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Role of F-box WD Repeat Domain Containing 7 in Type 1 Diabetes

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Abstract

Type I diabetes (T1DM) is a chronic immune system disease characterized by the devastation or injury of ß-cells in the Langerhans Island, resulting in insulin deficiency and hyperglycemia. This study determines the new marker F-box and WD repeat domain containing 7 (FBXW7). One hundred twenty type 1 diabetic patients from three different places (central child hospital, Alkindi center for diabetes and endocrinology, Children's Education Hospital) in Iraq during the period from (20 December 2021 to 25 March 2022) an age ranges of (4-17) years. The patient group consisted of being derived to three groups: group one healthy patient group (33) was included as healthy patient, group two (20) newly diagnosed T1DM and (67) type 1 diabetic with insulin treatment. The quantitative enzyme-linked immunosorbent assay (ELISA) biochemical parameters were used to quantify the protein FBXW-7 levels. FBG, Cholesterol, Triglyceride, HDL, LDL, VLDL, HbA1c, GOT, GPT, Total Oxidant status, and Total Antioxidant status were measured through spectrophotometry. Serum FBXW-7 protein levels were considerably elevated noticeably (p-value = 0.00). In terms of FBXW7 protein, there was a significant variation between the new and therapy groups. There was no significant variation in protein levels between the new compared to healthy groups. Serum FBXW-7 protein was positively correlated with FBG, TG, cholesterol, GOT, GPT, LDL, and VLDL, and was negatively correlated with HDL in the patient group. According to ROC analysis, the cutoff value for FBXW-7 protein was (1.9) in the newly group and (2.1) in the treatment group. Levels of FBXW-7 protein are elevated in DM patients. FBXW-

7 protein was significantly different in the treatment group but not different in the newly group when compared with the healthy group.

Keywords: FBXW7 protein, Newly diagnosis, Type 1 diabetes.

1. Introduction

Type 1 diabetes mellitus (T1DM), commonly known as autoimmune diabetes, is a chronic illness that causes hyperglycemia owing to insulin insufficiency caused by pancreatic beta-cell death. The pathophysiology of the disease is considered to be T-cell-mediated destruction of beta cells. T1DM pathophysiology may be classified into three phases based on the presence or absence of hyperglycemia and hyperglycemia-related symptoms (such as polyuria and thirst). Insulin is the primary therapy for all patients with T1DM. Patients with T1DM will require numerous daily injections at the time of diagnosis, as well as innovative insulin therapy techniques such as insulin pumps and continuous glucose monitoring [1]. Hypoglycemia, hyperglycemia, and diabetic ketoacidosis (DKA) are the most prevalent complications in children and adolescents with T1DM. Long-term consequences (such as retinopathy, nephropathy, neuropathy, and cardiovascular disease) begin to develop in childhood [2]. To lower the risk of long-term diabetes-related complications, the American Diabetes Association (ADA) treatment recommendations recommend a goal to be HbA1c less than 7.0% (53 mmol/mol) in people with T1D (7.5% for the patient with T1D) [3]. The F-box protein, Skp1, Cullin 1, and Roc1/Rbx1/Hrt1 are the primary components of the SKP1-CUL1-F-box (SCF) ubiquitin ligases. F-box/WD repeat-containing protein 7 (Fbxw7) belongs to the F-box protein family and serves as the substrate recognition component of the SCF E3 ubiquitin ligase [4]. FBXW7 is located in chromosome 4 and comprises 707 amino acids and has a molecular weight of approximately 79 KD [5]. There are three structures of proteins: FBXW7α, FBXW7β, and Fbxw7 γ [6]. It has a different cell location; FBXW7α found in the nucleoplasm, FBXW7β found in the cytoplasm, and Fbxw7 γ in nuclear [7]. HbA1c is the most often used marker for the diagnosis of prediabetes, coupled with fasting plasma glucose (FPG). FPG findings may change depending on food influences and fasting length. The oral glucose tolerance test used to diagnose IFG and IGT is difficult for patients to complete in a clinical environment since it is time-consuming and involves numerous blood samples [8]. The purpose of this study was to study the role of FBXW7 and type 1 diabetes and to compare the newly diagnosed patients that have taken insulin therapy.

2. Materials and methods

Patients

A case control study was conducted in Iraq to investigate FBXW-7 levels in individuals with type 1 diabetes. This research included 120 participants ranging in age from 4 to 17 years. They were classified into three groups: A total of 90 individuals with type 1 diabetes were recruited from (Central Child Hospital, Alkindi center for diabetes and endocrinology, Child Protection Teaching Hospital) during the period from 20 December 2021 to 25 March 2022. These patients were divided into two groups: group 1 included (67) individuals children (33 girls, 34 boys) of T1DM with insulin injection treatment, and group 2 included (20) child (9 girls, 11 boys) individuals of newly diagnosed. In addition (33) child (14 girls, 19 boys) as a control group.

Blood sample collection

The blood sample was taken following an overnight fast. 5 ml of blood was taken from every patients and control through venipuncture using a 10 ml syringe. Two milliliters of blood were discharged into a tube containing ethylene diamine tetracetic acid (EDTA), and this blood was used to calculate HbA1c. Serum was used to calculate Fasting blood glucose (FBG),Glutamic oxaloacetate transaminase (GOT), Glutamic pyruvic transaminase (GPT), Cholesterol, Triglyceride ,Oxidant status, Antioxidant oxidant status, oxidative stress index ,High density lipoprotein (HDL) ,Low density lipoprotein (LDL) ,Very Low density lipoprotein (VLDL) and F-box and WD repeat domain containing 7 (FBXW7) (FBXW-7 protein).

Determination of diabetic-related parameters

All of Serum glucose, cholesterol, triglyceride, and HDL were measured using a total enzymatic colorimetric technique from a kit supplied by LINEAR Chemicals, Barcelona, Spain. HbA1c was measured using a kit supplied from a RANDOX in the United Kingdom and the RX DAYTONA+ clinical chemistry analyzer programmer; this test is based on latex immunoagglutination. GPT was performed out with the use of a kit given by GenWay Biotech in the United States. GOT was performed out using a kit provided by Sigma-Aldrich in the United States. LDL is calculated using the equation LDL-C =cholesterol - (TG/5) - HDL-C, VLDL is also is calculated using the equation VLDL-C = TG / 5. TAS and Total oxidants status was determined by measuring absorbance using a spectrophotometer instrument, while OSI was computed using the equation OSI = TOS (mmol H2O2/L/TAS mmol vit.C/L).

Estimation of FBXW-7 protein

Protein was measured using sandwich enzyme immunoassay method and a kit provided by BioSource, USA. The samples were placed in the wells for one hour, and then washed three and a half times. The (HRP enzyme) conjugated was added to the washed well and waited for half an hour, then washed three and a half times. The TMB was introduced, and the color was created as a result of the reaction. Stop solution was added to the mixture, causing the color change to yellow and being measured at 450nm with a Human ReaderHS.

Statistical analysis

The findings were analyzed using a statistical analysis tool (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. Results were expressed as mean±SD spearman correlation was used to perform the correlation. Receiver operating characteristic curve (ROC) analysis were also utilized to determine the cutoff value for the parameters.

3.Results and Discussion

Statistical distribution of studied groups was presented in **Figure 1**, as shown the three groups were: G1 (control), G2 (DM without treatment) and G3 (DM with treatment), those groups were subdivided according to gender.

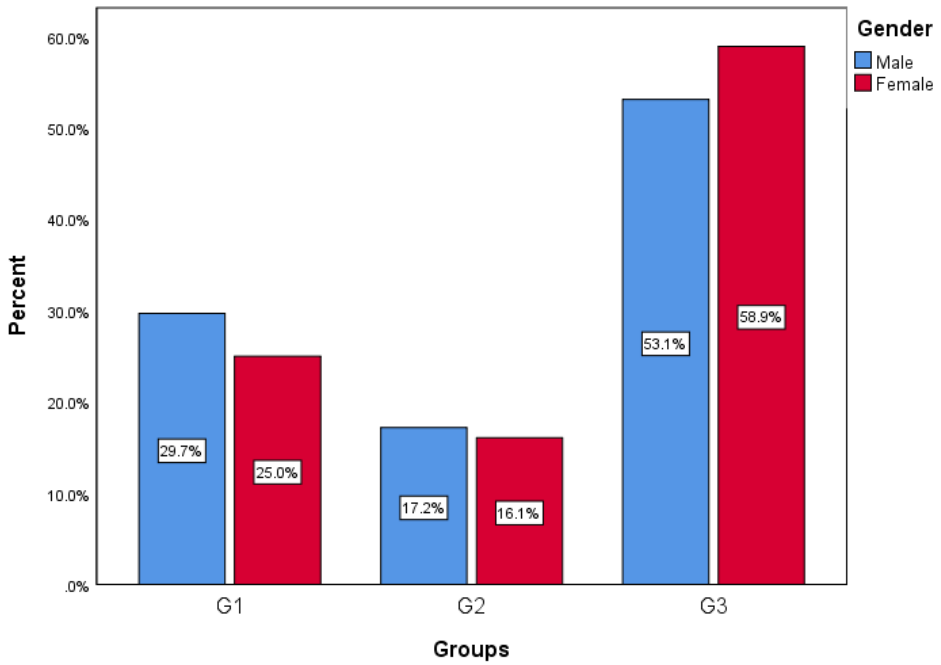

Figure1. groups distribution in the studied population

Anthropometric characteristics were measured it was illustrated in **Table 1**.

Table 1. Demographic factor distribution in studied groups

Parameters		Mean± SE	P-valu
Age	G1	9.9667±0.578	0.006
	G2	9.35±0.703[c]	
	G3	11.53±0.354[b,c]	
BMI	G1	16.51±0.530	0.002
	G2	16.36±0.715[c]	
	G3	18.37±0.325[b,c]	

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.

(K. O. Kyvik, 2004) pointed in there studied that type 1 diabetes is less common in adults than in children, and the range of occurrence is considerably narrower [9]. According to (Ferrara, 2017) study, having a high BMI is connected with an increased risk of type 1 diabetes development, but the effect varies by gender and age [10].

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. Clinical data in patient having diabetes mellitus and control (G1 control group, G2 newly diagnosis DM, G3 with treatment).

Parameters		Mean± SE	p-value
FBG	G1	108.43±2.55	0.00
	G2	124.86±6.13[a,c]	
	G3	153.12± 2.70[b,c]	
HbA1c	G1	4.69±0.05	0.00
	G2	10.62±0.54[a,c]	
	G3	9.18±0.20[b,c]	
TG	G1	147.61± 3.77	0.02
	G2	151.15±5.8	
	G3	163.05± 3.73[b]	
Cholesterol	G1	178.60± 3.56	0.00
	G2	192.2± 6.24[a,c]	
	G3	212.48± 2.14[b,c]	
HDL	G1	48.89± 0.94	0.00
	G2	46.59±1.56	
	G3	42.19± 0.84 [b,c]	
LDL	G1	100.18± 2.43	0.00
	G2	115.41± 5.09[a,c]	
	G3	137.67± 1.52[b,c]	
VLDL	G1	29.52± 0.75	0.02
	G2	30.23± 1.16	
	G3	32.61± 0.74[b]	
GOT	G1	29.86± 0.91	0.00
	G2	30.76± 1.49	
	G3	45.52± 0.83[b,c]	
GPT	G1	26.03± 0.88	0.00
	G2	26.37± 1.34	
	G3	39.50± 0.67[b,c]	

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)

Results of FBG, HbA1c, cholesterol, and LDL levels show significant difference between control and newly groups, control and treatment groups and between control and newly groups. Triglyceride and VLDL levels are significantly different between the control and treatment groups, but not different between the control and newly groups or between the treatment and newly groups. Results of HDL, GOT, and GPT levels showed significant different between the control and treatment groups, as well as between the treatment and newly groups, but not different significant between the control and newly developed groups.

The WHO Consultation concluded that HbA1c and FSG used as a diabetes diagnostic test [11]. (Vergès, 2009) pointed in there studied that T1DM patients also have lipid turbulence. Lipoprotein quantitative abnormalities are seen in T1D patients with poor glycemic control (raise plasma triglycerides and LDL cholesterol) or nephropathy (raise triglycerides and LDL cholesterol, low level of high density lipoprotein [HDL] cholesterol) [12]. (Valentina Guarnotta, 2018) pointed out

that IDM patients had a significantly higher BMI (p 0.012), LDL-cholesterol (p 0.027), GOT (p 0.005), and GPT (p 0.001), with concomitantly lower HDL-cholesterol (p 0.001) [13].

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. Mean ± SD value of TOS, TAS and OSI for the studied groups.

Parameters		Mean± SE	p-value
TOS	G1	0.04± 0.008	0.4
	G2	0.07± 0.01	
	G3	0.06± 0.01	
TAS	G1	1.61± 0.05	0.6
	G2	1.48± 0.10	
	G3	1.76± 0.21	
OSI	G1	0.059± 0.004	0.03
	G2	0.09± 0.01	
	G3	0.04± 0.01[c]	

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)

Our study agreed with the results of (Zainab M.etal,2022) for both TOS and TAS, which showed non-significant difference for type 1 diabetes patients [14].

Statistically there is significant in FBXW7 protein, the significant difference between control group and treatment group, also have different significant between newly and treatment groups as show in **Table 4**.

Table 4. Mean ± SD value of FBXW7 for the studied groups.

Parameter		Mean± SE	p-value
FBXW7_Protein	G1	1.99±0.041	0.00
	G2	2.24± 0.053	
	G3	4.66±0.17436[b,c]	

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)

The FBXW7 protein was chosen as the marker for the current study, and when compared to the previous study on DM with type 2, Suhayla K. Mohammed pointed out in their study that FBXW7 protein was highly associated with G1 (newly diagnosed patients), they suggested that serum FBXW7 may be used as an early diagnostic test in patients with DM, and metformin significantly reduces FBXW7 level [15]. In the first study of FBXW-7 protein in T1DM in 2021, clearly, the mechanism of EZH2/ZBTB16 in T1DM inhibition by FBW-7 is clarified, suggesting that FBW-7 is a promising target of T1DM therapy. Overexpression of FBW-7 in the nod mice inhibits pro-inflammatory cytosine release in the splenocyles and the apoptosis of islet beta cells [16].

The amount of serum FBXW7 protein and its relationship to other parameter in the examined population

In the patient group, we studied the connection between the FBXW7 protein level and numerous other parameters **Table 1**. In patients groups, FBXW7 serum have positive correlation with these parameter FBG, TG, Cholesterol, GPT, GOT, LDL, VLDL and have negative correlation with HDL. Moreover, FBXW7 have significant with all these parameter in patients. In control all have positive correlation. Moreover, we found significant correlation with all parameter in **Table 5**.

Table 5. Correlation analysis of variable associated with serum FBXW7 protein levels in the study populations.

Variable	Sample			
	Patients		Control	
	R	P	R	P
FBG	0.5	0.00	0.39	0.02
TG	0.3	0.00	0.61	0.0
Cholesterol	0.34	0.00	0.47	0.006
HDL	-0.3	0.00	0.4	0.006
GPT	0.5	0.00	-	-
GOT	0.5	0.00	-	-
LDL	0.4	0.00	-	-
VLDL	0.3	0.00	0.6	0.0

Roc curve of protein FBXW7

According to the Receiver Operating Characteristic (ROC), the best cutoff point in the FBXW-7 has a sensitivity of 80% and a 1-specificity of 48.8% in area 0.8 with cut off 1.9, the FBG has a sensitivity of 75% and a 1-specificity of 60% in area 0.68 with cut off 105, and the HbA1c has a sensitivity of 100% and a 1-specificity of 100% in area 1 with cut off 6.2 in the newly as show in **Table 6 Figure 1**.

Therefore, FBXW7 can be considered as an indicator because it gave a high sensitivity 80%.

Table 6. The ROC curve analysis of HbA1C, FBG and FBXW7 Protein using patients DM without treatment.

Parameters	**Area**	**Sensitivity**	**Specificity**	**Cutoff**	**Asymptotic 95% Confidence Interval**	
					Lower Bound	**Upper Bound**
FBXW7 Protein	0.80	80%	48.8%	1.9	0.69	0.92
FBG	0.68	75%	60%	105	0.51	0.84
HbA1C	1	100%	100%	6.2	1	1

Null hypothesis: true area = 0.5

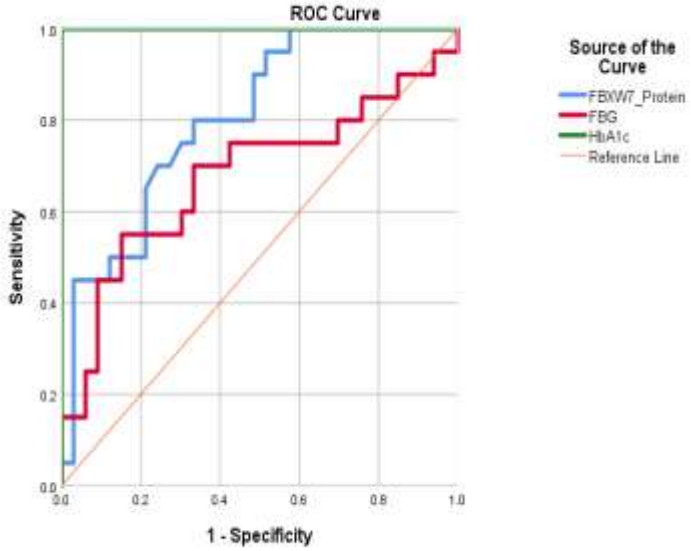

Figure 2. The ROC curve analysis of the prognostic value of blood FBXW-7 protein content in newly diagnosed diabetic patients.

While on insulin treatment, the FBXW-7 has a sensitivity of 100% and a 1-specificity of 98.5% in area 0.99 with cut off 2.1, the FBG has a sensitivity of 97% and a 1-specificity of 98% in area 0.95 with cut off 118, and the HbA1c has a sensitivity of 95% and a 1-specificity of 100% in area 0.99 with cut off 6 as show in **Table 7 Figure 2**.

Therefore, FBXW7 can be considered as an indicator because it revealed a high sensitivity 100% and high specificity 98.5%.

Table 7. ROC curve analysis of HbA1C, FBG and FBXW7 Protein using patients DM with treatment.

Parameters	Area	Sensitivity	Specificity	Cutoff	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
FBXW7 Protein	0.99	100%	98.5%	2.1	0.98	1
FBG	0.95	97%	98%	118	0.90	0.99
HbA1C	0.99	95%	100%	6	0.99	1

Null hypothesis: true area = 0.5

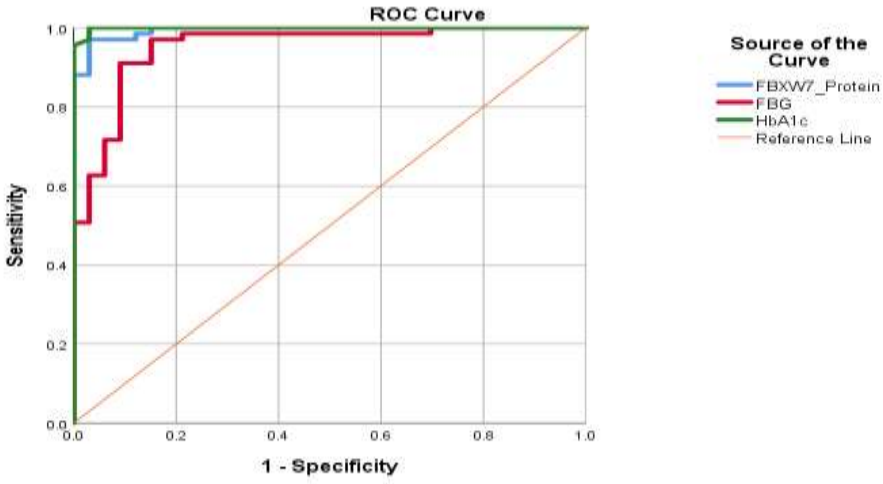

Figure 2. ROC curve analysis of the predictive value of serum concentration of FBXW-7 protein in insulin therapy patients with diabetes.

4. Conclusion

The FBXW-7 protein was high associated with G3 (treatment) in the area (0.99) as shown in the ROC curve, and also it was good associated with G2 (newly) in the area (0.80), also as shown in the ROC curve. So we suggested that FBXW7 used as indicator to diagnose in patient with DM. insulin not reduce FBXW7 level significantly.

5. Ethical approval

The studies have been approved by Committee of the University of Baghdad College of science for women, and have been performed in accordance with the ethical standard as laid down in the 1964, I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. 6. **Conflict of interest**

The authors announce that they have no contending interests.

7. Source of funding

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8. Guarantor Asst. Prof. Dr. Ekhlass M. Taha

9. 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 ; FBXW7, F-box and WD repeat domain containing 7.

References

1. Katsarou, A.; Gudbjörnsdottir, S.; Rawshani, A.; Dabelea, D.; Bonifacio, E.; Anderson, B.J.; Jacobsen, L.M.; Schatz, D.A.; Lernmark, A. Type 1 diabetes mellitus. *Nature reviews Disease primers.* **2017**, *3.1*: 1-17.
2. Levitsky, L.L.; Misra, M. Complications and screening in children and adolescents with type 1 diabetes mellitus. **2007** Up to date, *17.1*.
3. Scott, S.N.; Anderson, L.; Morton, J.P.; Wagenmakers, A.J.M.; Riddell, M.C. Carbohydrate restriction in type 1 diabetes: a realistic therapy for improved glycaemic control and athletic performance?. *Nutrients.* **2019**, *11.5*: 1022.
4. Cao, J.; Ge, M.H.; Ling, Z.Q. Fbxw7 tumor suppressor: a vital regulator contributes to human tumorigenesis. *Medicine* . **2016**, *95.7*.
5. Tejomayee, S. Functionalization of cancer-associated mutant alleles of human CDC4 (FBXW7) PhD[dissertation], *University of British Columbia*. **2013**.
6. Li, M.R.; Zhu, C.C.; Ling,T.L.; Zhang, Y.Q.; Xu, J.; Zhao, E.H.; Zhao,G. FBXW7 expression is associated with prognosis and chemotherapeutic outcome in Chinese patients with gastric adenocarcinoma. *BMC gastroenterology.* **2017**, *17.1*: 1-8.

7. Welcker, M.; Orian, A.; Grim, J.A.; Eisenman, R.N.; Clurman, B.A. A nucleolar isoform of the Fbw7 ubiquitin ligase regulates c-Myc and cell size. *Current Biology.* **2004**, *14.20*: 1852-1857.

8. Bae, Y.U.; You, J.H.; Cho, N.H.; Kim, L.E.; Shim,H.M.; Park, J.H.; Cho, H.C. Association of protein Z with prediabetes and type 2 diabetes. *Endocrinology and Metabolism* . **2021**, *36.3*: 637.

9. Kyvik, K.O.; Nystrom, L.; Songini, M.; Oestman, J.; Castell, C.; Green, A.; Guyrus, E.; Tirgoviste, C.I.; McKinney, P.A.; Michalkova, D.; Ostrauskas, R.; Raymond, N.T. The epidemiology of type 1 diabetes mellitus is not the same in young adults as in children. *Diabetologia*. **2004**, *47.3*: 377-384.

10. Ferrara, C. T.; Geyer, S. M.; Liu, Y. F.; Evans-Molina, C.; Libman, I. M.; Besser, R., ... & Type 1 Diabetes TrialNet Study Group. . Excess BMI in childhood: a modifiable risk factor for type 1 diabetes development?. *Diabetes care*. **2017**, *40(5)*, 698-701.

11. World Health Organization. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. *World Health Organization*, **2011**, No. WHO/NMH/CHP/CPM/ *11.1*

12. Bruno, V. Lipid disorders in type 1 diabetes. *Diabetes & metabolism*. **2009**, *35.5*: 353-360.

13. Guarnotta, V.; Vigneri, E.; Pillitteri, G.; Ciresi, A.; Pizzolanti, G.; Giordano, C. Higher cardiometabolic risk in idiopathic versus autoimmune type 1 diabetes: a retrospective analysis. *Diabetology & Metabolic Syndrome*. **2018**, *10.1*: 1-8.

14. Qassam, Z.M.; Taha, E.M. Study the Dynamic Thiol -Disulfide Homeostasis in patients with Diabetes type I and type 2. **2022**.

15. Mohammed, S.K.; Taha, E.M.; Muhi, S.A.; A case-control study to determination FBXW7 and Fetuin-A levels in patients with type 2 diabetes in Iraq. *Journal of Diabetes & Metabolic Disorders.* **2021**, *20.1*: 237-243.

16. Guo, Y.; Li, J.; Fan, S; Giordano, Q.H. Suppressive role of E3 ubiquitin ligase FBW7 in type I diabetes in non-obese diabetic mice through mediation of ubiquitination of EZH2. *Cell death discovery*. **2021**, *7.1*: 1-9.