and this blood was put to use to calculate HbA1c. Serum was used to calculate FBG, GOT, GPT, Cholesterol, Triglyceride, Oxidant status, Antioxidant oxidant state, oxidative stress index, HDL, LDL, VLDL, and Z protein.

Diabetic related parameters determination

For serum glucose, Serum cholesterol, Serum triglyceride and Serum HDL all these parameters, an enzymatic colorimetric approach was utilized with a kit provided by LINEAR Chemicals, SPAIN, Barcelona. HbA1c was determined using the RANDOX, UK kit and the RX DAYTONA+ clinical chemistry analyser programmer. GPT was performed using a kit provided by GenWay Biotech in the United States. GOT was performed using the kit supplied by sigma-aldrich, USA. LDL was compute using the equation LDL-C =cholesterol – (TG/5) – HDL-C, and VLDL is also compute using an equation VLDL-C = TG / 5. TAS and TOS was determined by measuring absorbance using a spectrophotometer instrument, while OSI was calculated using equation OSI = TOS (mmol H2O2/L/TAS mmol vit.C/L).

Z protein estimation

Protein was measured using sandwich enzyme immunoassay methods and a kit provided by BioSource, USA. The samples were placed in the wells for one hour. Then they were rinsed three and a half times. The conjugated (HRP enzyme) was added to the washed well and left for half an hour. Then it was rinsed three and a half times. The TMB was added, and the reaction resulted in the formation of the blue color. A stop solution was added to the mixture to cease the reaction, causing the color to turn yellow and be measured at 450nm with a Human ReaderHS.

3. Statistical analysis

The findings were analyzed using a statistical analysis program (SPSS 25). To explain the major findings, the General descriptive statistic was utilized, and the One-Way analysis of variance test was performed to compare groups. Spearman correlation was used to perform the correlation. Cluster analysis and receiver operating characteristic curve (ROC) analysis were also utilized to determine the cutoff value for the parameters.

4. Results and Discussion

The anthropometric features of the research population are depicted in (Table 1). Age and BMI showed statistically significant variations between research groups.

Table 1: Demographic factor distribution in studied groups

		Mean± SE	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
Age	G1	9.9667±0.578	8.7887	11.1446	4.00	17.00
	G2	9.35±0.703c	7.8776	10.8224	2.50	13.00
	G3	11.53±0.354b,c	10.8302	12.2444	4.00	17.00
BMI	G1	16.51±0.530	15.4332	17.5968	10.36	22.90
	G2	16.36±0.715c	14.8719	17.8661	11.60	25.14
	G3	18.37±0.325b,c	17.7234	19.0232	11.15	24.32
The mean difference is significant at the 0.05 level have been identified using ANOVA test.						

a. significant difference between G1 (control) and G2 (DM without treatment)
b. significant difference between control G1 (control) and G3 (DM with treatment)
c. significant difference between G2 (DM without treatment) and G3 9 DM with treatment)

Age and BMI showed a significant increase in DM patients who are under treatment G2 compared to control subjects G1, also significant increments were observed when comparing the treatment group G3 to the new diagnostic group G2.

They have shown that there is no significant difference between age of controls and patients with T1DM and T2DM (11). The normal BMI varies from 18.5 to 24.9 kg/m2, and obesity above 30 kg / m2 (15).BMI were found to be elevated in diabetic groups in current study which was in agreement with Fadhil et al who showed that BMI when compared to non-diabetic people, was considerably higher in diabetic patients. (12).

FSG, TG, Cholesterol, HDL, LDL, VLDL, GOT, GPT and HbA1c levels showed statistically significant variations between study groups, as shown in (table 2).

Table 2: ONEWAY ANOVA test for FBG, HbA1c, TG, Cholesterol, HDL, LDL, VLDL, GOT, GPT, TOS, TAS, OSI, Z-Protein, and FBXW7-Protein by Groups							
		Mean± SE	95% Confidence Interval for Mean		Minimum	Maximum	p-value
			Lower Bound	Upper Bound			
FBG	G1	108.43±2.55	103.2238	113.6365	90.15	149.56	0.00
	G2	124.86±6.13[a,c]	112.0195	137.7036	85.54	180.99	
	G3	153.12± 2.70[b,c]	147.7334	158.5161	98.56	212.09	
HbA1c	G1	4.69±0.05	4.5870	4.7948	4.30	5.90	0.00
	G2	10.62±0.54[a,c]	9.4840	11.7590	6.50	15.00	
	G3	9.18±0.20[b,c]	8.7723	9.5910	5.37	13.70	
TG	G1	147.61± 3.77	139.9309	155.2969	120.77	197.87	0.02
	G2	151.15±5.8	138.9217	163.3901	117.18	208.07	
	G3	163.05± 3.73[b]	155.5957	170.5185	108.63	232.52	
Cholesterol	G1	178.60± 3.56	171.3492	185.8640	141.51	217.00	0.00
	G2	192.2± 6.24[a, c]	179.1595	205.3125	147.03	238.07	
	G3	212.48± 2.14[b, c]	208.1939	216.7745	163.46	269.09	
HDL	G1	48.89± 0.94	46.9806	50.8149	40.78	62.94	0.00
	G2	46.59±1.56	43.3194	49.8631	37.53	61.82	
	G3	42.19± 0.84[b,c]	40.5122	43.8815	28.30	65.74	
LDL	G1	100.18± 2.43	95.2348	105.1375	75.72	121.45	0.00
	G2	115.41± 5.09[a, c]	104.7483	126.0789	86.07	150.64	
	G3	137.67± 1.52[b, c]	134.6281	140.7237	107.01	195.22	
VLDL	G1	29.52± 0.75	27.9862	31.0594	24.15	39.57	0.02
	G2	30.23± 1.16	27.7843	32.6780	23.44	41.61	
	G3	32.61± 0.74[b]	31.1191	34.1037	21.73	46.50	
GOT	G1	29.86± 0.91	28.0023	31.7337	19.22	42.15	0.00
	G2	30.76± 1.49	27.6246	33.8959	22.94	47.87	
	G3	45.52± 0.83[b, c]	43.8479	47.1990	23.24	64.59	
GPT	G1	26.03± 0.88	24.2319	27.8410	15.17	36.63	0.00
	G2	26.37± 1.34	23.5593	29.1899	20.62	41.06	
	G3	39.50± 0.67[b, c]	38.1629	40.8463	25.85	52.97	
The mean difference is significant at the 0.05 level have been identified using ANOVA test.							

a. significant difference between G1 (control) and G2 (DM without treatment)
b. significant difference between control G1 (control) and G3 (DM with treatment)
c. significant difference between G2 (DM without treatment) and G3 9 DM with treatment)

FBG, HbA1c, cholesterol, and LDL levels show significant differences between control and newly diagnosed groups, control and treatment groups, and between treatment and newly diagnosed groups. Triglyceride and VLDL levels are significantly different between the control and treatment groups. HDL, GOT, and GPT levels were significantly different between the control and treatment groups, as well as between the treatment and newly diagnosed groups.

Khursheed Muhammad Uttra in 2011 used diabetes types 1 and 2 as a model to define the pace and pattern of hyperlipidemia, he found Hyperlipidemia was found in patients suffer DM2 patients, was observed High triglycerides, high LDL, low HDL, and high cholesterol were the style of lipid abnormalities (13). The Diabetes Control and Multiples Trial proof has conclusively shown that stringent glycemic control in individuals with type 1 diabetes can both postpone the emergence of microvascular complications and slow down the rate of complications. Furthermore, a curvilinear link was found between the happening risk of multiples, such as retinopathy, and HbA1c levels, if HbA1c values shifted from 7-8% to higher levels (14). M Verma and colleagues (2014) noted in their research that GOT levels were considerably greater in type 1 diabetics aged 1-20 years. The findings of the researchers imply that salivary gland damage is caused by the same immunological onslaught that causes pancreatic cell damage and type 1 diabetes (15).

Regarding to oxidative stress markers, there is a non-significant in TOS and TAS, but OSI has a significant difference between the newly and treatment groups as shown in (table 3).

Table 3: ONEWAY ANOVA test for TOS, TAS, and OSI by Groups							
		Mean± SE	95% Confidence Interval for Mean		Minimum	Maximum	p-value
			Lower Bound	Upper Bound			
TOS	G1	0.04± 0.008	0.0322	0.0665	0.00	0.16	0.4
	G2	0.07± 0.01	0.0478	0.1015	0.00	0.18	
	G3	0.06± 0.01	0.0426	0.0930	0.00	0.55	
TAS	G1	1.61± 0.05	1.5087	1.7274	0.91	2.04	0.6
	G2	1.48± 0.10	1.2550	1.7050	0.32	2.19	
	G3	1.76± 0.21	1.3424	2.1814	0.13	14.21	
OSI	G1	0.059± 0.004	0.0506	0.0691	0.00	0.12	0.03
	G2	0.09± 0.01	.0550	0.1345	0.00	0.38	
	G3	0.04± 0.01c	.0244	0.0655	0.00	0.49	
The mean difference is significant at the 0.05 level have been identified using ANOVA test.							

d. significant difference between G1 (control) and G2 (DM without treatment)
e. significant difference between control G1 (control) and G3 (DM with treatment)
f. significant difference between G2 (DM without treatment) and G3 9 DM with treatment)

TAS was lower in type 1 diabetics compared to nondiabetic patients, there were associations between TAS and HbA1c and duration of diabetes (16). In 2010, Pilar Codoer-Franch pointed out that both children with T1D and obese children have oxidative stress. In T1D patients, glutathione peroxidase activity and exogenous antioxidants are dropped than in obese children. (17). In 2018, Rabia Alghazeer pointed out that these findings suggested that oxidative state and antioxidant levels were influenced in T1DM. The findings showed that indicators such as plasma levels of lipid peroxidation and antioxidants in newly diagnosed diabetics can be utilized to track the progression of diabetes complications. (18).

The results of the novel marker Z protein show Statistically significant difference between control and newly diagnosis also between control and treatment groups as show in (table 4).

Table 4: ONEWAY ANOVA test for Z-Protein by Groups

		Mean± SE	95% Confidence Interval for Mean		Minimum	Maximum	p-value
			Lower Bound	Upper Bound			
Z_Protein	G1	90.90±1.51	87.8241	93.9808	79.24	117.03	0.00
	G2	142.5±12.11131[a]	117.2325	167.9310	94.88	285.25	
	G3	152.36±8.94046[b]	134.5173	170.2177	99.57	333.13	

The mean difference is significant at the 0.05 level have been identified using ANOVA test.

a. significant difference between G1 (control) and G2 (DM without treatment)
b. significant difference between control G1 (control) and G3 (DM with treatment)
c. significant difference between G2 (DM without treatment) and G3 9 DM with treatment)

There is no enough research on Z protein with type 1 diabetes. Yun-Ui Bae in 2021 he linked between Z protein and type 2 diabetes, he found that Prediabetes individuals had lower plasma PROZ levels. (10). Fatima S. Al-Shaikh in 2013 pointed out in her study that after testing the genetic mutations for Z protein and ZPI protein, it was found that there are several genetic mutations that are partly involved in the formation of Z protein that contribute to reducing the level of Z protein in the blood, which plays a role in increasing the possibility of clots that may lead to recurrent miscarriage (19).

The amount of serum Z protein and its relationship to other parameters in the examined population

We studied the connection between Z protein level and numerous other parameters in patient and control groups (Table 5). In patients, serum Z have positive correlation with FBG, TG, Cholesterol, HDL, TAS and VLDL. In control group, serum z protein has positive correlation with FBG, TG, Cholesterol, HDL, LDL and VLDL, whereas GPT have negative correlation.

Table (5): Correlation analysis of variable associated with serum Z protein levels in the study populations.

	Sample			
	Patients		Control	
	R	P	r	P
FBG	0.3	0.00	0.49	0.003
TG	0.5	0.00	0.39	0.02
Cholesterol	0.2	0.04	0.53	0.001
HDL	0.2	0.02	0.68	0.0
GPT			-0.4	0.04
TAS	0.3	0.01		
LDL			0.4	0.02
VLDL	0.5	0.00	0.4	0.02

Cluster analysis of multivariate for all parameters in studied subjects

Cluster analysis groups variables by searching for related or interdependent variables, combining them in clusters or segments, and separating them from other, dissimilar, variables. Ward's approach is one of the most common type of cluster analyses 25. This method is typically displayed using a dendrogram. In this test, no prior assumption of the clustering is made.

In the present study, cluster analysis was used to discover the similarities in the studied variables. According to the coefficients, the variables in all the studied groups were distributed into 6-7 clusters with mild shifting in some groups. Fig. 1: a shows the multivariable cluster analysis results using patient data. The variables were identified and classified into 7 clusters. The first cluster included (TOS, OSI, TAS and HbA1c). Cluster second included (GOT, VLDL, GPT and HDL). Cluster third included (Z protein, LDL and FBG). Cluster fourth included (LDL and TG). Cluster fifth included (TG, and Cholesterol). Cluster sixth included (TAS and GPT) and the last cluster

included (TAS, GPT, LDL and TG).
A fusion of the cluster took place to form a single group. Fig. 1: b shows the multivariable cluster analysis results using control data. The variables were identified and classified into 6 clusters. The first cluster included (TOS, OSI, TAS, and HbA1c). Cluster second included (GOT, GPT, VLDL, and HDL). Cluster third included (FBG, LDL, and TG). Cluster fourth included (Z protein and Cholesterol). Cluster fifth included (TAS and VLDL). Cluster sixth included (LDL and Z protein), and the last cluster included (TAS, VLDL and cholesterol).
The difference between patients and control, in patient Z protein is associated with FBG and LDL, while in control Z protein is associated with LDL.

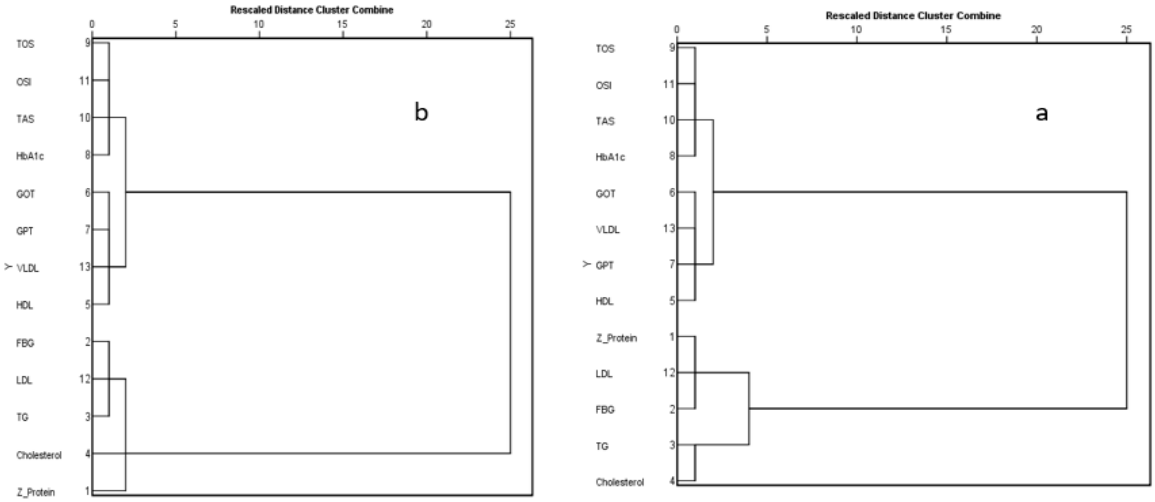

Fig. 1: The cluster analysis of multivariate for all parameters among patients in the group[a] and control [b].

Roc curve of protein Z

According the Receiver Operating Characteristic (ROC) the best cutoff point in Z protein shows sensitivity of 1 and 1- specificity of 0.82 in area 0.97, the FBG shows sensitivity of 0.55 and 1- specificity of 0.82 in area 0.67 and HbA1c shows sensitivity of 1 and 1- specificity of 1 in area 1 in the newly diagnosis fig 2.
In patients that taken insulin therapy, Z protein shows sensitivity of 0.89 and 1-specificity of 0.97 in area 0.97, the FBG shows sensitivity of 0.97 and 1-specificity of 0.85 in area 0.95, and HbA1c shows sensitivity of 0.98 and 1-specificity of 0.97 in area 0.99 in the fig 3.
HbA1c is considered a marker in diagnosing diabetes, and Z protein can also be considered a marker for diagnosing diabetes.

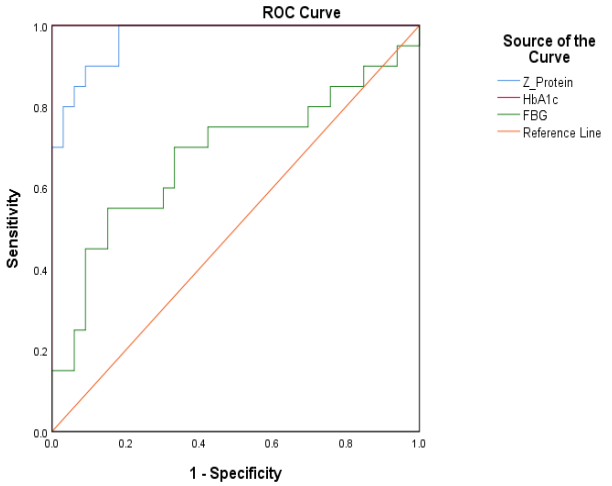

Fig 2 ROC curve analysis of the predictive value of serum concentration of Z protein in newly diagnosis patients with diabetes.

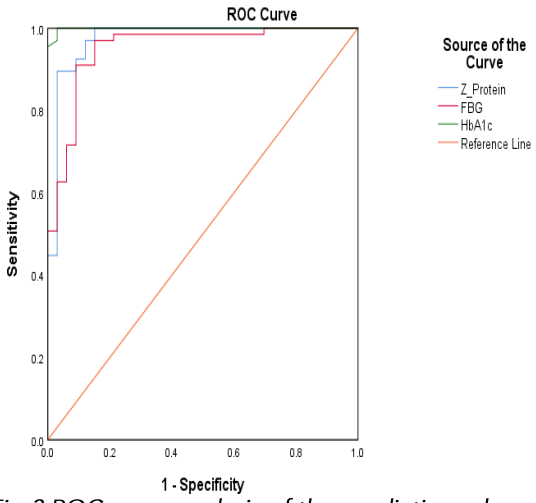

Fig 3 ROC curve analysis of the predictive value of serum concentration of Z protein in insulin therapy patients with diabetes.

5. Conclusion

PZ protein was perfectly associated with G2 (newly), so it may be used as an early diagnosis as show in the area (0.97) of the ROC curve that distinguishes between newly and healthy groups.
Guarantor Asst. Prof. Dr. Ekhlass M. Taha

Abbreviations

T1DM, Type 1 diabetes; FBG, Fasting blood glucose ; HbA1c, Glycated hemoglobin ; LDL, Low density lipoprotein ; HDL, High density lipoprotein ; VLDL, Very low density lipoprotein ; TC, Total cholesterol ; TG, Triglyceride ; GOT, Glutamic oxalocetic transaminase ; GPT, Glutamic pyruvic transaminase ; TOS, Total oxidant status ; TAS, Total antioxidant status ; OSI, Oxidative stress index ; DM, Diabetes mellitus .

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