IAA, GAD65 AND IA2 Antibodies In Type 1 Diabetes Mellitus Children And Adolescents

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Abstract

Objective: The objective of the study was tofind out the association ofInsulin Auto antibodies (IAA), glutamic acid decarboxylase (GAD65), and Insulinoma-Associated-2 Auto antibodies (IA-2A) antibodies in type 1 diabetes mellitus concern in gage, gender, and duration of disease.

Methods: This was a retrospective study conducted at the department of endocrinology and diabetes by using nonprobability consecutive sampling technique, after taking ethical approval. The duration of the study was one year. A total of 143 individuals (both children and adolescents up to the age of 18 years) clinically diagnosed with type 1 diabetes were selected for the study where as those who were having any congenital and any other endocrine disorder were excluded. All study subjects were investigated for pancreatic autoimmune markers (GAD65, IA-2A, IAAs). Pearson's Chi-square test was applied to evaluate the association.

Results: The study results showed that out of 143 individuals, 71 (49.7%) were males and 72 (50.3%) were females. Distribution of positivity of autoantibodiesshowed that 27 (18.9%) had AntiInsulin IgG, 63 (44.1%) had GAD65, 47 (32.9%) had IA-2A and only 5 (3.5%) had a grey zone. There was a significant association was reported ($p<0.001$) in the duration of diabetes and with IAA. Furthermore, there was a statistically significant association with ($p=0.003$) duration of diabetes and frequency of IA-2A.

Conclusion: This study concludes that the presence of autoantibodies such as insulinoma-associated antigen-2 autoantibodies, Insulin autoantibodies, and glutamic acid decarboxylase 65 predicts type-I diabetes mellitus in children and adolescents. Additionally, the presence of insulinoma-associated antigen-2 autoantibodies, Insulin autoantibodies are significantly associated with the duration of disease.

Keywords: Type 1 Diabetes Mellitus, autoantibodies, children, adolescent

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Introduction

The occurrence of type 1 Diabetes Mellitus (T1DM) is rapidlyincreasing worldwide. Itwas estimated about 1.48/1000 in the year 2001 that was increased up to 1.93/1000 in the year 2009[1]. As stated by International Diabetes Federation (IDF),T1DM is expected to increase up to 3% yearly and is likely to diagnose about 132,600 individual severy year having the age of < 20 years[2].

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Type 1 diabetes is a state that is described by a deficiency of insulin because of an autoimmune response mediated by the T cells of the immune system that obliterates the pancreatic beta cells of Islet[3]. One or more islet-specific autoantibodies for instance Islet Cell Cytoplasmic Autoantibodies (ICA), Insulinoma-Associated-2 Autoantibodies (IA-2A), Glutamic Acid Decarboxylase Autoantibodies (GADA), Insulin Autoantibodies (IAA), and Zinc Transporter-8 Autoantibodies (ZnT8A) are present in Type 1 diabetes[4].

Islet autoantibodies are indicators of self-reactive response to the islets in the pancreas, but they do not cause type 1 diabetes. Islet autoantibodies

are usually appeared at the time of diagnosis and can exist before detection of Type 1 diabetes, and its frequency can reduce above 5 to 10 years after detection of Type 1 diabetes[5].

Type 1 diabetes was formerly regarded as juvenile or insulin-dependent diabetes but has been recognized asa completed eficiency of insulin[6]. One or more of the islet autoantibodies will be developed in about 95% of individuals affected at the time of early diagnosis, as autoimmune type 1 diabetes is present. On the other hand, autoantibodies are usually absent in type 2 diabetes[6].

Approximately, 10% of cases of diabetes are type 1 (autoimmune) and most of these cases are detected in individuals under the age of 20 years. Though, people of any age can develop type 1 diabetes[7]. Symptoms of diabetes are polyuria, feeling of dehydration, loss of weight and poor healing of the wound, which appearsas approximately 80-90% of the person's beta cells have been damaged and are incapable to develop insulin[8,9].

Testing of Islet autoantibody is mainly used to assistin differentiating type 1 diabetes from the other causes of diabetes10.Presence of Islet autoantibodies like ICA, GADA, IA-2A, and/or ZnT8A confirms the diagnosis of type 1 diabetes and they are absent in those diabetic cases that are caused by the non-autoimmune response[11,12].

One study reported that IAA and ZnT8 usually occur in children less than 10 years of age, while the incidence of GAD65 and IA2 occurs in children greater than 10 years of age. It has also been revealed that GAD is also very common in females[13].

Although appropriate treatments are available proper dose of insulin, balanced diet, and physical exercises together with regular monitoring of blood glucose is mandatory. However, frequent observation of the management of diabetes improves glycemic control, while lower levels of Hemoglobin A1c (HbA1c) minimize the complications of diabetes[14].

In Pakistan, there is deficient data available on the prevalence of these autoantibodies in type 1 Diabetes mellitus. Therefore, this study was conducted to evaluate the prevalence of GADA, IA2A, and IAA concern in gage, gender, and duration of diabetes in T1DM children and adolescents in Pakistan

Patients and Methods

This was a retrospective study conducted at the Department of Endocrine and Diabetes by using non probability consecutive sampling technique, after taking ethical approval. The duration of the study was one year. A total of 143 individuals with clinically diagnosed type 1 diabetes ≥(1year to18 years of age were included in the study,while patients with hormonal disorders like thyroid diseases or any other congenital disorders were excluded from the study. Assent has been taken from participants where possible and permissionhas beentaken from the parents/legal guardianof all the participants.

All the patients were examined for pancreatic autoimmune markers i.e., GAD65, IA-2, IAAs. Further, GADA and IA-2A were assessedthrough enzyme-linked immunosorbent assay (ELISA). The ELISA test kit gives a quantitative in vitro test for autoantibodies against glutamic acid decarboxylase and tyrosine phosphatase in serum.

The demographic details such as age at the time of diagnosis, duration, and history of diabetes were documented. The collected data were then entered and evaluated using SPSS version 20. Frequency and percentages were calculated for constant variables like age, gender, and duration of diabetes. Pearson's Chi-square test was applied for the evaluationof association. A P-value of ≤ 0.05 was considered significant.

Results

A total of 143 individuals diagnosed with type 1 diabetes were enrolled for this study where in 71 (49.7%) were males and 72 (50.3%) were females. Regarding affected age, 9 (6.3%) children had <2 years of age, 24(16.8%) had 2-5 years of age, 42 (29.4%) had 6-10 years of age, 47 (32.9%) had 11-

15 years of age and 21 (14.7%) had > 15 years of age. Disease duration was observed <1 month in 68 (47.6%) children, 2-6 months in 18(12.6%) children, 7 months to 1 year in 12 (8.4%) children, 2-3 years in 15 (10.5%) children, 4-5 years in 18(12.6%) children, >5 years in 12 (8.4%) children. The distribution of positivity of autoantibodies showed that 27 (18.9%) had AntiInsulin IgG, 63 (44.1%) had GAD65, 47 (32.9%) had IA-2A and only 5 (3.5%) had grey zone, as shown in Table I.

Regarding the association of prevalence of Anti-Insulin IgG (IAA) with demographic characteristics, there was an insignificant association was found between age and IAA while it was positive in 7 (25.9%) in 2-5 years of age, 7 (25.9%) in > 15 years of age. Male patients had a higher prevalence of IAA than females that were observed in >5 years of duration of diabetes. However, there was no significant difference was observed in the positivity of IAA between female and male patients (p=0.268). Furthermore, there was a significant association was reported (p<0.001) in the duration of diabetes and prevalence of IAA, as shown in Table II.

As far as the association of prevalence of AntiGAD65 with demographic characteristics are concerned, there was an insignificant association was reported between age and AntiGAD65 while it was positive in 11 (17.5%) in 2-5 years of age, 19 (30.2%) in 6-10 years of age and 23 (36.5%) in 11-15 years of age. Male patients had a higher prevalence of AntiGAD65 than females with an insignificant association between them (p=0.112). In addition, there was an insignificant association was reported (p=0.585) in the duration of diabetes and prevalence of AntiGAD65, as shown in Table III.

About association of prevalence of Insulinoma-associated Antigen2 (IA-2A) with demographic characteristics, there was an insignificant association was shown between age and IA-2A,while it was positive in 12(25.5%) in 2-5 years of age, 15(31.9%) in 6-10 years of age, and 13(27.7%) in 11-15 years of age whereas 3(60.0%) patients were falling in the grey zone. Male patients had a slightly higher prevalence of IA2 than females and 4(80%) were in the grey zone with an insignificant association between them (p=0.352). Likewise, there was a significant association was reported (p=0.003) in the duration of diabetes and prevalence of IA-2A, as shown in Table IV.

Discussion

The present study demonstrated that T1DMin children and adolescentshad a higher occurrence of autoantibodies (GAD, IA-2A, and IAA) within the period of the disease.

One study reported the presence of GADA, IA-2A, or both in 89.4% of T1DM in children in the first year of their diagnosis and their occurrence was about 70% in the initialthree years. Hence, the existence of both IA-2A and GADA gives the highest predictive significance in T1DM[15]. Our study is inconsistent with the above-mentioned study and reported that presence of GADA in 27(42.9%) within 1 month after diagnosis, IA-2A in 24(51.1%) within 1 month after diagnosis, and IAA in 8(29.6%) > 5 years after diagnosed T1DM.

Table 1. Demographic Profile of participants (n=143)

Characteristics	n	%
Age		
< 2 year	9	6.3
2 - 5 year	24	16.8
6 -10 year	42	29.4
11 - 15 year	47	32.9
16 to 18 years	21	14.7
Gender		
Male	71	49.7
Female	72	50.3
Disease Duration		
< 1 Month	68	47.6
2-6 Months	18	12.6
7 - 1 year	12	8.4
2 - 3 year	15	10.5
4 - 5 year	18	12.6
> 5 year	12	8.4
AntiInsulinIgG		
Negative	116	81.1
positive	27	18.9
AntiGAD		
Negative	80	55.9
positive	63	44.1
InsulinomaAg		
Negative	91	63.6
positive	47	32.9
Grey Zone	5	3.5

Table 2. Association of Ant-insulin IgG (IAA) with demographic characteristics

Ant-insulin IgG	Negative		Positive		Pearson Chi-Square	df	p-value
Characteristics	n	%	n	%			
Age					6.603	4	.158
< 2 year	8	6.9	1	3.7			
2 - 5 years	17	14.7	7	25.9			
6 - 10 years	36	31.0	6	22.2			
11 - 15 years	41	35.3	6	22.2			
> 15 years (16-18)	14	12.1	7	25.9			
Gender					1.229	1	.268
Male	55	47.4	16	59.3			
Female	61	52.6	11	40.7			
Duration of DM					27.303	5	<0.001
< 1 Month	64	55.2	4	14.8			
2-6 Months	13	11.2	5	18.5			
7 - 1 year	10	8.6	2	7.4			
2 - 3 year	12	10.3	3	11.1			
4 - 5 year	13	11.2	5	18.5			
> 5 year	4	3.4	8	29.6			

Table 3. Association of AntiGAD65 with demographic characteristics

AntiGAD65	Negative		Positive		Pearson Chi-Square	df	p-value
Characteristics	n	%	n	%			
Age					1.908	4	0.753
< 2 year	6	7.5	3	4.8			
2 - 5 years	13	16.2	11	17.5			
6 - 10 years	23	28.8	19	30.2			
11 - 15 years	24	30.0	23	36.5			
> 15 years (16-18)	14	17.5	7	11.1			
Gender					2.529	1	0.112
Male	35	43.8	36	57.1			
Female	45	56.2	27	42.9			
Duration of DM					3.759	5	0.585
< 1 Month	41	51.2	27	42.9			
2-6 Months	8	10.0	10	15.9			
7 - 1 year	5	6.2	7	11.1			
2 - 3 year	7	8.8	8	12.7			
4 - 5 year	11	13.8	7	11.1			
> 5 year	8	10.0	4	6.3			

Table 4. Association of Insulinoma-associated Antigen2 (IA-2A) with demographic characteristics

Insulinoma-associated Antigen2	Negative		Positive		Grey Zone		Pearson Chi-Square	df	p-value
Characteristics	n	%	n	%	n	%			
Age									
< 2 year	9	9.9	0	0.0	0	.0%			
2 - 5 years	11	12.1	12	25.5	1	20.0%			
6 - 10 years	24	26.4	15	31.9	3	60.0%	13.190	8	0.105
11 - 15 years	34	37.4	13	27.7	0	.0%			
> 15 years (16-18)	13	14.3	7	14.9	1	20.0%			
Gender									
Male	43	47.3	24	51.1	4	80.0%	2.089	2	0.352
Female	48	52.7	23	48.9	1	20.0%			
Duration of DM									
< 1 Month	42	46.2	24	51.1	2	40.0%			
2-6 Months	8	8.8	10	21.3	0	.0%			
7 - 1 year	5	5.5	7	14.9	0	.0%	26.646	10	0.003
2 - 3 year	14	15.4	1	2.1	0	.0%			
4 - 5 year	12	13.2	3	6.4	3	60.0%			
> 5 year	10	11.0	2	4.3	0	.0%			

Insulin autoantibodies IAA.Normal range (>2.4 Positive) AntiGAD65 Normal range (>5.0 Positive)

Insulinoma-associated antigen-2 autoantibodies Normal range (8-10 Grey Zone, >10 Positive)One cohort study by Cheng BW, et al. on patients with T1D reported that there was no significant difference was found in both gender, butthe occurrence of GADA was observed more evident in females[16]. while no difference was reportedby other studies in gender.[17] Our study was inconsistent with the above-cited studies and reported that the frequency of GAD was more obvious in males than females but there was an insignificant association was observed in gender with GAD.

Similarly, one research identifiedthat there was no association was seen between the incidence of GADA and age at the time of diagnosis[16]. Moreover, no further analysis has been performed on Asian studies to the best of our review[18]. Our study is consistent with the above-said studies and revealed that there was no association was found in the prevalence of GADA and age at diagnosis.

Similarly, previous research showed that GADA and IA-2A autoantibodies both were present in 48.8% of patients, which shows the positivity of the said autoantibodies associated with each other. The disease duration in that study was 1.4 years. This recommends that the results of this study may more preciselyrespondto the accurateassociation between GADA and IA-2A16.A study of Northern Taiwan revealed that in a total of 174 patients with T1D, only 15.5% had both GADA and IA-2A which is considerably lower than the study by Cheng et al. and the disease duration in that study was 4.7 years that was significantly longer than the said study19. Our study was inconsistent with the above-cited studies and revealed that 63(44.1%) had GAD and 47(32.9%) had IA-2A autoantibody in TIDM while 68(47.2%) cases were diagnosed in <1 month of duration.

The presence of autoantibodies (GAD65, IA-2A, IAAs, ZnT8, or ICA) activates an autoimmune response of ß-cell devastationinthe pancreasthat signifies the severity of the condition, therefore the prevalence of many autoantibodies render the greatest positive diagnostic value for T1DM9,20. This also supported our study that showed the presence of one or more autoantibodies for instanceGAD65, IA-2A, and IAA triggers the autoimmune response against ?-cell and indicates the condition 's severity.

One more research revealed that the occurrence of GAD65 in T1DM was shown to have depended on gender and age at the time of diagnosis. The study reported that the diabetic females with positive GAD65 antibodies had higher levels of GAD65 antibodies and showed more destruction of ß-cell function compared with males[21]. As far as our study is concerned, it was shown that the presence of GAD65 in T1DM was not dependent on age, gender, and duration of diabetes. There was an insignificant association observed in presence of GAD65 with age, gender, and duration of diabetes. Moreover, males have higher GAD65 levels than females.

In another cohort study, a higherincidence of GAD65 antibodies was found in diabetic patients with the start of disease between the ages of 14 to 19 years (66.4%)[22]. Our study was inconsistent with the above-cited study and proved that the highest occurrence of GAD65 antibodies was reported in 23 (36.5%) patients with the commencement of disease between 11-15 years of age.

Similarly, one more research reported that the prevalence of ZnT8 autoantibody was found in 17.5% (32 of 183) individuals with type 1 diabetes with a mean duration of disease was 7.00 [2.00; 11.0] years[23].Our study was inconsistent with the above-mentioned study because our study reported the frequency of GAD 65, IA-2A, and IAA auto antibodies instead of the frequency of ZnT8 auto antibody with the age, gender,and duration of the disease.

The study has showed that the data has been collected from not a very large cohort of type 1 diabetes mellitus. Hence more prospective studies in this area are needed to develop strong causal association between the anitbodies and type 1 diabetes among children so that the disease may be predicted and diagnosed earlier in these children which may be helpful in preventing diabetes related complications in these children.

Conclusion

This study concluded that the presence of autoantibodies such as Insulinoma-associated antigen-2 autoantibodies, Insulin autoantibodies, and glutamic acid decarboxylase 65 are present in type I diabetes mellitus in children and adolescents and may predict type-I diabetes among children. Furthermore, these autoantibodies were found to be predominant in males than females although the frequency of these antibodies were not significantly associated with age and gender. Additionally, the presence of Insulinoma-associated antigen-2 autoantibodies, Insulin autoantibodies are alsosignificantly associated with the duration of disease.

Conflict of Interest

Authors have no conflict of interest and no grant/funding from any organization.

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