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RESEARCH ARTICLE

Pancreatic Autoantibodies in Sudanese Children with Newly Diagnosed Type 1 Diabetes Mellitus

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Abstract

Background: Immunepositive type one diabetes mellitus is the commonest cause of diabetes in children worldwide. Seronegative cases are said to be more common among black Africans. In a previous study in Sudan 46% of cases were found to have positive GAD antibodies.

Objectives of the study: The aim of this study was to find out the prevalence of pancreatic autoantibodies among multiethnic group of newly diagnosed Sudanese children by testing for multiple antibodies and to see how common are seronegative cases as reported in black Africans.

Subjects and methods: Eighty newly diagnosed children aged (1-18 years) of multiethnic groups with clinical diagnosis of type 1 diabetes mellitus were tested for 3 islet cell antibodies Glutamic Acid Decarboxylose (GADA), insulin autoantibodies (IAA) and Zinc Transporter (ZNT8A) using ELISA (ZNT8M GADA) and Radioimmunoassay (RIA) for IAA.

Demographic and clinical data were obtained from the records - clinical presentations of the….seronegative cases was compared with the seropositive cases.

Results: A positive result for one or more antibodies was found in 73 (91.2%) and negative in 7 (8.8%) of the cases- No ethnic variation was demonstrated. There was no difference in the clinical presentation of the two group. IAA was positive in 29 (36.3%), GADA in 62 (77.5%) and ZNT8A in 13 (16.3%). On testing for two antibodies, the best yield was on combining GADA and IAA 61 (76.3%).

Conclusion: Unlike the previous study from Sudan most (91.2%) of Sudanese children with Type 1 diabetes have got one or more pancreatic antibodies at onset. However 8.2% are seronegative. Further studies to find the causes in this seronegative group is needed.

Keywords

Pancreatic, Autoantibodies, Sudanese children

Abbreviations

BMI: Body Mass Index; CDC: Center of Diseases Control; C-peptide: Connecting peptide; DM: Diabetes Mellitus; ELISA: Enzyme Linked Immunosorbent Assay; GADA: Glutamic Acid Decarboxylase Autoantibodies; IAA: Insulin Autoantibodies; MODY: Maturity Onset Diabetes of the Young; T2: Type 2 Diabetes Mellitus; TIDM: Type 1 Diabetes Mellitus; WHO: World Health Organization; ZNT8A: Zinc Transporter & Autoantibodies

Introduction

Type 1 diabetes mellitus accounts for over 90% of childhood diabetes in the world including Africa [1] and Middle Eastern countries [2]. The incidence varies from 0.1% of cases to as high as 64/100000 in children under 15 years [3]. In a recent study we have found it to be 10.1/100000 in children under 18 years in Sudan [4]. Studies have shown that up to 90% of those of Northern European origin have raised levels of at least one antibody at diagnosis while they are less frequently found in black Africans or African Americans [5-10]. In one study from Sudan using Glutamic Acid Decarboxylase antibodies only 46% were found to be seropositive [10].

The aim of this study was to see the prevalence of pancreatic autoantibodies in children with type 1 diabetes using three tests to find out whether the low prevalence reported before was due to using

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one antibody or whether this is related to the ethnic population of Sudan.

Methods

All Sudanese children aged 1-18 years who were diagnosed clinically as having type 1 diabetes mellitus by their treating endocrinologist within 6 months of onset and were attending the three main childhood diabetes clinics in Khartoum State (Sudan Childhood Diabetes Center, Omdurman Children's' Hospital and Ahmed Gasim Children's Hospital) between July 2011 and May 2012 were included in the study. Clinical data including age, sex, tribe, socioeconomic status, clinical presentation at onset, weight, height, BMI, were obtained from the records. CDC BMI charts were used to classify BMI..Children who were diagnosed clinically or by investigations to have other forms of diabetes e.g type 2, MODY or neonatal diabetes were excluded.

Auto antibodies assay

3 ml of venous blood were collected to a plain tube from each patient. Blood was centrifuged and separated and serum stored at -20 °C till testing.

Insulin auto antibodies were tested by radio immunology assay (RIA) using RIA Kits (1125RIA system -Wuhan Elabscience Company (www.elabscience.com). The test was considered positive if the binding rate was > 5%. The specificity and sensitivity of this test is 36% and 95% respectively.

The anti-Zn T8 (Zinc Transporter 8) antibodies were tested with ELISA using methods as described by the manufacturer (Euro immune company L TO-UK) The cut-off point for positive test was 15 units per million (IU/MI) with specificity and sensitivity of 99% and 68% respectively.

Glutamic Acid Decarboxylase (GADA) antibodies were also tested with ELISA Kits using methods as described by the manufacturer (Euro immune company LTO-UK) with 10 IU/MI being the cut-off point for being positive, the test having specificity and sensitivity of 98% and 92% respectively.

Statistical Analysis

Data were analyzed using SPSS version 17 (Chicago IL USA) Comparison between groups was done by one way ANOVA test or Chi-square test as appropriate. Correlation analysis was done for variables. One-tailed p. value < 0.05 was considered statistically significant.

Results

A total of 80 patients, 42 males (52.5%) and 38 females (47.5%) were included in the study. The mean age was 9.7 years (± 4.3) with a range of 13-17 years. Fifteen patients (18.8%) were of pure African origin and the rest (81.2%) were of Arab or African-Arab mixture.

The mean duration of symptoms at diagnosis was 2.2 weeks (± 0.073). Fifty-five patients (68.8%) presented at onset with DKA. Two children were obese, three (3.9%) were overweight and 26 (32.9%) had BMI below the 5th centile. Eleven patients (13.8%) and 29 (36.3%) gave family history of type 1 and type 2 diabetes mellitus respectively. In addition, 4 (5%) and 25 (31%) gave family history of coeliac disease and autoimmune thyroiditis respectively. The mean duration of the disease at testing was 7.6 weeks (± 7.l).

Table 1: The prevalence of pancreatic auto antibodies among the study population: (n = 80).

Autoantibodies	Number	%
GADA	62	77.5
IAA	29	36.5
ZNT8A	13	16.3
One or more antibody	73	91.2
Negative for all antibodies	7	8.75

Table 2: The value of combining testing for 2 antibodies.

Antibodies	Number	%
GADA + IAA	70	87.5
GADA + ZNT8A	65	81.3

P < 0.05.

Table 3: Correlation of IAA frequency with age and sex (n = 29).

Group	Positive n (%)	Negative n (%)	P value
Age (years)			
1-5	9 (60)	6 (40)	
5-10	6 (26.1)	17 (73.9)	0.177
10-15	12 (34.3)	23 (65.7)	
> 15	2 (28.6)	5 (71.4)	
Sex			
Male	17 (40.5)	25 (59.5)	0.408
Female	12 (31.6)	26 (68.4)	

The prevalence of autoantibodies in the study group is shown in Table 1. Seven patients (8.8%) were negative for all antibodies and 73 (91.2) were positive for one or more antibodies. The commonest was GADA antibodies (77.5%) and the least was ZNT8A (16.25%).

Table 2 shows the value of combining testing for another antibody with GADA. 1AA seems superior to ZNT8A 70 (87.9%) versus 65 (82.3%). p < 0.05.

Insulin auto antibodies were found in 29 (36.5%) of the patients. Table 3 shows the frequency of 1AA among various age groups and sex. There was no significant difference between males and females and the highest frequency was among the 10-15 years age group.

GADA antibodies frequencies are shown in Table 4. They were positive in 77.5% of the cases. The highest frequency was among the 5-10-year age group, but there was no statistically significant difference between the various age groups or sex. Those with duration of

the disease of more than 30 days showed significant increase in antibody positivity.

Table 5 shows the frequency of ZNT8A among the study population- There was no significant difference among various age groups or sex.

Table 6 shows the prevalence of autoantibodies among different ethnic groups. Fifty cases (62.S%) came from tribes of Arab origin, I5 (18.8%) mixed ethnical groups and 15 (18-8%) were of pure African descent No significant difference in prevalence of antibodies was demonstrated among the various ethnic groups.

Four patients had age below 2 years and their weight for height was normal, as CDC charts don't have BMI for below 2 yrs. The mean BMI of the rest was I5.7 (± 3.7), 3.9% were overweight and 2.6%) obese. Whereas (39.2%) had BMI below the third centile. There was no significant difference in the prevalence of antibodies between those with BMI above the 85th and those below it.

Comparison of the clinical presentation between the seronegative and seropositive groups is shown in Table 7. There was no significant clinical difference between the two groups. In particular we didn't encounter any case that showed a fulminant course with exocrine and endocrine dysfunction. One of the seronegative cases gave a typical family history of MODY, but no genetic testing or c-peptide levels were done on them.

None of the seronegative patients was obese or overweight or had acanthosis nigricans to suggest type 2 diabetes.

Table 4: Correlation of GADA antibodies with age and sex (n = 62).

Group	Positive n (%)	Negative n (%)	P value
Age (years)			
1-5	10 (66.7)	5 (33.3)	
5-10	21 (91.3)	2 (8.7)	0.209
10-15	25 (71.4)	10 (28.6)	
> 15	6 (85.7)	1 (14.3)	
Sex			
Male	31 (73.8)	11 (26.2)	
Female	31 (81.6)	7 (18.4)	

Table 5: Correlation of ZNT8A with age and sex (n = 13).

Group	Positive n (%)	Negative n (%)	P value
Age (years)			
1-5	2 (13.3)	13 (86.7)	
5-10	3 (13)	20 (87)	0.885
10-15	7 (20)	28 (80)	
> 15	1 (14.3)	6 (85.7)	
Sex			
Male	5 (11.9)	37 (88.1)	0.268
Female	8 (21.1)	30 (78.9)	

Table 6: The prevalence of antibodies among different ethnic groups.

Ethnic Group	Positive n (%)	Negative n (%)	Total
African	12 (80)	3 (20)	15 (100)
Arab	48 (96)	2 (4)	50 (100)
Others (Mixed)	13 (86.7)	2 (13.3)	15 (10)

P = 0.296.

Discussion

This is the first study from Sudan to use multiple antibodies-testing including ZNT8A in patients clinically diagnosed as having type 1 Diabetes mellitus and to look into the prevalence of antibodies-negative type 1 diabetes in this country. Unlike the findings on one previous study from Sudan [10] where only 46.1% of newly diagnosed type 1 children with diabetes were found to have positive GADA antibodies, we have found that 91.2% of our cases were having one or more antibodies and only 8.8% were tested antibodies negative indicating that most cases of type I diabetes in this multiethnic population have immune mediated disease. This shows the importance of using multiple antibodies testing before ruling out immune mediated type 1 diabetes mellitus. Our finding is similar to a figure of 90.7% that has been reported from Tunisia [11]

Table 7: Comparison of clinical data between antibodies positive and negative groups.

Clinical data	Seropositive	Seronegative	P value
Number of patients	73	7	
Age (years)	9.61 (± 1.03)	11.3 (± 27)	0.327
Male	40	2	0.184
Female	33	5	
BMI (kg/m^2)	15.55 (± 0.75)	17.3 (± 5.1)	0.249
Polyuria	71	7	0.832
Polydipsia	71	7	0.832
Weight loss	58	6	0.557
DKA at onset	49	6	0.311
Duration of symptoms before diagnosis	15.82 (± 1.3)	14 (± 3.1)	0.388

but a bit less than what was reported from European countries 95.2% [12]. Using multiple antibodies testing however might be expensive and if at all one is to use two antibodies rather than three, we have found that combining 1AA with GADA is sensitive than combining it with ZNT8A. This is similar to a report from Tunisia [11]. During SEARCH study [13] 15.1% of type 1 diabetes patients were autoantibody negative. These findings may reflect variation in assay standardization, number of auto antibodies measured, variation in population studied or existence of other forms of non-immune mediated diabetes.

IAA assay has a sensitivity of 95% and specialty of 36%. Insulin auto antibodies were detected in 36% of our patients and this falls in the lower limit of figures of 40-70% reported in literature [5-8,14,15]. It is similar to data from Saudi Arabia [16] and Japan [17] but lower than figures of 69% [18] and 50% reported from UK and Tunisia [11,18] respectively. This wide range in the frequency of IAA may be due to heterogeneity of IAA repertoire recognizing different epitopes of Insulin [19]. The highest prevalence of IAA was among the 1-5 years age group (60%) followed by the 10-15 years group (34.3%). However, difference between various age groups was not statistically significant. This is similar to data from Saudi Arabia [16] and Indo- Aryan population [20] but lower than figures of 90% reported from Australia for this age group [18]. There was no significant difference among males (41.4%) and females (58.6%). This is similar to study from Japan [17] but different from Australia where the frequency was higher among males (82%) compared to females (60%) [21]. However some of these IAA could be due to exogenous insulin and some authors prefer IA2A antibodies than IAA as a more specific marker of islet cell antibodies.

GADA antibodies were present in 77.5% of the study population. This is higher than the previous findings of (46.1%) from Sudan [10], similar to others [21] and lower than those reported from Caucasians (80%) and Japanese (82%) [22]. It is however higher than some regional figures reported among Syrian (34%), Jordanians [23], Saudis [16] and Tunisians [11]. The highest frequency (71%) was found among the 10-15 year - age group though the difference between age groups was not significant. This is similar to what has been reported from Sudan and other countries [9,10,24] but in Japanese it peaked among the 13-19 years age group [17]. There was no difference among males and females as has been reported from Japan [17]. However, in Caucasians there was higher frequency in females (88%) compared to males (71%) [25].

Zink transporter 8 auto antibodies (ZnT8A) assay has a sensitivity of 68% and specificity of 99%. No data about this assay is available from Sudan, or regional countries. Therefore, our study is the first to report about it. In this study ZnT8A were present in 16.5% of cases. It is lower than the frequency of 60-80% reported in Caucasian population [26]. However, it is similar to figure reported from Chinese (24.1%) and Japanese [27]. The levels peaked among age group 10-15 years but the difference between various age groups was not significant. This is in contrast to findings of Lin Yang, et al. in Japan [27] where the peak was around the age of 9 years with significant difference among various age groups. There was no significant difference between males and females similar to the findings of Lint Young, et al. [27]. The ZNT8A has 3 polymorphism at the amino acid position 325 with R, W and Q. The Q isoform is minor mostly present in black people whereas many of the commercial kits are focused towards testing for the the R and W isoforms which are found in the Whites and Asians. So, these variations could be related to types of kits. Kits containing the Q isoform could give higher results in blacks. We did not look into this issue in our study.

The mean age of diagnosis of seronegative group was 11.3 years (± 2.7), which was higher than that of the seropositive group 9.6 (± 1.03), but this was not statistically (2.15) significant. This is contrary to the results reported by Pinero-Pilon, et al. [28] which showed that the age of diagnosis of seronegative cases is higher than the sero-positive group. In this study there was no significant difference between males and female's. This is different from what has been reported by Umpierrez among African-Americans [29] who found significantly higher prevalence among males. Though most of our seronegative cases were from on-Arab African group (42.9%), the difference from Arab ethnic group was not statistically significant. This is in contrast to the higher prevalence previously reported among African Americans [29]. This could be due to more mixed ethnic groups that we encounter in Sudan the mean BMI (17.3 ± 5.1) kg/m^2 was higher among the seronegative but this difference was not statistically significant. This is similar of study from India [30], but lower than data reported by Pinero-Pilon, et al. from Dalas [28] and Ji Hae, et al. from Korea [31].

There was no statistically significant difference in duration of symptoms between the seronegative and seropositive group similar to what has been previously reported by Krishna Murthy, et al. from India [30]. Though 85.7% of seronegative group had DKA at presentation compared to 67.1% in seropositive group '. ~ This difference was not statistically significant. This is similar to was reported before [32]. Almost 28.6% of antibody negative group were exclusively breast - fed compared to 4.1% of seropositive group. This could possibly support the hypothesis of protective of effect of breast feeding [33].

In conclusion by testing for three islet auto antibodies we have shown that in 91.2% of Sudanese children with type 1 diabetes, the disease is immune

mediated. This figure could have been higher if a fourth antibody like IA2 was used. In this multiethnic African population idiopathic (or non-immune) type 1 is not as common as in the Black African Americans and even in the seronegative group the clinical presentation is not significantly different from the seropositive group. The fulminant course of the seronegative group as seen in Japan has not been demonstrated among our seronegative population. Further studies with larger numbers and from other parts of the country are needed to validate our findings and to see whether the antibody negative group is having other forms of diabetes such as fibrocalculous pancreatitis, MODY, type 2 diabetes or other forms of monogenic diabetes as was discussed and shown in other recent studies [1,34,35].

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Ethical Approval

A written consent was obtained from parents and the study was approved by the Ethical Boards of the concerned hospitals and Sudan Medical Specialization Board.

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