Evaluation of Some Immunological Aspects and Lipid Profile Among Type I and Type II Diabetes Mellitus in Iraqi Patients of Basrah Province

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Abstract
Diabetes mellitus is a long-standing metabolic disorder due to insufficient insulin secretion or no response for insulin result in increased blood glucose level leading to multiple signs and symptoms. One hundred sixty nine participants were enrolled in this study from May 2022 to August 2022. The sample had been taken from patients attended diabetic unit in Basrah city, Iraq. Serum samples were tested to investigate and evaluate the level of lipid profile and anti-GAD 65 by ELISA technique. The levels of all biochemical markers that were found statistically significant differences when compared between type 1 and its control, except HDL. While, the only biochemical markers levels of FBS and HbA1c that were found statistically significant differences when compared between type 2 and its control. Anti-GAD 65 was found to be elevated (11.64±3.59), (12.28±4.42) in patients with T1DM and T2DM respectively when compared with controls group for each patient groups with highly significant differences, the aim of the present study is to compare the role of anti-GAD 65 and the level of lipid profile between patients and control groups.
Keywords: Diabetes Mellitus, lipid profile, Anti-Gad-65, Auto-immune diseases.

1. Introduction

Diabetes mellitus is a long-term metabolic disorder caused by insufficient insulin secretion or no insulin response, resulting in a high blood glucose level (1,2). From etiological aspect Diabetes mellitus divide into four classes, class 1diabetes mellitus (T1DM), class 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM) and other class of diabetes. Diabetic classes 1 and 2 are the most common (3,4).
T1DM develops as a result of pancreatic β-cell destruction. However, is usually mediated by an autoimmune reaction (5,6). Reasons for development of T1DM are still unclear but may be due to changes in environmental risk factors or viral infections (7). The onset of T1DM primarily occurs suddenly during childhood or young adulthood. T2DM is caused by insulin insensitivity or insulin resistance combined with a failure of insulin secretion to overcome this by hypersecretion. The term gestational diabetes mellitus refers to early pregnancy glucose intolerance (8). Insulin deficiency causes excessive metabolism of free fatty acids, this may lead to a disorder in lipid metabolism (9). A low level of serum HDL-C is a key feature of type 2 diabetes. Diabetes being one of the strongest risk factors with associated age adjusted risk ratios of about 2.2 for men and about 3.7 for women (10). An increase in body fat leads to less action of insulin as well increases several other toxic substances in the body.
There are 366 million diabetics worldwide, half of whom are unaware that they have the disease. Six of the ten countries with the highest diabetes prevalence are in the Middle East (Kuwait, Lebanon, Qatar, Saudi Arabia, Bahrain, and the United Arab Emirates). Nearly 20.5 million people have diabetes in the 20 Arab countries with data available, and another 13.7 million live with impaired glucose tolerance, called prediabetes (11).
An immune response to type 1 diabetes causes the release of pancreatic islet autoantibodies (12). Among type 2 diabetes (T2D) patients, 10 percent have antibodies against glutamic acid decarboxylase 65 (GADA), which become insulin-dependent antibodies as insulin levels rise (13). A glutamic acid decarboxylase autoantibody was first identified in patients suffering from stiff man syndrome, a rare neurological condition with neither beta cell nor islet specific autoantibodies (14). The glutamic acid in neurons and beta cells is converted to g-aminobutyric acid (GABA) by the enzyme glutamic acid decarboxylase (15). A measurement of which can be performed by a variety of methods to evaluate human disease (16). In this study we compare result of anti- GAD 65 detected by ELISA between patients and control.

2. Methods

This study was conducted from May 2022 to August 2022on a total number of 169 subjects including 98 diabetes mellitus patients and 71 control group. All samples were collected from Basrah city, Iraq.
A blood sample of 5 mL was taken from patients and a control group, placed in Gel tubes, left for 30 minutes at room temperature, centrifuged for 5 minutes at 3000 rpm to obtain serum, which was utilized for calculating the lipid profile and glucose levels, and portions were kept in the freezer for detection the levels of (GAD65-Ab) by ELISA test.

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Estimation of anti- Gad

The concentrations of Anti- Gad were determined using enzyme-linked immune sorbent assay (ELISA) kit based on biotin double antibody sandwich technology, ELISA kit (China/SunLogbiotechSL2489Hu) according to the manufacturer's instructions.

Statistical Analysis

Analysis of data was carried out using the available statistical package of SPSS-26(Statistical Packages for Social Sciences-version 26). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of the difference of different means (quantitative data) was tested using Students t-test for difference between two independent means or Paired t-test for difference of paired observations (or two dependent means). Mann-Whitney U Test was applied to test for the statistical significance of differences in non-parametric quantitative data. Statistical significance was considered whenever the p- value was less than 0.05.

3. Results

Table (1) shows One hundred sixty-nine subjects participated in this study: ninety eight subjects were diabetic patients which is divided in two group forty nine with type 1 diabetic mellitus (23 males and 26 females) compare with thirty one healthy control subject (18 males and 13 females) and forty nine patients with type 2 diabetic mellitus (21 males and 28 females) compare with forty healthy control subject (22 males and 18 females) Age ranges for patients and controls of T1DM were 5-30, 5-28 years respectively while in patients and control of T2DM 30-74.

Table (1): Sex and age distribution of the study population according to study groups

Variable		Type 1 DM No. (%)	Control 1 No. (%)	Type 2 DM No. (%)	Control 2 No. (%)
Total		49	31	49	40
Sex	Male	23 (46.9)	18 (58.1)	21 (42.9)	22 (55)
	Female	26 (53.1)	13 (41.9)	28 (57.1)	18 (45)
Age (Year)	Min.-Max.	5-30	5-28	30-74	30-74

It is clear from Table (2) that the presence of positive family history of diabetes was statistically significant associated with type 2 diabetes, wherever no significant differences with type 1.

Table (2): The existence of positive family history of diabetes in both types of diabetes compared with their controls

		Type of diabetes		Total	P-value
		Type 1	Control 1		
Family history of diabetes	Yes	12	9	21	0.653*
		24.5%	29.0%	26.3%	
	No	37	22	59	
		75.5%	71.0%	73.8%	
Total		49	31	80	
		100.0%	100.0%	100.0%	
		Type 2	Control 2		
Family history of diabetes	Yes	30	8	38	0.0001*
		61.2%	20.0%	42.7%	
	No	19	32	51	
		38.8%	80.0%	57.3%	
Total		49	40	89	
		100.0%	100.0%	100.0	

Biochemical analysis and comparison between the studied groups are shown in Table 3. The levels of all biochemical markers that were found statistically significant differences when compared between type 1 and its control, except HDL. While, the only biochemical markers levels that were found statistically significant differences when compared between type 2 and its control, were the FBS and HbA1c.

Table (3): Comparison of fasting blood sugar, lipid profile indices, and HbA1c levels between types of diabetes mellitus with their controls

Variable	DM Type 1 (N= 49)		Control 1 (N= 31)		P-value*
	Mean ± SD	Median (Min.-Max.)	Mean ± SD	Median (Min.-Max.)	
FBS	263.71±114.32	220 (90-561)	92.90±8.32	92 (78-110)	0.0001
Chol.	155.20±37.77	153 (80-250)	120.32±40.39	112 (70- 216)	0.0001
TG	137.98±51.32	123 (71-270)	105.84±38.06	90 (55-233)	0.005
HDL	44.37±13.37	40 (27-95)	42.84±8.05	40 (27-60)	0.956
LDL	90.76±34.75	95 (18-188)	56.64±38.25	52.2 (12.8-143.6)	0.0001
VLDL	27.36±9.74	24 (14-48.8)	21.07±7.39	18 (11-44.6)	0.003
HbA1c	11.12±2.16	11 (7.2-16.8)	5±0.43	5 (4.2-5.7)	0.0001
Variable	DM Type 2 (N= 49)		Control 2 (N= 40)		P-value*
	Mean ± SD	Median (Min.-Max.)	Mean ± SD	Median (Min.-Max.)	
FBS	265.86±114.68	261 (103-568)	103.85±15.38	102.5 (78-135)	0.0001
Chol.	178.84±48.33	178 (84-293)	166.85±36.88	173 (90-237)	0.337
TG	191.43±93.05	183 (59-473)	164.67±71.74	152.5 (71-480)	0.180
HDL	39.55±10.52	38 (17-67)	37.47±5.93	37 (27-58)	0.272
LDL	107.72±39.26	106 (23-188)	96.08±31.26	101.3 (19-153.8)	0.306
VLDL	38.28±18.63	36.6 (12-95)	32.48±14.57	30.4 (14.2-96)	0.126
HbA1c	10.21±2.41	9.9 (6.6-16.8)	5.73±0.68	5.9 (2.2-6.3)	0.0001

Anti-GAD 65 was found to be elevated (11.64±3.59), (12.28±4.42) in patients with T1 DM and T2DM respectively when compared with controls group for each patient groups with highly significant differences as seen in table (4) and figure (1)

Table (4): The levels of serum anti-Gad in patients and healthy groups.

Anti-Gad 65	DM I	DM I Control	DM II	DM II Control
Mean±SD	11.64±3.59	0.34±0.32	12.28 ± 4.42	0.32 ± 0.29
Median (Min.-Max.)	11.87 (6.32-22.87)	0.23 (0.01-1.09)	11.89 (3.98-23.98)	0.26 (0.02-0.99)
P-value*	0.0001		0.0001	

* Mann-Whitney U Test

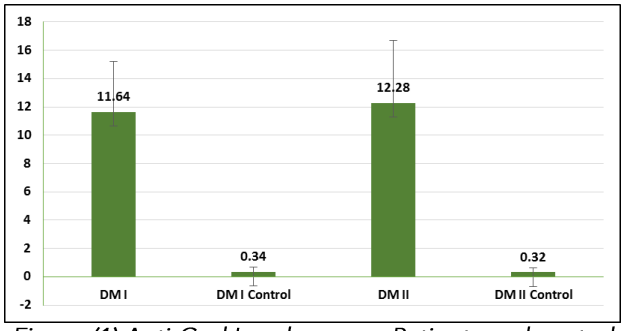

Figure (1) Anti-Gad Levels among Patients and control groups.

4. Discussion

Among the major demographic and socioeconomic factors influencing diabetes mellitus, gender and age were highlighted in this study, more women participated than males, this is in agreement with UNRWA's estimate of 62% participation among women in 2007 (17). In this respect Azimi-Nazhad (2008) that show type 2 diabetes is slightly more prevalent in females than in males (5.8% vs. 5.1%) (18). This study attempt to assess the association between serum lipids (including Chol, TG, LDL, HDL and VLDL) and degrees of glucose intolerance. From the data obtained, the means and standard deviation of all the lipid parameters except HDL showed significant difference between patients with T1DM and its controls, this was in agreement with Gordon et. al (19).

Significant linear trends for glucose tolerance status were observed for high Chol, high Tg and low HDL. In addition, high levels of TG in combination with low levels of HDL showed the highest association with T2DM and prediabetes that seen in table 3.

In diabetes many factors may affect blood lipid levels, this is because carbohydrates and lipid metabolism are interrelated to each other if there is any disorder in carbohydrate metabolism it also leads disorder in lipid metabolism so there is high concentration of cholesterol and triglycerides and due to this there is reduction in HDL cholesterol levels (20).

A common marker of autoimmune diabetes is GADA, which is an antigen-specific autoantibody marker (21). The present findings of serum(Anti-GAD65) among patients with type 1 and type II Diabetes was elevated in concentration (11.64±3.59, 12.28 ± 4.42) than its control groups (0.34±0.32, 0.32 ± 0.29) respectively, with highly significant difference (p≤0.0001) among four groups, table (4). Interestingly, this result was consistent with Basu et al. (2020) who reported high levels of anti-GAD serum in 79.3% of diabetes patients with type I diabetes which it is caused by an autoimmune disorder that results in a loss of peripheral tolerance that leads to the loss of pancreatic beta cells (T1D), and a combination of mature CD4+ and CD8+ cytotoxic T cells destroys beta cells, with the assistance of innate immunity (23).

References

1. Jayakumar A. Greenspan' s Basic and Clinical Endocrinology. The Yale Journal of Biology and Medicine. 2012 Dec;85(4):559.
2. American Diabetes Association. Standards of medical care in diabetes--2014. Diabetes care. 2014 Jan;37: S14.
3. Abusaib M, Ahmed M, Nwayyir HA, Alidrisi HA, Al-Abbood M, Al-Bayati A, Al-Ibrahimi S, Al-Kharasani A, Al-Rubaye H, Mahwi T, Ashor A, Howlett H, Shakir M, Al-Naqshbandi M, Mansour A. Iraqi Experts Consensus on the Management of Type 2 Diabetes/Prediabetes in Adults. Clin Med Insights Endocrinol Diabetes. 2020 Aug 19; 13:1179551420942232.
4. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee (2013). Definition, diagnosis and classification of diabetes mellitus prediabetes and metabolic syndrome 2013; 37: S8- S11.
5. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014;383(9911):69-82.
6. Kerner W and Brückel J; German Diabetes Association. Definition, classification and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes. 2014;122(7):384-386.
7. International Diabetes Federation. (2015). Diabetes Atlas (7thed.). Retrieved from International Diabetes Federation website: http://www.diabetesatlas.org/.
8. American Diabetes Association. (2016b). Standards of Medical Care in Diabetes: Classification and diagnosis of diabetes. Diabetes Care,39(1), S13–22.
9. Godkar P and Godkar D . Textbook of medical laborarty technology.Ed.2 chemistry of carbohydrates (Bhalani publishing house) s. 2003; 176-233.
10. Rosenson, R.S.: HDL-C and the diabetic patient: target for therapeutic intervention. Diabetes Res. Clin.Pract., 2005; 68 Suppl 2: S36-S42.
11. Mansour AA, Al-Maliky AA, Kasem B, Jabar A, Mosbeh KA. Prevalence of diagnosed and undiagnosed diabetes mellitus in adults aged 19 years and older in Basrah, Iraq. Diabetes Metab Syndr Obes. 2014; 7:139-144.

12. George W. Burke, Gaetano Ciancio, Mahmoud Morsi, Jose Figueiro, Linda Chen, Francesco Vendrame, Alberto Pugliese Chapter 24 - Recurrence of type 1 diabetes following simultaneous pancreas-kidney transplantation,Editor(s): Giuseppe Orlando, Lorenzo Piemonti, Camillo Ricordi, Robert J. Stratta, Rainer W.G. Gruessner,Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas, Academic Press,2020: 295-311.
13. Thijail H.A. and Mousa H. M.. "Comparative Study Of Some Immunological Aspects Between Type I And Type II Diabetic Mellitus In Iraqi Patients Of Thi-Qar Province.". European Journal of Molecular & Clinical Medicine, 7, 1, 2020, 3537-3546.
14. De Aizpurua HJ, Wilson YM, Harrison LC. Glutamic acid decarboxylase autoantibodies in preclinical insulin-dependent diabetes. Proceedings of the National Academy of Sciences. 1992 Oct 15;89(20):9841-5.
15. Al-Abady HL, Mahdi NK, Al-Naama LM, Mahdi JK. The prevalence of autoantibodies among relatives for type 1 and 2 diabetic patients. J Pak Med Assoc. 2016;66(9):1064-1067.
16. Pihoker C, Gilliam LK, Hampe CS, Lernmark Å.Autoantibodies in diabetes. Diabetes. 2005 Dec 1;54 (suppl 2): S52-61.
17. UNRWA, (2007). "Annual Report of the Department of Health": Disease Prevention and Control. P. 88.
18. Azimi-Nezhad M, Ghayour-Mobarhan M, Parizadeh MR, Safarian M, Esmaeili H, Parizadeh SM, Khodaee G, Hosseini J, Abasalti Z and Ferns G (2008). Prevalence of type 2 diabetes mellitus in Iran and its relationship with gender, urbanisation, education, marital status and occupation. Singapore Medical Journal.49(7):571-576.
19. Gordon L, Ragoobirsingh E, Errol Y St A Morrison, McGrowder D, and Martorell E. Lipid profile of type 2 diabetic and hypertensive patients in the Jamaican population. Journal of Laboratory Physicians,2010; (2): 1 ; 25-30.
20. Sapna Smith,Alok M Lall. A Study on Lipid Profile Levels of Diabetics and Non-Diabetics Among Naini Region of Allahabad, India. Turk J Biochem 2008; 33 (4) ; 138–141.
21.Towns R. and Pietropaolo M. (2011). GAD65 autoantibodies and its role as biomarker of Type 1 diabetes and Latent Autoimmune Diabetes in Adults (LADA). Drugs Future. Nov; 36(11): 847.
22. Basu, M., Pandit, K., Banerjee, M., Mondal, S. A., Mukhopadhyay, P.,&Ghosh, S. (2020). Profile of autoantibodies (Disease related andother) in children with type 1 diabetes. Indian Journal ofEndocrinology an Metabolism, 24(3), 256d.
23. Christoffersson, G., Rodriguez-Calvo, T., & Herrath M. V. (2016). Recent advances in understanding Type 1 Diabetes. F1000Research, 5: 1-9 .