Molecular and Biochemical Diagnosis (MBD)
Vol 2, No 1, 2016
Original Article

Biochemical markers could predict type-1 diabetes mellitus

Ragaa H. M. Salama[1*], Yasser F. Abd-Elraheem[2], Khaled H. Mahmoud[2], Jalal A. Bilal[2], Almutlaq M. Abdullah[3], Aya A. A. Alsanory[4], Tasnim A. A. Alsanory[5]

1. Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt
2. Pediatric, College of Medicine, Qassim Universitym, KSA
3. Maternal Childhood Hospital, Qassim, KSA
4. Medical Students, Assiut University, Egypt
5. Pharmacy Students, Assiut University, Egypt

Abstract

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by selective destruction of pancreatic beta cells.

Methods: The study included 80 children, 20 of them have T1DM, 40 children were selected from first degree relatives to the same child and 20 healthy children serve as control. Body mass index (BMI) was calculated, random blood glucose and glycosylated hemoglobin A1c (GHbA1c) were measured. The following biochemical markers were measured in sera of all subjects by ELISA kits: Human insulin ,C-peptide, human islet cell antibody (ICA), insulin auto antibodies (IAA) and antiglutamic acid decarboxylase (anti-GAD) antibodies.

Results: This study showed that diabetic children had high level of ICA (65%), IAA (55%), anti-GAD antibodies (50%) and decrease in C-peptide (60%). Whereas the relatives showed high level of anti-GAD antibodies (30%), IAA(25%), ICA(2.5) and decrease in C-peptide (30%). Anti-GAD antibodies were significantly higher among the relatives of the diabetics compared to the healthy controls.

Conclusions: The strongest predictors of diabetes were C- peptide and islets cell antibodies, which had odd ratio 4.7 and 3.1, respectively. Autoantibodies could distinguish T1DM patients from healthy control subjects and they may also identify individuals at high risk during progression from pre-diabetes to overt disease.

Keywords: Human islet cell antibody (ICA), Insulin auto antibodies (IAA) , Antiglutamic acid decarboxylase (anti-GAD) antibodies

Introduction

Type 1 Diabetes (T1D) is the most severe type of diabetes with prolonged and variable latent period that culminates in the destruction of pancreatic β -cells and the development of hyperglycemia, leading to lifelong dependency on daily insulin injections. This autoimmune disorder develops as a consequence of a synergistic combination of genetic pre-disposition, largely unknown environmental triggers, and immunologic events (Lebastchi and Herold, 2012). T1DM is a serious health problem in Saudi Arabia. Disease prevalence among Saudi children and adolescents is on the

*Corresponding author. **Ragaa H. M. Salama, PhD**
Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt; P.O. Box: Assiut 71526
Tel. 00201063492008; Fax. 002088-2080278
Email: ragaa_2002@yahoo.com

rise, standing at 109.5 per 100,000 (AL-Herbish et al., 2008). The reported disease incidence in the Middle East ranges between 2.62 and 20.18 per 100,000 (Soltesz etal., 2007) which is comparable to that in North America (Bell etal., 2009). In general, the incidence of T1DM is increasing globally (Karvonen et al., 2000).

Auto-antibodies to islet cell antigens often present at the disease onset. Islet cell auto-antibody (ICA) is the first to be identified, followed by other auto-antibodies specific to tissue antigens. These include antibodies to glutamic acid decarboxylase (GAD), Protein tyrosine phosphatase like protein (IA2) antibodies, and insulin antibodies (IAA).The presence of ICA and or GAD antibodies at the time of diagnosis identifies 85% of T1DM (Borg et al., 1997). Therefore, the initial screening in T1DM prediction studies recommends testing for both auto-antibodies. Diabetes risk is related to the magnitude and maturity of these autoantibody responses (Siljander et al., 2009).

Understanding the biochemical processes that underlie T1DM and identifying diagnostic biomarkers predict the onset of the disease in the relatives and other members of child's family are very important issues that need to be investigated. This will help to detect more accurately individuals with prediabetes to expedite targeting for prevention and intervention strategies.

Patients and Methods

This is a case-control hospital-based study. The study was approved by the Regional Research Ethics Committee, Qassim Province, Ministry of Health, and KSA. Written informed consent was obtained from the parents of each participant after an informed sheet was provided. The total sample (n=80) was divided into three groups. Group 1: known diabetics (n=20) using insulin injection; group 2 first degree relatives of the diabetics (n=40) and group 3 (n=20) as healthy controls with no family history of DM and matched in age, sex and body mass index with groups 1&2. The twenty diabetic children had onset of diabetes before age of 15 years and fulfillment of the of the Expert Committee of Diabetes criteria for diagnosis of type 1 diabetes mellitus (Imagawa et al., 1996). Patients with autoimmune diseases were excluded. For all 80 children, a questionnaire was used to collect demographic and clinical data. Three ml of blood was collected from each participant; centrifuged and the serum was kept at -80ºC for biochemical analysis. Immunospecific insulin and C-peptide quantitative sandwich enzyme immunoassay Kits (Cat# E29-072& E29-071, USA) were used to estimate human insulin and C-peptide in serum. Human islet cell antibody (ICA), insulin auto antibodies (IAA) and antiglutamic acid

decarboxylase (anti-GAD) were estimated using ELISA kits purchased from WKEA MED supplies, USA. In ICA and IAA, human purified antigens were coated to the wells, whereas in anti-GAD kit human purified antibodies were coated. Random blood glucose and glycosylated hemoglobin A1c (GHbA1c) were measured as routine investigations in the Maternal Childhood Hospital (MCH).

Statistical Analysis

Data were analyzed using SPSS version 16. Values were expressed as mean ± SD. Results considered statistically significant at $p \leq 0.05$. Differences between values were assessed by Mann Whitney U-test. A multiple regression test was conducted to determine the impact of 5 variables. Odd's ratio was calculated to determine the risk of development T1 DM in the relatives.

Results

In this cross-sectional hospital-based study we recruited a total of 80 children. Their mean age was 7.9±2.0 years with a median and mode of 8 years and a range of 4-12 years. There was no significant gender difference using X 2test ($p>0.05$). There was no significant differences of ages in the different groups ($p=0.37$). Also, there was no statistically significant differences in gender, parents education and consanguinity, using X 2 test ($p>0.05$).

Table1: Biochemical markers in different groups expressed as mean ± SD

Characteristic	Diabetics (n=20)	Relatives (n=40)	Control (n=20)	*P
Glucose	160.1±21.26	124.6±14.0	111.0±7.2	0.00
glycosylated haemoglobin A1-A1c	6.87±0.43	4.9±0.53	4.99±0.50	0.00
Insulin hormone	8.57±4.19	6.33±2.31	6.37±4.48	0.05
C-peptide	0.62±0.19	1.03±0.26	1.42±0.24	<0.001
Insulin-autoantibody	23.38±12.89	19.49±3.62	22.43±7.6	0.15
Islet cell antibody	2.82±0.96	1.78±0.32	1.67±0.25	0.003
Anti-GAD	99.01±46.99	78.36±40.99	43.12±5.59	<0.001

Anti-GAD: antiglutamic acid decarboxylase. Blood glucose is random sample. *P value compare between diabetic and control groups.

Diabetics have significantly higher value for anti GAD antibodies and islet cell antibody as well as for glucose and gAC1 level (table 1). However, insulin hormone level is higher in DM because the patients received exogenous insulin. C- Peptide was found to be significantly higher among controls compared to diabetics ($p<0.001$).

A multiple regression was conducted to determine if these markers were well significantly predicting the blood glucose level. For all variable higher scores correspond to C-peptide, glycosylated hemoglobin and blood glucose level. The results indicated that these predictors in aggregates were significant predictors of the

blood glucose level, explaining 44.7% of the variance, f (6) =11.6, P<. 001. When the variables were examined for their individual contribution to the model, it was found that insulin hormone level, islet cell antibodies and glycosylated hemoglobin AC1 were the significant predictors of the blood glucose level (p=0.003, 0.040, 0.001; respectively). However, Anti- GAD, C-peptide and insulin antibodies were not significant predictors of blood glucose level (p = 0.288, 0 .88, 1.06; respectively).

Table 2: Biochemical and immunological markers levels among different groups at specific cut off value

Markers	Diabetics No (%)		Relatives No (%)		Controls No (%)	
	Normal	High	Normal	High	Normal	High
Insulin hormone (5-20 pmol/L)	17 (85%)	3 (15%)	40 (100%)	0 (0%)	14(70%)	6 (30%)
C- peptide (0.7-1.9 ng/ml)	8(40%)	12 (60%)*	28(70%)	12(30%)*	19(95%)	1(5%)*
Insulin auto-antibodies (5-20 pmol/L)	9(45%)	11(55%)	30(75%)	10(25%)	19(95%)	1(5%)
Islet cell antibodies (1-2.5 iu/L)	7(35%)	13(65%)	39(97.5%)	1(2.5%)	20(100%)	0(0%)
Anti-GAD antibodies (≤50pmol/L)	10 (50%)	10(50%)	28(70%)	12(30%)	18(90%)	2(10%)

*C peptide is decreased. Glycosylated hemoglobin (HbA1c 6-8%) is within normal range in all groups. Anti-GAD: antiglutamic acid decarboxylase

A logistic regression was conducted to determine what impact the 5 variables have on the likelihood of developing diabetes. These independent variables were anti-GAD; C-peptide, insulin autoantibody, insulin hormone and islets cell antibodies. The full model containing all predictors was statistically significant X2 = 37.167 (p < 0.001) indicating that the model was able to distinguish between diabetics and non-diabetics. The total model explained between 37.2% (Cox and Snell) and 55% (NagelKerke R squared) of the variants in diabetics. The strongest predictors of diabetes were C peptide, which had an odd ratio of 4.7 and islets cell antibodies which had an odd ratio of 3.1. This indicates that children who had islets cell antibodies were almost 3 times more likely to have diabetes than those who did not had islets cell antibodies. A unit decrease in C- peptide level is 5 times likely to increase the likelihood to develop diabetes.

This study showed that diabetic children had high level of ICA (65%), IAA (55%), anti-GAD antibodies (50%) and decrease in C-peptide (60%). Whereas the relatives showed high level of anti-GAD antibodies (30%), IAA (25%), ICA (2.5) and decrease in C-peptide (30%). Anti-GAD antibodies were significantly higher among the relatives of the diabetics compared to the healthy subjects as shown in table 2.

Discussion

Type 1 diabetes is preceded by the development of auto antibodies to multiple islet antigens. Glutamic acid decarboxylase (GAD) is expressed in human β cells (but also by α, and δ cells of the islets) and represents the major target autoantigen in type 1 diabetes (Yu et al., 2012& Hanzu and Gomis 2008). In our study, 50% of the diabetic group was found to have significantly higher value for anti GAD antibodies when cutoff value ≤50pmol/L was used compared to 73.2% and 65.1% in another studies (Sabbah et al., 1999; Fakhfakh et al.,2008). Antibodies to GAD could be detected in the sera of 75% of new-onset T1D patients (Hanzu and Gomis 2008). This is in agreement with Torn et al., 2008 who reported that high GAD antibody at diagnosis was a risk factor for a decrease in β cell function. These findings could be attributed to the fact that the 2003 Expert Committee on the diagnosis and classification of diabetes divided type 1 diabetes into type 1 A (immune mediated) and type 1 B (idiopathic). Moreover, not all cases in this study were newly diagnosed. Autoantibody may also become negative after many years of diabetes. GAD autoantibodies were 40% in longstanding type 1 diabetes vs. 55% in new onsets (Imagawa et al., 1996). In this study, 30% of the relatives of the diabetics had high serum level of Anti-GAD antibodies whereas 90% of the controls had normal values. The risk for IDDM increases as the number of autoantibodies increases, and that relatives with three autoantibodies (IA-2A, GADA, and IAA) had a 100% estimated risk of contracting IDDM within 5 years (15). Anti- GAD level, in our study, was not a significant predictor of blood glucose level (p=0.288). This is in accordance with Sabbah et al., 1999 who found that there was no significant difference between the GADA-positive and negative subjects in the metabolic characteristics at diagnosis.

Stimulation of C-peptide secretion with glucose, a mixed meal, or arginine provides the most sensitive and clinically validated method to evaluate β cell function (Greenbaum et al., 2004). In our study 60% of diabetic group of children had abnormally low levels of C peptide in comparison to 30% of the patients' relatives when used normal value (0.7-1.9 ng/ml). This is in agreement with Tsai et al., 2006 and Sosenko et al., 2006 who identified a progressive decline in C-peptide responses that are relatively modest during the prediabetic period compared with the changes after diagnosis with hyperglycemia. Interpretation of the changes in C-peptide responses may be more complicated in children because there is an age-related increase in C-peptide levels (Torn et al., 2000). In our study, a significant correlation between the decrement in C-peptide serum level and the likelihood to develop diabetes was found. Similar findings

were reported in diabetic children at diagnosis and during follow-up, and the degree of decline in C- peptide level was inversely related to the levels of autoantibodies in these patients. This can be explained by the fact that patients with autoimmune diabetes have a continuous process of destruction of β cells, probably caused by a combination of humoral and a cellular activity (Torn et al., 2000).

IAA was the first autoantigen identified in T1D and is still the only β cell-specific. Autoantibodies react to mature insulin, whereas specific proinsulin autoantibodies have been difficult to demonstrate (Stadinski et al., 2010). IAA is more common in younger children with new-onset T1DM before insulin therapy (approximately 60% positive) than in adults (Palmer et al., 1983). In our study, 55% of diabetics were found to have significantly higher serum value for insulin autoantibodies in comparison to 25% of relatives based on cut off value (5-20 pmol/L). Antibodies against insulin (IAA) are ones of the earliest clinical markers of prediabetes (Stadinski et al., 2010). The presence of IAA is inversely correlated with age of diabetes onset, with almost 100% of newly diagnosed children less than 5 years of age expressing IAA, compared to less than 20% of those diagnosed after 15 years of age. Moreover, the levels of IAA are associated with the rate of the autoimmune destruction of the β cells making their detection an important aspect of diagnosis and prevention in those who are in high risk and relatives of type 1 diabetics (Winter and Schatz 2011).

Islet cell autoantibody (ICA) is directed specifically against auto antigens of the insulin secreting β cells. et al., 2009) in our study 65% of diabetic children had high IC antibody. ICA was detected in 70% to 80% of individuals with new-onset TIDM and that ICA positivity declines after the diagnosis. Auto-antibodies to islet cell antigens often present at disease onset, Islet cell antibody (ICA), 70%–80%; insulin autoantibodies (IAA), 60%; tyrosine phosphatase like insulinoma antigen 2 (IA2), 60%; and glutamic acid decarboxylase (GAD), 70%–80%. Secondary screening for antibodies to cytoplasmic ICA in GAD and IA2 Ab-positive first-degree relatives of persons with T1D can detect individuals at greater risk for T1D (Watkins et al., 2014). Moreover, 60-75% of new-onset T1D patients have autoantibodies against the islet cell antigen 512(ICA512 or IA-2), compared to only 2% of healthy controls (Kantarova et al., 2012). Risk score using panel of biochemical markers may be used to understand onset, progression and prevention of T1DM in the relatives of diabetics.

Conclusion

Diabetic children had high levels of anti-GAD antibodies, insulin autoantibodies and islet cells antibodies but low level of C-peptide.

Anti-GAD antibodies were significantly higher among the relatives of the diabetics compared to healthy controls. C-peptide and blood glucose are significant predictors of high blood glucose level. A unit decrease in C- peptide and a unit increase in islet cell antibodies levels increase the likelihood of developing diabetes. Autoantibodies can distinguish responses in T1D patients from healthy control subjects and they may also identify individuals at the highest risk for progression from prediabetes to overt disease.

Acknowledgments

We would like to thank the participating parents and their children for their cooperation. Special appreciation goes for the staff of the Maternity and Children Hospital, Buraidah for contribution in collecting the blood samples. This work was supported by a grant from the Scientific Research Deanship, Qassim University, and KSA. (Grant# 692).

Conflicts of interest

The authors declare that there is no conflict of interest.

References

[1] AL-Herbish AS, EL-Mouzan MI, Al-Salloum AA, Al-Qurachi MM, Al-Omar AA. 2008. Prevalence of type 1 diabetes mellitus in Saudi children and adolescents. Saudi Med J, 29(9):1285-8.

[2] Bell RA, Mayer-Davise EJ, Beyer JW, D'Agodino RB, Lawerence JM, et al. . 2009. Diabetes in non-Hispanic white youth: Prevalence and clinical characterization. Diabetes Care, 32: 102-11.

[3] Borg H, Fernlund P, Sundkvist G. 1997. Protein tyrosine phosphatase like protein IA2-antibodies plus glutamic acid decarboxylase 65 antibodies (GADA) indicates autoimmunity as frequently as islet cell antibodies assay in children with recently diagnosed diabetes mellitus. Clin. Chem, 43: 2358-63.

[4] Concannon P, Rich SS, Nepom GT. 2009. Genetics of type 1A diabetes. N Engl J Med, 360: 1646–54.

[5] Fakhfakh R, Haddouk S, Hadj Hamida YB, Kamoun T, Ayed MB, et al. 2008. Pancreatic autoantibodies in Tunisian children with newly diagnosed type 1 diabetes. Pathol Biol, 56(3): 130-2.

[6] Greenbaum C, Seidel K, Pihoker C. 2004. The case for intravenous arginine stimulation in lieu of mixed-meal tolerance tests as outcome measure for intervention studies in recent-onset type 1 diabetes. Diabetes Care, 27: 1202-4.

[7] Hanzu F, Gomis R. Autoimmune diabetes mellitus. In: Shoenfeld Y, Cervera R, Gerschwin ME (eds). Diagnostic criteria in autoimmune diseases. Totowa; Hamana

Press, 2008; p: 241-9.

[8] Imagawa A, Itoh N, Hanafusa T, Katsura H, Yamaguchi A, et al. 1996. High prevalence of antobodies to glutamic acid decarboxylase in comparison to islet cell antibodies in patients with long standing insulin dependent diabetes mellitus. Res Commun Mol Pathol Pharmacol, 92: 43-52.

[9] Kantarova D, Vrlik M, Buc M. 2012. Genetic determinationand immune-pathogenesis of type 1 diabetes mellitus in humans. Acta Martniniana, 12(2): 19-31.

[10] Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, et al. 2000. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care, 23: 1516-26.

[11] Lebastchi J, Herold KC. 2012. Immuno-logic and metabolic biomarkers of β-cell destruction in the diagnosis of Type 1 diabetes. Cold Spring Harb Perspect Med, 6: 2(6). a007708. doi: 10.1101/cshperspect. a007708.

[12] Michels AW, Eisenbarth GS. Immunologic endocrine disorders. 2010. J Allergy Clin Immunol, 125(2): S226-37.

[13] Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, et al. 1983. Insulin antibodies in insulin dependent diabetics before insulin treatment. Science, 222: 1337-39.

[14] Peng H, Hagopian W. Environmental factors in the development of Type 1 diabetes. 2006. Rev Endocr Metab Disord, 7: 149-62.

[15] Sabbah E, Savola K, Kulmala P, Veijola R, Vähäsalo P, et al. 1999. Diabetes-associated autoantibodies in relation to clinical characteristics and natural course in children with newly diagnosed type 1 diabetes. J Clin Endocrinol Metab, 84: 1534-9.

[16] Siljander HT, Simell S, Hekkala A, Lahde J, Simell T, et al. 2009. Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population. Diabetes, 58: 2835-42.

[17] Soltesz G, Patterson CC, Dahlquist G. 2007. Worldwide childhood type 1 diabetes incidence-what can we learn from epidemiology? Pediatric Diabetes, 8(6): 6-14.

[18] Sosenko JM, Palmer JP, Greenbaum CJ, Mahon J, Cowie C, et al. 2006. Patterns of metabolic progression to type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care, 29: 643-9.

[19] Stadinski B, Kappler J, Eisenbarth GS. 2010. Molecular targeting of islet autoantigens. Immunity, 32: 446-56.

[20] Yu L, Herold K, Lachin J, Bundy B, Pugliese A, Krischer J, Eisenbarth G, Pescovitz M, Type 1 Diabetes TrialNet Anti-CD20 Study Group. 2011. Rituximab selectively suppresses specific islet

antibodies. Diabetes, 60: 2560-5.

[21] Torn C, Landin-Olsson M, Lernmark A, Palmer JP, Arnqvist HJ, et al. 2000. Prognostic Factors for the Course of b Cell Function in Autoimmune Diabetes. The Journal of Clinical Endocrinology & Metabolism, 35(12): 4619-23.

[22] Torn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ. 2008. Participating Laboratories. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia, 51(5): 846-52.

[23] Tsai EB, Sherry NA, Palmer JP, Herold KC. 2006. The rise and fall of insulin secretion in type 1 diabetes mellitus. Diabetologia, 49: 261-70.

[24] Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, et al. 1996. Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes, 45: 926-33.

[25] Watkins RA, Evans-Molina C, Blum JS, DiMeglio LA. 2014. Established and emerging biomarkers for the prediction of type 1 diabetes: a systematic review. Transl Res, 164(2): 110-21.

[26] Winter WE, Schatz DA. 2011. Autoimmune Markers in Diabetes. Clinical Chemistry, 57(2): 168-75.

[27] Yu L, Boulware DC, Beam CA, John C. Hutton JC, Wenzlau JM, Greenbaum CJ, Bingley PJ, Krischer JP, Sosenko JM, Skyler JS, Eisenbarth GS, Mahon JL and the Type 1 Diabetes TrialNet Study Group. 2012. Zinc Transporter-8 Autoantibodies Improve Prediction of Type 1 Diabetes in Relatives Positive for the Standard Biochemical Autoantibodies. Diabetes Care, 35: 1213-8.