

Frequency of C-peptide and antibody levels (anti GAD, Islet cell antibodies, insulin auto antibodies) in children of Pakistan with Type-1 diabetes

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ABSTRACT

Background and Objective: The autoimmune mechanism in T1DM causes gradual loss of pancreatic β -cell, which progresses to hyperglycemia and ultimate reliance on consistent insulin therapy. T1DM has been the commonest type of diabetes in children and this study will help in refining indulgent towards the problem and its pathophysiology in our people. The objective was to find out the prevalence of C-peptide and antibody levels (anti GAD, ICA, IAA and IA2) in children and adolescents of Pakistan with T1DM.

Methods: We conducted this cross-sectional study at Department of Pediatric Endocrinology, National Institute of Child Health, Karachi between August 2019 to February 2020 and included 98 children who had T1DM for more than one month. Subjects whose GFR was $<30\text{ml/min}$ were omitted from the study. Among those registered subjects, C-peptide, human islet cell antibody (ICA), insulin auto antibodies (IAA) and anti-glutamic acid decarboxylase were assessed. Demographical and laboratorial facts were noted on a pre-constructed proforma.

Results: There were 77(78.3%) cases who had level of C-peptide <0.8 and anti-GAD was found in 47(48%) subjects. 35(35.7%) cases found positive for IA2 .and 7(7.1%) patients had insulin auto antibodies positive while ICA was negative in total 98(100%) subjects.

Conclusion: Children with T1 DM possessed increased levels of anti-GAD antibodies, insulin autoantibodies and anti (IA2) but islet cells antibodies were negligible in our population when checked at a point of time. C-peptide may be normal in some, but its level declines with long duration of diabetes in children.

KEYWORDS: Type-1 DM, Insulin autoantibodies, C-peptide, Anti GAD antibodies.

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INTRODUCTION

Type-1 diabetes, an autoimmune disease is said to be the commonest type of diabetes in children, and its incidence is on the rise.¹ It is predicted that in under five years European children, it will increase two-fold in less than 20 years. According to one meta-analysis, the frequency of T1DM in Asia, Africa, Europe, and America was 15 per 100,000, eight per 100,000, 15 per 100,000 and 20 per 100, respectively. While its worldwide prevalence in these regions was, 6.9 per 10,000, 3.5 per 10,000, and 12.2 per 10,000, respectively.² Though males more commonly present with this illness in initial adult life, the incidence of T1DM is equivalent in both genders during childhood.³ Onset of T1DM usually depends on environmental triggers, recruitment and activation of immune cells and genetic predisposition.⁴

Type-1 diabetics are unable to produce insulin, therefore level of C-terminal cleavage product (C-peptide) produced during processing of the insulin pro-hormone to the mature insulin molecule is found

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low in them.⁵ Usually, in T1DM, C-peptide secretion decreases with time and the rate of decline is faster in younger age of onset.⁶ Data from Pakistan suggests that C-peptide levels maintains itself in young people with T1DM.⁷ C-peptide levels differ in various populations at the time of diagnosis but generally it correlates with type of diabetes, duration of disease, and age at the time of diagnosis. Multiple islet autoantibodies are found in the large majority of children who develop Type-1 diabetes, Type-1 diabetes in adulthood often present with only GAD antibodies.⁵ As per western literature, the prevalence of GAD autoantibodies, IA-2 autoantibodies, and ZnT8 autoantibodies are 70%-80%, 60%-70%, and 60%-80%, respectively in children with new onset T1DM.⁶

According to a local study, among 99 subjects with T1DM 16 (16.2%) and 53 (53.5%) were positive for IA2 and GAD-65 respectively, while 10 (10.1%) tested positive for both autoantibodies.⁷ However, there are other antibodies like insulin autoantibodies (IAA) and islet cell antibody (ICA) which have not been looked for in Pakistani children. There are two recent studies discussing that the existence of anti-Glutamic Acid Decarboxylase (anti GAD) with various islet cell antibodies in children is concomitant with leisure advancement of Type-1 diabetes.⁸ Being the largest in province, we get referrals from different regions of the country, hence this study will help us more to understand the autoimmunity pattern of Pakistani children in developing diabetes. Also, future studies among first degree relatives of diabetics can be conducted to identify pre-clinical diabetes, allows for less diabetic keto-acidosis, early initiation of insulin therapy, and the potential to delay or prevent diabetes onset. With the advent of safe and specific therapies to block disease specific immune cells our study would provide major ground to use the new drugs in prolonging the disease onset. Moreover, this study opens doors for the detection of other organ specific antibodies like thyroid as the prevalence of thyroid antibodies among anti-GAD positive patients is almost twice as high as among anti-GAD negative.⁹

The rationale of our study is to evaluate the pattern of antibodies in our population of diabetic children so to expedite targeting for prevention and recent intervention in pre diabetics of our country. Moreover, the preservation of C-peptide secretion prevents or delays diabetes related complications¹⁰. so, its level will help us to identify the population more at risk of developing T1DM related complications like vasculo-pathy and nephropathy.

METHODS

This cross-sectional study was conducted at Department of Pediatric endocrinology, National Institute of Child Health, Karachi during August 2019 to February 2020. Children from age two years to fifteen years having T1DM for more than a month period were enrolled in the study. There were 98 patients fulfilling the inclusion criteria.

Ethical Approval: After approval from IERB (Ex-01/2021; dated February 16th, 2021) informed written consent was taken before enrolment.

Immuno specific insulin and C-peptide quantitative sandwich enzyme immunoassay Kits (Cat# E29-072& E29-071, USA) were used to estimate C-peptide in serum. Human islet cell antibody (ICA), insulin auto antibodies (IAA), glutamic acid decarboxylase (anti GAD) and Insulinoma associated antigen (IA2) were valued using ELISA. Demographical information, clinical and biochemical information were noted by the principal investigator on a pre-designed proforma. SPSS version 21 was used for data analysis. Frequencies and percentages were computed for qualitative variables like gender, family history, Anti GAD, islet cell antibody, insulin autoantibody (positive/negative). Quantitative variables were presented as mean \pm standard deviation for variables like age, duration of Type-1 DM, level of C-peptide, antibody levels. Effect modifiers like age, gender, family history, and duration of T1D were controlled through stratification. Chi-square test and fishers exact test were used where applicable, p -value ≤ 0.05 was considered as significant.

RESULTS

Out of 98 type-1 diabetic patients 47 (48%) were female and 51 (52%) were male. Although, not statistically significant level of C-peptide was low in around two third of our study group (Table-I) and has a significant reciprocal relationship with duration of diabetes ($p=0.006$) (duration: minimum > one month to maximum 10 years after diagnosis). Age at the time of diabetes has no significant impact on C-peptide level. (Table-I). Anti- GAD was the most frequent antibody found in our subjects (48%), followed by IA2(35.7%) and insulin autoantibodies (7.1%). The whole cohort was negative for islet cell antibody (ICA). (Table-II). Only IAA showed significant association with age ($p=0.046$). Other effect modifiers, like gender and duration of diabetes were not significantly associated with antibodies positivity.

DISCUSSION

We evaluated the frequency of different Type-1 diabetes related autoantibodies and their relationship with the age at onset and duration of diabetes in children. At least one antibody was found positive in a round 90% of the children affected with Type-1 DM. Data on β -cell antibodies from Qatar showed 75.5% positivity¹¹ and from Iran reported to be 80% at first presentation, which is then declined as the beta cells of the pancreas decrease in number.¹²

A multicenter study from China reported to have 61.01% of their T1DM children positive for beta cell antibodies at initial diagnosis. However, they only checked for anti-GAD, IAA, and ICA.¹³ Studies from India proved that antibody positivity reaches from 55% when only Anti GAD and IA2 were checked, while it reached up to 96% when all five antibodies against Type-1 diabetes were estimated.⁹ The reported prevalence of

Table-I: Patient characteristics and association of c-peptide level.

Study Outcome		Frequency	Percent
C-Peptide	< 0.8	77(78.3%)	
	≥0.8 (Normal)	21	21.4
Total		98	100
		C-peptide	P-value
< 0.8		≥0.8 (Normal)	
Gender	Female	34(72.3%)	13 (27.7%)
	Male	43(84.3%)	8 (15.7%)
Duration of Type-1 DM	< 5years	57 (73.1%)	21 (26.9%)
	≥ 5 years	20 (100.0%)	0(0.0%)
Age groups (in years)	< 5 years	19 (76.0%)	6 (24.0%)
	≥ 5 years	58(79.5%)	15(20.5%)

idiopathic Type-1 diabetes (iAb-) is high (30%) in one study¹⁴ but remains low (2.2%) only if all five antibodies are estimated.^{14,15}

Among 98 Type-1 diabetics 47 were females and 51 were males similar to a local study. Mean age at diagnosis was 11.0 ± 5.2 years (range 1.6-21.7 years) in the mentioned study, that is also similar to our data where two third of the children were above five years of age.⁷ Our data represented that antibodies in T1 diabetic children are more likely to be positive if checked within five years, than after five years of establishing a diagnosis. This is similar to other studies where median duration of diabetes was 34.0 (12.7-69.7) months.¹² Another report also showed significant positive antibodies titer within 11 months of diagnosing diabetes.

We found Anti-GAD antibody among most of diabetic children and adolescents followed by Insulinoma-associated antigen 2(IA2 and insulin autoantibodies (IAA). This is similar to available local data where 53 (53.5%) were GAD-65 positive and 16 (16.2%) were IA2 positive.⁷ one international studies also supports same findings where anti-GAD and anti-IA2 auto-antibodies were found in 76.92% and 62.82% respectively in a mix group of newly and established diabetic children.¹⁶ Hence, these two antibodies can detect patients early and can persist several years after diagnosis of T1D. This is also alike to the global data where IA2 found to be the most frequent antibody in T1DM.¹⁷

Our data represents IAA to be detected in 7(7.1%) in contrast to positivity for this antibody from India (63%) 9(Qatar (40.08%))¹¹ and Iran (21.8%)¹² respectively. This

may be due to differences in genetic patterns, age of onset and duration of diabetes.

Our cohort was negative for islet cell antibodies, this is in contrast to the data from a nearby country where ICA was the most commonly found antibody in 66.2%, followed by anti-GAD (56.3%), IA-2A (40.1%) in 142 subjects with different ages and duration of diabetes.¹² Another study from Qatar reported ICA to be positive in 53.4% of T1DM.¹¹

Recent Meta analyses states that the number of iAb determined is more predictive of development of

Table-II: Level of antibodies against islet cells in Type-1 Diabetic Pakistani children.

Study Outcome	Frequency (%)
Anti-GAD	Negative 51 (52%)
	Positive 47 (48%)
Total	98 (100%)
Insulinoma-associated antigen 2(IA2)	Negative 63 (64.3%)
	Positive 35 (35.7%)
Insulin auto antibodies	Negative 91 (92.9%)
	Positive 7 (7.1%)
Islet Cell antibody	Negative 98 (100%)
	Positive 0 (0%)
Total	98 (100%)

Type-1 diabetes rather than the specific iAb profile.^{13,17} Though, we did not look for znt8 antibodies but international studies support its presence to be (40-60%) in newly diagnosed diabetics. However, a study from India shows only 20.08% positive znt8 antibodies. Mean duration of 6.3 ± 4 years after diagnosis of diabetes could be an explanation to this difference.⁹ Hence the author recommends complete antibody profile including znt8 antibody.

We estimated c-peptide levels in our targeted population and did not find significant differences in relation to gender and age groups. However, its levels significantly declined with the increasing duration of diabetes. This is similar to a recent local study in which C-peptide levels were decreased with longer duration of T1DM but not statistically significant.⁷ Our results are supported by Leighton E et al where C-peptide has been shown to correlate with longevity of disease.⁶ Few other studies from the world also emphasize on stabilization of c-peptide levels more than five years after diagnosis.^{18,19}

Limitations: Due to financial constraints; we could not perform the C-peptide level at the time of diagnosis and then follow it to see the pattern of its decline. Further, znt8 antibody could not be assessed which affects the overall positivity of the T1DM antibodies in children as per literature. Despite that, this is probably the first study conducted here with more than two antibodies detection. Previously published local study did not incorporate IAA and ICA in our population. Hence, the authors recommend complete antibodies profile along with c-peptide value when T1DM is diagnosed, as well as on long term follow-up for better understanding of changes in immune function and stronger evidence of beta cells preservice.

CONCLUSION

Diabetic children had high levels of anti-GAD antibodies, insulin autoantibodies and anti (IA2) but islet cells antibodies were negligible in our population when checked at a point of time. C-peptide may be found normal in a portion of diabetic children but its level declines with increasing duration of diabetes and this fall is steeper in younger children.

Recommendation: There is other disease associated antibodies which need to be performed like antibodies thyroid gland and celiac antibodies which we could not perform to avoid financial burden on the family. Other than that, we recommend detection of antibodies in non-diabetic siblings of patients as well.

REFERENCES

- Krzewska A, Ben-Skowronek I. Effect of Associated Autoimmune Diseases on Type 1 Diabetes Mellitus Incidence and Metabolic Control in Children and Adolescents. *Biomed Res Int.* 2016;2016:6219730. doi: 10.1155/2016/6219730
- Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojzadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health Promot Perspect.* 2020;10(2):98-115. doi: 10.34172/hpp.2020.18
- Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. *Pediatr Diabetes.* 2018;19(3):346-353. doi: 10.1111/pedi.12597
- Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. *Lancet (London, England).* 2016;387(10035):2340-2348. doi: 10.1016/S0140-6736(16)30507-4
- Singh GC, Ahmed M, Zaid M, Hasnain S. Biochemical, serological, and genetic aspects related to gene HLA-DQB1 and its association with type 1 diabetes mellitus (T1DM). *Mol Genet Genomic Med.* 2020;8(5):e1147. doi: 10.1002/mgg3.1147
- Leighton E, Sainsbury CA, Jones GC. A Practical Review of C-Peptide Testing in Diabetes. *Diabetes therapy: research. Diabetes Ther.* 2017;8(3):475-487. doi: 10.1007/s13300-017-0265-4
- Fawwad A, Waris N, Askari S, Ogle G, Ahmedani MY, Basit A. Relationship of C-peptide levels to duration of Type 1 diabetes - A study from Sindh, Pakistan. *Pak J Med Sci.* 2020;36(4):765-769. doi: 10.12669/pjms.36.4.1531
- Ziegler AG, Bonifacio E. Why is the presence of autoantibodies against GAD associated with a relatively slow progression to clinical diabetes? *Diabetologia.* 2020;63(8):1665-1666. doi: 10.1007/s00125-020-05175-8
- Basu M, Pandit K, Banerjee M, Mondal SA, Mukhopadhyay P, Ghosh S. Profile of Auto-antibodies (Disease Related and Other) in Children with Type 1 Diabetes. *Indian J Endocrinol Metab.* 2020;24(3):256-259. doi: 10.4103/ijem.IJEM_63_20
- Souto SB, Campos JR, Fangueiro JF, Silva AM, Cicero N, Lucarini M, et al. Multiple Cell Signalling Pathways of Human Proinsulin C-Peptide in Vasculopathy Protection. *Int J Mol Sci.* 2020;21(2):645. doi: 10.3390/ijms21020645
- Alyafei F, Soliman A, Alkhalaf F, Sabt A, De Sanctis V, Elsayed N, et al. Prevalence of β -cell antibodies and associated autoimmune diseases in children and adolescents with type 1 diabetes (T1DM) versus type 2 diabetes (T2DM) in Qatar. *Acta Biomed.* 2018;89(55):32-39. doi: 10.23750/abm.v89i54.7359
- Zamanfar D, Aarabi M, Amini M, Monajati M. Prevalence of autoantibodies in type 1 diabetes mellitus pediatrics in Mazandaran, North of Iran. *J Pediatr Endocrinol Metab.* 2020;33(10):1299-1305. doi: 10.1515/jpem-2019-0396
- Hou L, Li X, Liu L, Wei H, Xiong F, Du H, et al. A Multicenter Survey of Type 1 Diabetes Mellitus in Chinese Children. *Front Endocrinol.* 2021;12, 583114. doi: 10.3389/fendo.2021.583114
- Vipin VP, Zaidi G, Watson K, G Colman P, Prakash S, Agrawal S, et al. High prevalence of idiopathic (islet antibody-negative) type 1 diabetes among Indian children and adolescents. *Pediatr Diabetes.* 2021;22(1):47-51. doi: 10.1111/pedi.13066
- Hameed S, Ellard S, Woodhead HJ, Neville KA, Walker JL, et al. Persistently autoantibody negative (PAN) type 1 diabetes mellitus in children. *Pediatr Diabetes.* 2011;12(3Pt1):142-149. doi: 10.1111/j.1399-5448.2010.00681
- O Belhiba, Z Aadam, L Jeddane, R Saile, H Salih ALJ, AA Bousfiha, et al. Research of anti-GAD and anti-IA2 autoantibodies by ELISA test in a series of Moroccan pediatric patients with diabetes type 1. *Afr Health Sci.* 2020;20(3):1337-1343. doi: 10.4314/ahs.v20i3.40
- Ross C, Ward ZJ, Gomber A, Owais M, Yeh JM, Reddy CL, Atun R. The Prevalence of Islet Autoantibodies in Children and Adolescents with Type 1 Diabetes Mellitus: A Global Scoping Review. *Front Endocrinol.* 2022;13:815703. doi: 10.3389/fendo.2022.815703
- Shields BM, McDonald TJ, Oram R, Hill A, Hudson M, Leete P, et al. C-Peptide Decline in Type 1 Diabetes Has Two Phases: An Initial Exponential Fall and a Subsequent Stable Phase. *Diabetes Care.* 2018;41(7):1486-1492. doi: 10.2337/dc18-0465
- Hao W, Gitelman S, DiMeglio LA, Boulware D, Greenbaum CJ, Type 1 Diabetes TrialNet Study Group. Fall in C-Peptide During First 4 Years from Diagnosis of Type 1 Diabetes: Variable Relation to Age, HbA1c, and Insulin Dose. *Diabetes Care.* 2016;39(10):1664-1670. doi: 10.2337/dc16-0360

Authors' Contribution:

MR designed and did statistical analysis & editing of manuscript, is responsible for integrity of research.
MA conceived designed and helped in data collection.
ZAK did data collection and manuscript writing.
MNI did review and final approval of manuscript.