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Evaluation of Some Proteins as Potential Markers in Different Durations of Diabetic Patients Type 2

Sarah Sameer Sami[1,2*] and Ekhlass M. Taha[2]
[1]Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq.
[2]Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

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Abstract

Type 2 diabetic mellitus (T2DM) is growing rapidly around the world, due in large part to significant changes in the modern human lifestyle. T2DM and its complications are affected by a complex interplay of genetic, life course, ecological, cultural, socioeconomic, cognitive-psychological-behavioral, and access to care factors that influence regional variations in disease burden and care quality by gender. This study aims to investigate the role of laminin subunit alpha 2 (LAMA2), mixed lineage leukemia 4 (MLL4), plexin domain containing 2 (PLXDC2), and Protein Z (PORZ) as potential biomarkers for T2DM at different durations of the disease. These biomarkers were assessed using an enzyme-linked immunosorbent assay. While biochemical parameters (FSG) and lipid profiles were measured spectrophotometrically. HbA1C using HPLC. The volunteer individuals were subdivided into five groups: group I: DM with duration of disease less than 1 year, group II: DM with duration of disease from 1-5 years, group III: DM with duration of disease from 5-10 years, group IV: DM with duration of disease more than 10 years, group V: healthy control. Results showed a significant difference among the studied groups in LAMA2 and PLXDC2. In contrast, no significant difference was observed in MLL4 and POR Z. It has been concluded that LAMA2 and PLXDC2 are involved in the duration of the disease.

Keywords: Laminin subunit alpha 2, Mixed lineage leukemia 4, Plexin domain containing 2, Protein Z, Type 2 diabetes.

1. Introduction

Type 2 diabetic mellitus (T2DM) is growing rapidly around the world, due in large part to significant changes in the modern human lifestyle (1). Diabetes mellitus is a complicated long-term condition linked to elevated blood glucose levels, known as hyperglycemia, resulting from insufficient insulin production, function, or both. To enhance the management of DM and ensure adequate care for adults with the condition, it is crucial to gain a better comprehension of how age at diagnosis and duration of diabetes relate to diabetes-related outcomes. Individuals who are diagnosed with diabetes at different ages exhibit distinct characteristics both initially and as time progresses. A person diagnosed with diabetes at a specific age may differ significantly from another individual diagnosed at a different age in terms of their baseline attributes and how their condition evolves (2-4). Numerous studies

have demonstrated the impact of lifestyle modifications, such as regular physical activity and healthy eating habits, in preventing or delaying the onset of T2MD. The Diabetes Prevention Program, a landmark clinical trial, showed that lifestyle interventions, including a modest weight loss and increased physical activity, can reduce the risk of developing T2DM by up to 58% in high-risk individuals (5-7). Out of the various conditions referred to as "diseases of civilization," DM currently ranks high in terms of the rapid increase in rates of illness, disability, and death (8). Globally, there are currently more than 460 million individuals living with diabetes. However, according to the projections of the International Diabetes Federation, that number is expected to increase to 642 million by the year 2040 (9,10). Many factors contribute to the development of T2DM (11). Moreover, to identify genetic and protein markers for future T2DM, significant efforts have been made. Therefore, protein markers are the most common and have been successful in diagnosing diabetes, though not specifically at the beginning of the disease or in the early stages of its progression (12). It has been found that the laminin subunit alpha 2 (LAMA2) mutation has been associated with merosin-deficient congenital muscular dystrophy (13). According to studies, mixed lineage leukemia 4 (MLL4) collaborates with transcription factors to control the function of islet β-cells (14). As the nervous system develops, the regulation of differentiation and proliferation is attributed to the known role of plexin domain containing 2 (PLXDC2). The protein Z-dependent protease inhibitor (POR Z), a powerful down-regulator of coagulation factor Xa, has recently been demonstrated to require POR Z as a crucial cofactor. Consequently, it is suggested that protein Z deficiency causes a prothrombotic condition (15). This study aims to investigate the role of LAMA2, MLL4, PLXDC2, and PORZ as potential biomarkers for T2DM at different durations of the disease.

2. Materials and Methods

2.1. Study groups

The current study was included 100 patients with T2DM that divided to five subgroup GI: DM with duration of disease less than 1 year, GII: DM with duration of disease from 1-5 years, GIII: DM with duration of disease from 5-10 years, GIV: DM with duration of disease more than 10 years, GV: healthy control with age ranging from 30-65 years old. The study was conducted at the National Diabetes Center at Mustansiriyah University in Baghdad, Iraq, from January to March 2022, and involved the recruitment of volunteers.

2.2. Exclusion criteria

The diabetic patients included in the study did not have any preexisting conditions such as heart disease, liver disease, kidney disease, or hypertension. Additionally, individuals who were smokers or had other complications related to diabetes were not included in the study. Healthy individuals, without diabetes, hypertension, or any other acute illnesses, were chosen as the control group. Additionally, they had no previous history of smoking or drinking alcohol.

2.3. Blood sampling

After a 10 to 12-hour overnight fast, each patient and control had their blood drawn. The blood sample was separated into a gel tube to separate the serum, then the serum was used to measure the levels of LAMA2, MLL4, PLXDC2, and PROZ. The HLC-723GX automated glycohemoglobin analyzer from Tosoh was used to measure glycated hemoglobin (HbA1c). Bio System Spain supplied kits for the enzymatic colorimetric measurement of serum fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL). LAMA2 and PLXDC2 quantities were assessed using sandwich enzyme

immunoassay methods with a CUSABIO kit from the USA. Serum MLL4 levels were determined through competitive enzyme immunoassay techniques using a kit from Biosource, USA, while PROZ was supplied from the UK, Houston.

2.4. Statistical analysis

The analysis of the outcomes was conducted using the statistical analysis program SPSS 25. The main findings were presented using descriptive statistics, and group comparisons were performed using the One-Way ANOVA test (16).

3. Results

Some biochemical characteristics were measured in the study population; the results are illustrated in **Table 1**. Statistically significant differences appeared between the study groups, depending on the duration of the disease, for all included parameters: age and BMI.

Also, the results showed significant differences between groups in **Table 2** for FSG and HbA1c. Moreover, TC, TG, LDL, VLDL, and AIP were significantly elevated in all DM patient groups compared to the control, as shown in **Table 3**.

Also, all the parameters showed significant increments between groups of DMS in different durations.

In **Table 4**, the results revealed a considerable effect in four groups compared to the control in LAMA2, MLL4, PLXDC2, and PROZ levels

Table 1. Distribution of mean age and BMI for all studied groups.

Parameter	Mean±SD				
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Age (Years)	47.0 ± 9.0^{a}	51.0 ± 6.0^{b}	51.0 ± 7.0^{c}	53.0 ± 6.0^{d}	42.0 ± 9.0
		p- value = 0.0001			
BMI (Kg/m^2)	30.0 ± 7.0^{a}	33.40 ± 5.0^{b}	29.40 ± 4.0^{c}	29.90 ± 5.90^{d}	25.90 ± 3.0
		p- value = 0.0001			

*Significantly using ONEWAY-ANOVA and at the 0.05 level, a) Indicate significant difference between G5 and G1, b) Indicate significant difference between G5 and G2, c) Indicate significant difference between G5 and G3, d) Indicate significant difference between G5 and G4.

Table 2. Distribution of mean HbA1c and FSG for all studied groups.

Parameter	Mean±SD				
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
HbA1c (%)	9.20 ± 2.40^{a}	8.90 ± 2.0^{b}	9.40 ± 2.40^{c}	8.40 ± 1.80^{d}	5.20 ± 0.30
			p-value = 0.0001		
FSG (mg/dL)	203.0 ± 10.40^{a}	207.60 ± 8.90^{b}	218.20 ± 7.29^{c}	188.80 ± 5.44^{d}	88.10 ± 6.30
			p-value = 0.0001		

*Significantly using ONEWAY-ANOVA and at the 0.05 level, a) Indicate significant difference between G5 and G1, b) Indicate significant difference between G5 and G2, c) Indicate significant difference between G5 and G3, d) Indicate significant difference between G5 and G4.

Table 3. Distribution of mean lipid profile for all studied groups.

Parameter	Mean±SD				
	Group 1	Group 2	Group 3	Group 4	Group 5
Cholesterol (mg/dL)	198.0 ± 4.70[a]	189.0 ± 5.70[b]	213.0 ± 6.30[c]	207.0 ± 4.60[d]	124.0 ± 2.30
			p-value = 0.001		
TG (mg/dL)	157.0 ± 6.90[a]	176.50 ± 7.90[b]	167.6±57[c]	165.30 ± 6.70[d]	95.60 ± 2.30
			p-value = 0.001		
LDL (mg/dL)	111.70 ± 5.30[a]	116.20 ± 5.70[b]	134.8± 67[c]	132.60 ± 4.0[d]	50.20 ± 1.90
			p-value = 0.001		
HDL (mg/dL)	49.60 ± 16.0[a]	41.80 ± 16.0[b]	53.3± 31[c]	39.30 ± 9.0[d]	51.90 ± 1.0
			p-value = 0.05		
VLDL (mg/dL)	32.0 ± 14.0[a]	36.10 ± 16.0[b]	33.40 ± 11.0[c]	33.0 ± 13.0[d]	19.90 ± 4.70
			p-value = 0.001		
AIP	0.49 ± 0.20[a]	0.60 ± 0.20[b]	0.52 ± 0.20[c]	0.59 ± 0.22[d]	0.28 ± 0.04
			p-value = 0.012		

*Significantly using ONEWAY-ANOVA and at the 0.05 level, a) Indicate significant difference between G5 and G1, b) Indicate significant difference between G5 and G2, c) Indicate significant difference between G5 and G3, d) Indicate significant difference between G5 and G4.

Table 4. Distribution of mean LAMA, MLL4, PLXDC2, and PROZ for all studied groups.

Parameter	Mean±SD				
	Group1	Group2	Group 3	Group 4	Group 5
LAMA4 (ng/mL)	9.70 ± 4.10[a]	13.10 ± 4.80 [b]	11.20 ± 4.70[c]	13.60 ± 5.30[d]	4.50 ± 0.80
			p-value = 0.001		
MLL4 (ng/mL)	2.40 ± 1.20[a]	2.20 ± 0.70[b]	2.20 ± 1.10[c]	2.40 ± 0.90[d]	1.60 ± 0.40
			p-value = 0.21		
PLXDC2 (pg /mL)	1083.0 ± 31.60[a]	1256.0 ± 38.60[b]	1345.0 ± 30.60[c]	1182.0 ± 31.70[d]	644.0 ± 21.10
			p-value = 0.001		
PROZ (ng/mL)	21.90 ± 6.0[a]	23.8 ± 5.60[b]	21.70 ± 5.60[c]	23.40 ± 5.0[d]	62.8 ± 5.20
			p-value = 0.12		

*Significant using ONEWAY-ANOVA and at 0.05 level, a) Indicate significant difference between G5 and G1, b) Indicate significant difference between G5 and G2, c) Indicate significant difference between G5 and G3, d) Indicate significant difference between G5 and G4.

4. Discussion

The duration of T2DM can significantly impact various factors, such as FSG, HbA1c, and lipid profile. Studies have shown that as the duration of T2DM increases, there is a gradual deterioration in glycemic control, as evidenced by higher levels of FSG and HbA1c. A study (17-20) observed that individuals with longer DM durations had elevated HbA1c levels, indicating poorer long-term glucose management (21, 22). This finding aligns with the results presented in **Table 2**. Moreover, as shown in **Table 3**, statistically significant differences were found between the study groups, depending on the duration of the disease, as well as between healthy controls and the other groups. Furthermore, an extended duration of T2DM has been associated with dyslipidemia, characterized by increased levels of TC, LDL, and TG, alongside decreased HDL cholesterol levels; TG levels increased, and glucose metabolism was impaired. Even if the effects of weight gain are kept under control, abnormalities in glucose metabolism, along with those of glucose and HbA1c, persist (23-26). Additionally, while the rise in LDL-C levels vanished, the increase in serum TG levels in diabetic patients persisted. Even if there wasn't much physical activity throughout the epidemic, diabetic patients may find it difficult to concentrate because of their impaired

perception. Additionally, T2DM patients' cognitive function reportedly suffered due to physical inactivity (27).

There is no known correlation or direct association between the LAMA2, MLL4, PLXDC2, and PROZ genes and T2DM. In contrast, T2DM is a multifactorial metabolic condition influenced by a combination of genetic, environmental, and lifestyle factors. While several genes have been identified as playing a role in the development and progression of T2DM, they are primarily involved in insulin secretion, insulin sensitivity, and glucose metabolism (28). They have concerns about T2DM or any genetic conditions; it is recommended to consult with healthcare professionals, such as physicians or genetic counselors. They can provide personalized guidance and information based on the specific situation, including appropriate screening, prevention strategies, and management approaches, as noted in previous outcomes (29).

5. Conclusion

The duration of the disease appears to affect some parameters but not others. MML4 and PROZ are not affected by the duration of the disease, while LAMA4 and PLXDC2 are affected by prolonged disease duration. Whereas FBG, HbA1c, and lipid profile are affected by the duration of the disease.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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None.

Ethical Clearance

The Scientific Committee approved this study in the College of Science for Women, and this is consistent with the instructions of the Iraqi Ministry of Health and Environment. A verbal agreement was obtained from each person included in the survey No. 22/6435 on 13/12/2021.

References

1. Chen X, Merovci A, DeFronzo RA, Tripathy D. Chronic physiologic hyperglycemia impairs insulin-mediated suppression of plasma glucagon concentration in healthy humans. Metabolism. 2023; 142: 155512. https://doi.org/10.1016/j.metabol.2023.155512.
2. Patel MK, Ramachandran A, Babulal MK, Vasanthi RK. Will hyperglycemia influence the cardiorespiratory endurance and other determinants among community-dwelling type 2 Dm patients? A cross-sectional study. JPSP 2022; 6(4):10403-10408.
3. Gerstein HC, Santaguida P, Raina P, Morrison KM, Balion C, Hunt D, Yazdi H, Booker L. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. Diabetes Res Clin Pract. 2007; 78(3):305-312. https://doi.org/10.1016/j.diabres.2007.05.004.

4. King P, Peacock I, Donnelly R. The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. Br J Clin Pharmacol. 1999; 48(5):643-648. https://doi.org/10.1046/j.1365-2125.1999.00092.x.
5. Bellary S, Kyrou I, Brown JE, Bailey CJ. Type 2 diabetes mellitus in older adults: Clinical considerations and management. Nat Rev Endocrinol. 2021; 17(9):534-548. https://doi.org/10.1038/s41574-021-00474-4.
6. Ergashev UY, Ernazarov KI, Zohirov AR, Alzabni ID. Complex treatment of experimental model of diabetic foot syndrome. Am J Med Sci. 2022; 12(4):471-480. https://doi.org/10.5923/j.ajmms.20221204.02.
7. American Diabetes Association. Standards of Medical Care in Diabetes. Diabetes Care. 2021; 44(S1): S1-S232. https://doi.org/10.2337/dc21-S001.
8. Rafiqovich ZA. Study of the effect of lipid peroxidase analysis on the body in diabetic foot syndrome. Conferencea. 2023; 76-82. https://conferencea.org/index.php/conferences/article/view/1990.
9. Ergashev UY, Zokhirov AR, Minavarkhujaev RR. Study and treatment of changes in biochemical processes in complications of diabetes mellitus. 2023.
10. Ogurtsova K, Guariguata L, Barengo NC, Ruiz PLD, Sacre JW. IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. Diabetes Res Clin Pract. 2022; 183:109118. https://doi.org/10.1016/j.diabres.2022.109118.
11. Broze GJ, Miletich JP. Human protein Z. J Clin Invest. 1984; 73(3):933-938. https://doi.org/10.1172/JCI111987.
12. Kaiafa G, Veneti S, Polychronopoulos G, Pilalas D, Daios S, Kanellos I. Is HbA1c an ideal biomarker of well-controlled Diabetes? Postgrad Med J. 2021; 97(1148):380-383. https://doi.org/10.1136/postgradmedj-2020-137861.
13. Takahashi E, Okumura A, Unoki-Kubota H, Hirano H, Kasuga M. Differential proteome analysis of serum proteins associated with the development of type 2 diabetes mellitus in the KK-A(y) mouse model using the iTRAQ technique. J Proteomics. 2013; 84:40–51. https://doi.org/10.1016/j.jprot.2013.03.014.
14. Scoville DW, Cyphert HA, Liao L, Xu J, Reynolds A, Guo S. MLL3 and MLL4 methyltransferases bind to the MAFA and MAFB transcription factors to regulate islet beta-cell function. Diabetes. 2015; 64(10):3772–3783. https://doi.org/10.2337/db15-0281.
15. Miller-Delaney SF, Lieberam I, Murphy P, Mitchell KJ. Plxdc2 is a mitogen for neural progenitors. PLOS One. 2011; 6(1):e14565. https://doi.org/10.1371/journal.pone.0014565.
16. Daniel WW. Biostatistics: A foundation for analysis in the health sciences. Wiley; 2018, 11th ed.
17. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982; 28(10):2077-2080. PMID: 6812986.
18. Zoungas S, Chalmers J, Neal B, Billot L, Li Q, Hirakawa Y, Arima H, Monaghan H, Joshi R, Colagiuri S, Cooper ME, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Lisheng L, Mancia G, Marre M, Matthews DR, Mogensen CE, Perkovic V, Poulter N, Rodgers A, Williams B, MacMahon S, Patel A, Woodward M; ADVANCE-ON Collaborative Group. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. N Engl J Med. 2014; 371(15):1392-1406. https://doi.org/10.1056/NEJMoa1407963.
19. Alicic RZ, Neumiller JJ. Incretin therapies for patients with type 2 diabetes and chronic kidney disease. J Clin Med. 2023; 13(1):201. https://doi.org/10.3390/jcm13010201.
20. Guo F, Moellering DR, Garvey WT. Use of HbA1c for diagnoses of diabetes and prediabetes: comparison with diagnoses based on fasting and 2-hr glucose values and effects of gender, race, and age. Metab Syndr Relat Disord. 2014; 12(5):258-268. https://doi.org/10.1089/met.2013.0128.
21. Palladino R, Tabak AG, Khunti K, Valabhji J, Majeed A, Millett C, Vamos EP. Association between pre-diabetes and microvascular and macrovascular disease in newly diagnosed type 2 diabetes. BMJ Open Diabetes Res Care. 2020; 8(1):e001061. https://doi.org/10.1136/bmjdrc-2020-001061.
22. Hopp A, Jones K, Meng H, Ott E, Basuni N, Axon SP. 184 Defining the pathological natural

history of LAMA2 muscular dystrophy. Neuromuscul Dis. 2022; 32(1):S121-S121. https://doi.org/10.1016/j.nmd.2022.07.327.

23. Sadiq CH, Hussein RH, Maulood IM. Ghrelin and leptin and their relations with insulin resistance in diabetes mellitus type 2 patients. Baghdad Sci J. 2022; 19(1):33-42. https://doi.org/10.21123/bsj.2022.19.1.0033.
24. Espeland MA, Lipska K, Miller ME, Rushing J, Cohen RA, Verghese J, McDermott MM, King AC, Strotmeyer ES, Blair SN, Pahor M, Reid K, Demons J, Kritchevsky SB; LIFE Study Investigators. Effects of physical activity intervention on physical and cognitive function in sedentary adults with and without diabetes. J Gerontol A Biol Sci Med Sci. 2017; 72(6):861-866. https://doi.org/10.1093/gerona/glw179.
25. Triggle CR, Mohammed I, Bshesh K, Marei I, Ye K, Ding H, MacDonald R, Hollenberg MD. Metformin: Is it a drug for all reasons and diseases? Metabolism. 2022; 133:155223. https://doi.org/10.1016/j.metabol.2022.155223.
26. Mohammed SK, Taha EM, Muhi SA. A case-control study to determination FBXW7 and fetuin-A levels in patients with type 2 diabetes in Iraq. J Diabetes Metab Disord. 2021; 20(1):237-243. https://doi.org/10.1007/s40200-021-00738-x.
27. American Diabetes Association. 5. Prevention or delay of type 2 diabetes: standards of medical care in diabetes-2018. Diabetes Care. 2018; 41(Suppl 1):S51-S54. https://doi.org/10.2337/dc18-S005.
28. Bouman K, Gubbels M, van den Heuvel FMA, Groothuis JT, Erasmus CE, Nijveldt R, Udink Ten Cate FEA, Voermans NC. Cardiac involvement in two rare neuromuscular diseases: LAMA2-related muscular dystrophy and SELENON-related myopathy. Neuromuscul Disord. 2022; 32(8):635-642. https:// doi.org/ 10.1016/j.nmd.2022.06.004.
29. Santacroce R, Sarno M, Cappucci F, Sessa F, Colaizzo D, Brancaccio V, Grandone E, Margaglione M. Low protein Z levels and risk of occurrence of deep vein thrombosis. J Thromb Haemost. 2006; 4(11):2417-2422. https://doi.org/10.1111/j.1538-7836.2006.02186.x.