Effects of Duration of Diabetes and Diabetes Therapy (Metformin) on Fbxw7 Levels in Iraqi Type II Diabetic Patients

Suhayla K. Mohammed[1], Hayyan I. Al-Taweil[2], NouraKaram M. Salih[3], Samer Abdulhasan Muhi[4], Ekhlass M. Taha[5]

[1]Instractor, Department of Chemistry College of Science for women University of Baghdad, Al-Jadriya Baghdad Iraq, [2]Assist. Prof., Department of Clinical Laboratory Science. Mohammed Al-Mana College for Medical Sciences. Al-Khobar, Eastern Province, Kingdom of Saudi Arabia, [3]Assist. Prof., Department of Science, Faculty of Engineering, Infrastructure University KualaLumpur, Jl-nikram/Uniten, Kajang, 43000, Selangor Darul El Ehsan, Malaysia, [4]Lecturer, Al-yarmouk Teaching Hospital, Al-Yarmouk Baghdad Iraq, [5]Assist. Prof., Department of Chemistry College of Science for women University of Baghdad, Al-Jadriya Baghdad Iraq

Abstract

Background: This study concentrated on evaluation the FBXW7 levels and other markers in T2DM patients related to the duration of metformin monotherapy and duration of T2DM.

Method: The current study includes 60 diabetic patients type II and30 control subjects. The groups of patients were subdivided in to two groups: (group I) (30) Newly diagnosed T2DM which also subdivided to two groups(> 1 year, and <1 year). Group II(30) T2DM (who were treated with metformin monotherapy) which also subdivided into two group (> 1 year and <1 year). In the present study FBXW7, FetuinA, pentraxin3, Nitric oxide and insulin were determined bythe enzyme linked immune sorbent assay (ELISA). Biochemical markers, fasting serum glucose, and lipide profile were determined through spectrophotometere technique in patients and control groups.

Result: The levelsof glucose, HbA1c and IR significantly affected byincrease the duration of diseaseWhile each of fbxw7, pentraxin 3,NO, and fetuin A not influenced by increase duration of disease. There was non-significant effect of metformin therapy duration on levels of Fbxw7,fetuinA, pentraxin3 and lipid profile. While IR showed significant decrease when the duration of treatment increase.

Conclusion: Fbxw7, fetuinA, pentraxin3 and NO arenot affected by long duration of therapy or by duration of disease while glucose, HbA1c, and IR are affected by increase duration of diseaseonly. Whereas insulin and IR are affected by both; duration of disease and therapy.

Keywords: *FBXW7, pentraxin3, nitric oxide, Type II diabetes, metformin, duration.*

Introduction

Among the most challenging health problems of the (21st century) is diabetes mellitus [1]. It is a chronic condition characterized by high levels of glucose in blood, resulting in significant damage to multiple organs including the heart,kidney,liver,eyes and vessels[2]. Weak glycemic regulation is known to be correlated substantially with a longer period of diabetes . Diabetes

Corresponding Author:
Ekhlass M. Taha
Assist. Prof., Department of Chemistry College of Science for women University of Baghdad, Al-Jadriya Baghdad Iraq
e-mail: ikhlasmb_chem@csw.uobaghdad.edu.iq

is a progressive disease and as glucose levels increase, more medicinesare required to regulate it [3]. Metformin monotherapy is first treatment in diabetes type 2 patients which initiated when non-pharmacological therapy has failed to reach sufficient glycemic regulation[4]. Data from other studies showed that drug therapy of T2DM becomes more complex in longer duration of disease[5]. Fbxw7 is one of Fbox proteins and an important part of SCF E3 ligas complex that target proteins for thedegradation[6][7] Fbw7 protects against hyperglycemia, insulin resistance, and the glucose intolerance. Previous studies in obese patients showed hepatic down regulation of fbxw7[8].Fetuin A, named also α-2 Hermans-Schmid glycoprotein AHSG) is an acidic glycoprotein with molecular weight 64 KD [9]. It is belongs to proteases inhibitor cystatinesuperfamily[10]. Fetuin A is associated with high risk of diabetes, especially in people with high plasma glucose[11,12]. Studies to identify the markers for diabetes2 and its associated cardiovascular complications are important to control the disease, prevent the onset and progression of lethal complications at early stage[13].Pentraxin3are an acute phase protein superfamily which induce each of short pentraxin like c reactive protein (CRP) and long pentraxin like pentraxin3 (PTX3)[14]Actually, serum PTX3 level in patients with diabetes has been shown to be positively associated with atherosclerotic markers[15,16]. Nitric oxide (NO) is one of most common substances in the mammals which is considered as an internal gaseous free radical[17]. Previous studies suggested an alteration in serum NO levels in T2DM [18]. Some studies documented raise in NO rates in patients with diabetes while others reported lower in levels[19,20]. In the present study, weexamined the effectof *duration* of T2DM disease and duration Therapy on fbxw7 and other parameters.

Materials and Method

Serum sample from Ninety subjects were selected to perform this project, agerage between 37 to 69 years. They were divided in to 3 groups as follows: A control group consisted of 30 apparently healthy individuals. Group1 included 30 newly diagnosed (T2DM) without treatment, and group 2 included 30 patientson metformin monotherapy. Each groups of patients' sub divided into two group,> 1 year and <1 year. Bothgroups collected from National Diabetes Center for Treatment and Research at Al Mustansiriya University/Iraq.Patients with heart disease, liver disease, hypertension, kidney disease and smoker patients were excluded.

Blood samples were taken after overnight fasting. From each patient and control, 10 ml of blood was obtained. Two ml was dispensed in ethelendiaminetetracetic acid(EDTA) tube. This blood was used for HbA1c estimation. Serum that obtained was used in determination of other parameters. Serum fetuin A was estimated be quantitative sandwich enzyme immunoassay technique, using the kit fromcusabio, china. FBXW7 was determined by competitive enzyme immunoassay technique using the kit fromBiosource,USA. Enzymatic colorimetric method was used for serum glucose determination using the kit supplied by Randox UK.Insulin was determined by ELISA technique,using the kit supplied by Demeditec,Germany.Serum cholesterol and triglyceride were measured using the kit supplied by Spinreact,Spain. Whileserum HDL was determined by the kit supplied by Randox UK. Results were summarized as mean±SD. Statistical analysis program (SPSS 25) have been performed for analysisof the results. Student t-test has been used to identify the significant differences. Dendrogram have been used to identify the similarity between variables.

Results and Discussion

Effect of disease duration on diabetesrelated parameters: Analysis of baseline characteristics table 1, showed that glucose level, HbA1c and insulin resistance IR significantlyaffected by the duration of disease,glucose level and IR showed significant increase in group of patient with duration of disease > 1 year, also HbA1c show significant decrease in patient group > 1 year. While BMI,WHtR, AI,and lipid profile showed non-significant difference between patient groups.A relation between FBS and duration of diabetes in this study is in agreement with the results obtained by a previous study which reported that the incidence of severe hyperglycemia increased with age and duration [21].Long duration of Diabetes is known to be associated with poor control, possibly due to decreasesecretion of insulin over time due to B cell failure, which makes the response to diet alone or oral agents unlikely[22]. The levels of Fbxw7 significantly increase in newly diagnosed DM as compared to control but the increase was non-significant with prolong the duration of disease as shown in Table 2. This increase may be due to damage tissues caused by diabetes lead to decrease fbxw7 in tissues and increase secretion in blood. While each of pentraxin 3,NO, and fetuin A not influenced by increase duration of disease.

Table 1. Baseline characteristics of studed subjects

Parameters	Control Group I	Newly DM (without therapy) Mean±Sd		P Value
		Group II (<1 year)	Group III (>1 year)	
BMI Kg/m²	26.7±2.87	30±4.6a	28.7±2.8	a0.041
WHtR	0.52±0.04	0.59 ± 0.058a	0.58±0.037	a0.015
FSG(mg/dl)	89.1±3.82	173.35±75.78a	197.20±28.83b,c	a0.0001 b0.0001 c 0.282
HbA1c%	5.26±0.44	9.34±2.15a	8.42±1.50b,c	a0.0001 b0.0001 c 0.17
Insulin(μU/ml)	13.67±8.11	12.32±4.18	19.24±17.4	>0.05 ns
HOMA-IR	2.98±1.8	5.08±2.42a	9.8±8.67 b,c	a0.04 b0.001 c0.003
TG(mg/dl)	114.15±20.7	160.82±126a	129.4±58.3	a 0.05
TC(mg/dl)	164.7±20.46	179.94±43.8	198.2±58.2	>0.05 ns
LDL-C(mg/dl)	96.4±15.69	110.15±32.9	128.4±53.4	>0.05 ns
HDL-C(mg/dl)	45.4±12.8	37±9.5a	44±8.4	a 0.047
VLDL-C (mg/dl)	22.7±4	32.1±25a	25.8±11.7	a0.047
Atherogenic index	0.4±0.16	0.57±0.24a	0.42±0.29	a0.033

P-value was calculated between groups and presented by the symbols below:

Group I (control) and group II (Newly diagnosed <1 year) represented by symbol (a)

Group III (Newly diagnosed >1 year) represented by symbol(b)

Group II and group III represented by symbol (c).

Table 2. Effect of duration of disease on levels of FBXW7, Fetuin A, pentraxin3 and nitricoxide for newly diagnosed DM group

Parameters	Control Group I	Newly DM (without therapy) Mean±SD		P value
		Group II (<1 year)	Group III (>1 year)	
FBXW7	1.12±0.9	2.56±1.18a	2.65±0.82b	a0.0001 b0.005
FetuinA	178.9±85.63	210±153	283.5±95.6	>0.05 ns
Pentraxin3	574.5±263.9	718.7±184	746.7±85.8	>0.05 ns
Nitricoxide	7.6±2.6	13.3±4.97	18.3±16.7	>0.05 ns

*The mean difference is significant at the 0.05 level.

p-value was calculated between groups and presented by the symbols below:

Group I (control) and group II (Newly diagnosed <1 year) represented by symbol (a)

Group III (Newly diagnosed >1 year) represented by symbol (b)

Group II and group III represented by symbol (c).

Effect of duration of metformin therapy on diabetic-related parameters: Metformin is an antidiabetic medication that decreases peripheral resistance to insulin, increase peripheral uptake of glucose and reduces liver gluconeogenesis[23,24]. The results in the present study investigated the effects of duration of metformin therapy on fbxw7 and other diabetic- related parameters. The result in table 3 revealed that insulin and IR significantly influenced by duration of therapy and their levels decrease significantly by increase the duration of therapy, while there was non-significant difference in BMI, WHtR, AI,glucose, HbA1c, and lipid profile between groups of patient with treatment < 1 year and >1 year. Such finding are consistent with Delman Najim et al[25], which revealed metformin as an antidiabetic agent to decrease blood insulin levels and insulin resistance.

Also table 4 showed that the levels of Fbxw7, fetuin A, pentraxin3, and NO not influenced by the duration of therapy and there were non-significant difference in these parameters between patient groups themselves.

Table 3. Effect of duration of metform in treatment on parameters.

Parameters	Control Group I	DM with metformin Mean±SD		P Value
		Group II (<1 year)	Group III (>1 year)	
BMI Kg/m²	26.7±2.87	34.3±6.09a	31.2±5.57b	a0.000b0.002
WHtR	0.52±0.04	0.63±0.07a	0.60±0.11b	a0.002b0.001
FSG(mg/dl)	89.1±3.82	136.5±16.8a	134.19±38.6b	a0.011 b0.001
HbA1c%	5.26±0.44	7.65±.85a	7.38±1.19b	a0.0001b0.0001
Insulin(μU/ml)	13.67±8.11	20.01±11a	10.18±5 c	a0.049 c0.002
HOMA-IR	2.98±1.8	6.82±4.13a	3.34±1.8c	a0.005c0.003
TG(mg/dl)	114.15±20.7	139.75±60.4	116.4±52.66	>0.05 ns
TC(mg/dl)	164.7±20.46	174.7±38.2	172.3±40.81	>0.05 ns
LDL-C(mg/dl)	96.4±15.69	103.53±33.5	106.6±39.20	>0.05 ns
HDL-C(mg/dl)	45.46±12.8	43.46±10.9	43.66±14.61	>0.05 ns
VLDL-C (mg/dl)	22.7±4	27.87±11.8	23.25±10.43	>0.05 ns
Atherogenic index	0.4±0.16	0.48±0.27	0.41±0.26	>0.05 ns

*The mean difference is significant at the 0.05 level.

p-value was calculated between groups and presented by the symbols below:

Group I (control) and group II (Newly diagnosed <1 year) represented by symbol (a)

Group III (Newly diagnosed >1 year) represented by symbol (b)

Group II and group III represented by symbol (c).

Table 4. Effect treatment duration (metformin) on levels of FBXW7, Fetuin A,pentraxin3 and nitricoxide for T2DM on metformin monotherpy group.

Parameters	Control Mean±SD	DM with metformin		P Value
		Group II (<1 year)	Group III (>1 year)	
FBXW7	1.12±0.9	0.77±0.61	1.07±1.18	>0.05 ns
FetuinA	178.9±85.6	170.5±65.8	166±97.60	>0.05 ns
Pentraxin3	574.5±263.9	595.3±188	602.7±224.3	>0.05 ns
Nitricoxide	7.6±2.6	10.8±6.49	27.1±32.2b	b0.002

*The mean difference is significant at the 0.05 level.

p-value was calculated between groups and presented by the symbols below:

Group I (control) and group II (Newly diagnosed <1 year) represented by symbol (a)

Group III (Newly diagnosed >1 year) represented by symbol (b)

Group II and group III represented by symbol (c).

Conclusion

Duration of disease and treatment appear effect on some parameters and not effected other parameters Fbxw7, fetuin A, pentraxin3 and nitric oxide are not affected by the long duration of therapy or by the duration of disease while glucose, HbA1c, and IR are affected by increase duration of disease only. Whereas insulin and IR are affected by both; duration of disease and therapy.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

1. International Diabetes Federation. IDF Diabetes Atlas, 6th ed. Brussels, Belgium: International Diabetes Federation;(2013). http://www.idf.org/diabetesatlas.
2. C.Mátyás, Pathophysiology and pharmacology of nitric oxide-cyclic guanosine monophosphate signaling in diabetic cardiomyopathy. Doctoral dissertation, Budapest: Semmelweis University;(2017).
3. M.Badedi, Y.Solan, H.Darraj, A.Sabai, M. Mahfouz, S.Alamodi, A.Alsabaani, Factors Associated with Long-Term Control of Type 2 Diabetes Mellitus. Journal of Diabetes Research(2016), http://dx.doi.org/10.1155/2016/2109542
4. M.Janić, S.Volčanšek, M.Lunder, A.Janež, Metformin: from mechanisms of action to advanced clinical use. ZdravniskiVestnik. (2017) 1;86
5. Y.O.Yaseen, J. J. Atyia, Facts about type 2 diabetes mellitus and its control in Misan governorate: singlecenter experience. The medical journal of Basrah university, (2018)36.
6. Q.Li, Y.Li, J.Li, Y.Ma, W.Dai, S.Mo, Y.Xu, X.Li, S.Cai, FBW7 suppresses metastasis of colorectal cancer by inhibiting HIF1α/CEACAM5 functional axis. . International Journal of Biological Science, (2018) 14(7): 726–735.doi: 10.7150/ijbs.24505
7. M.Takada, M.Zhuang, H.Inuzuka, J.Zhang, G.Zurlo, J.Zhang, EglN2 contributes to triple negative breast tumorigenesis by functioning as a substrate for the FBW7 tumor suppressor. Oncotarget, (2017)8:6787.doi: 10.18632/oncotarget.14290
8. J.ZhaoX., Xiong, Y.Li, X.Liu, T.Wang, H.Zhang, Hepatic F-box protein FBXW7 maintains glucose homeostasis through degradation of fetuin-A, Diabetes. (2018)67;818-30.doi: 10.2337/db17-1348. Epub 2018 Feb 23.
9. S.Sindhu, A.Nadeem, A.Rasheed, Fetuin a (AHSG) in metabolic and inflammatory diseases: a foe or a friend, Diabetes Obesity International Journal, (2016)1:1-5.
10. R.S. Komsa-Penkova, G.M. Golemanov, Z.V. Radionova, P.T. Tonchev, S.D. Iliev, V.V. Penkov, Fetuin-A–Alpha2-Heremans-Schmid Glycoprotein: From Structure to a Novel Marker of Chronic Diseases Part 1. Fetuin-A as a Calcium Chaperone and Inflammatory Marker. Journal of Biomedical and Clinical Research, (2017)10;90-7.
11. C.L. Udeh, Fetuin-A: A Biomarker for Type II Diabetes Mellitus. International Journal of Biomedical Materials Research, (2017)5; 72-77DOI: 10.11648/j.ijbmr.20170506.12
12. Q.Sun, M.C.Cornelis, J.EManson, F.B.Hu, Plasma levels of fetuin-A and hepatic enzymes and risk of type 2 diabetes in women in the U.S. Diabetes, (2013)62: 49–55.doi: 10.2337/db12-0372.
13. G.Lowe, M.Woodward, G.Hillis, A.Rumley, Q.Li, S.Harrap, M.Marre. Circulating inflammatory markers and the risk of vascular complications and mortality in people with type 2 diabetes and cardiovascular disease or risk factors: the ADVANCE study, Diabetes, (2014)163;1115-23. doi: 10.2337/db12-1625.
14. F.Bonacina, A.Baragetti, A.L.Catapano, G.D.Norata, Long pentraxin 3: experimental and clinical relevance in cardiovascular diseases. Mediators of inflammation. (2013)2013doi: 10.1155/2013/725102
15. Y.Takashi, M.Koga, Y.Matsuzawa, J. Saito, M.Omura, T. Nishikawa, Circulating pentraxin 3 is positively associated with chronic hyperglycemia but negatively associated with plasma aldosterone Concentration, PLoS ONE, (2018)13 https://doi.org/10.1371/journal. pone.0196526
16. A.Shindo, H.Tanemura, K.Yata, K.Hamada, M. Shibata, Y.Umeda, & W.Taki, Inflammatory biomarkers in atherosclerosis: pentraxin 3 can become a novel marker of plaque vulnerability, PloS one, (2014)9, e100045doi: 10.1371/journal.pone.0100045
17. N.N.Oudah, Purification and Characterization of Arginase and Measuring the Nitric Oxide Levels in Iraqi Diabetes Mellitus Type II Patients. Doctoral dissertation, Baghdad University, Baghdad, (2018).
18. O. Ozcelik, S. Algul, Nitric oxide levels in response to the patients with different stage of diabetes. Cell MolBiol, (2017)63; 49-52. doi:10.14715/cmb/2017.63.1.10

19. P.Tessari, D.Cecchet, A.Cosma, M.Vettore, A.Coracina, R.Millioni, Nitric oxide synthesis is reduced in subjects with type 2 diabetes and nephropathy. Diabetes, (2010)59;2152-9DOI: 10.2337/db09-1772.
20. K Maejima, S Nakano, M Himeno, S Tsuda, H Makiishi, T Ito, A Nakagawa, T Kigoshi, T Ishibashi, M Nishio, K Uchida, Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. Journal of Diabetes Complications, (2001)15;135-43doi: 10.1016/s1056-8727(01)00144-1.
21. J.S. Yun, S.H.Ko, Risk factors and adverse outcomes of severe hypoglycemia in type 2 diabetes mellitus. Diabetes MetabolismJournal, (2016)40; 423-432. https://dx.doi.org/10.4093%2Fdmj.2016.40.6.423
22. UK Prospective Diabetes Study (UKPDS) Group. Intensive bloodglucose control with sulphanylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes. Lancet, (1998)352; 837−853.https://doi.org/10.1016/S0140-6736(98)07019-6
23. M. P. van der, M. A. Elst, E. M. van de Garde, E. G . van Mil, C .A. J Knibbe, M .M. van der Vorst, Long-term treatment with metformin in obese, insulin-resistant adolescents: results of a randomized double-blinded placebo-controlled trial. Nutrition & diabetes, (2016)6; e228doi: 10.1038/nutd.2016.37..
24. Z.Polianskyte-Prause, T.A. Tolvanen, S.Lindfors, V. Dumont, M.Van, H. Wang, S. N. Dash, M. Berg, J.Naams, L.C.Hautala, H.Nisen, T.Mirtti, P.Groop, K.Wähälä, J.Tienari, and S.Lehtonen. Metformin increases glucose uptake and acts renoprotectively by reducing SHIP2 activity. The FASEBjournal, (2019)33; 2858-2869.
25. H.D. Najim, I.A. Majeed, A.M.Rahmah. Effects of Metformin &/or Glimepiride on Resistin Level and Related Biochemical Markers in Type 2 Diabetes Mellitus. Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS),(2014)14;78-88.