Age of Appearance of Diabetes Autoantibodies in Type 1a Diabetics and Their Siblings in Côte D'Ivoire

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Abstract Diabetes autoantibodies, which are important markers of classification and prevention of type 1 diabetes, are absent in the diabetes care in Côte d'Ivoire. The study aim was to determine the age at which these autoantibodies were at their highest levels in type 1A diabetic patients (T1D) and their siblings in Côte d'Ivoire. The study population is composed of 28 T1D patients and 59 siblings, aged from 5 to 21 years. T1D were followed up in the U.H.C Endocrinology departments of Yopougon and Treichville, in the District of Abidjan. Anti-GAD and anti-IA2 autoantibodies were researched by ELISA and anti-ICA autoantibodies by indirect immunofluorescence on monkey pancreas. The peaks of anti-GAD and anti-IA2 autoantibodies were observed respectively at 9 years old (604.30 ± 521.70 IU/ml) and 10 years old (125.10 ± 90.10 IU/ml) in T1D. In siblings, the peaks of anti-GAD and anti-IA2 autoantibodies were observed respectively at 13 years old (137 IU/ml) and 10 years old (53 ± 40 IU/ml). The peak of diabetes autoantibodies in T1D and their siblings in Côte d'Ivoire is at a very young age noted. This could explain the high mortality rate among T1D youth in Côte d'Ivoire.

Keywords Diabetes Autoantibodies, Type 1A Diabetes, Age of Peak of Autoantibodies, Côte d'Ivoire

1. Introduction

Type 1A diabetes or juvenile insulin-dependent diabetes is a disease that affects more than 31 million people worldwide [1]. In Africa, 39,100 children aged from 0- to 14 years have type 1A diabetes in 2013 [2] and 0.4% of children and adolescents in Côte d'Ivoire [3]. Type 1A

diabetes is an autoimmune disease, the final consequence of a slow and gradual process of pancreatic islet Langerhans cells leading β cells destruction, in the absence of treatment, to ketoacidosis [4]. β cells destruction affects insulin production, and begins the initiation of the autoimmune reaction triggered by certain environmental factors and, after several years of evolution, leads to the clinical signs of the disease when the mass of β cells becomes insufficient to regulate blood glucose [5]. Thus, the development of the autoimmune reaction, characterized by the progressive infiltration of Langerhans islets, would be carried out without destruction of the β cells. Subsequently, under the effect of a hypothetical environmental triggering factor, the effectors responsible for the destruction of these β cells would be established. During this longer or shorter period, which can be defined as a pre-diabetic stage, certain signs indicative of β -cell lesion can already be demonstrated, in particular anti-pancreas autoantibodies [6]. Type 1A diabetes or juvenile insulin-dependent diabetes is a major public health problem due to the often early onset of the disease, increasing incidence in most populations, lack of curative treatment and vascular complications related to residual hyperglycemia despite insulin injections in treated patients [7]. It is an incurable disease that is gaining momentum in Africa and particularly in Côte d'Ivoire. Today, the diagnosis of type 1A diabetes is biological (fasting glucose greater than 1.26 g / l) and requires the search for autoantibodies of diabetes as a confirmation marker for autoimmune process [8-10]. According to several authors, these autoantibodies of diabetes are present in siblings of type 1A diabetics (T1D) and are markers of the imminence of diabetes in this population at risk [11-13]. In Côte d'Ivoire, there is an absence of diagnosis of diabetes autoantibodies in the diabetes care, resulting in incomplete

care. The search for autoantibodies is essential in the prevention of type 1A diabetes in the siblings of patients, but also in the classification of diabetics, some of whom are taken as type 2 diabetics when they are actually type 1A. It is therefore important to diagnose diabetes as early as possible in order to avoid the rapid evolution of the disease and its aggravation in the long term. The aim of this work is to show the presence of diabetes autoantibodies in T1D and their siblings and to determine the peak of these autoantibodies in T1D and their siblings aged from 5 to 21 years in Côte d'Ivoire.

2. Material and Methods

Material

Study Population

The study population was composed of 87 people. It included 28 known T1D patients aged from 5 to 21 years and followed up in two diabetes care centers in Abidjan district, Côte d'Ivoire. The Endocrinology department of the University Hospital Center (U.H.C) of Yopougon and the Diabetes Clinic of U.H.C of Treichville. Some T1D have been recruited from two NGOs, the Diabetics Association of Côte d'Ivoire (DIACI) and the New Diabetic Association of Côte d'Ivoire (NDIACI). The study population was also composed of 59 apparently healthy consanguineous siblings of the selected T1D patients, also aged from 5 to 21 years. The sex ratio of the whole study population (28 patients and 59 healthy controls) was 0.89 (41 boys and 46 girls); the mean age of T1D was 12.62 ± 2.75 years and that of diabetic siblings was 12.13 ± 4.94 years. This cross-sectional study started in January 2014 and ended in April 2016.

Selection criteria

1. Inclusion criteria

The T1D selected were aged from 5 to 21 years and declared diabetics between 2007 and 2016. They had at least one non-diabetic sibling aged from 5 to 21 and a complete medical folder of T1D.

2. Non-inclusion criteria

HIV-positive patients undergoing antiretroviral therapy were excluded from the study because of the impact of ARVs that would lead to hyperglycemia.

Biological Material

The biological material was composed essentially of serum. These sera were obtained after centrifugation at 3000 rpm for 3 minutes of venous blood collected on dry tubes in T1D and their siblings.

Methods

Selection of Type 1A Diabetics and Their Siblings

The project was approved by the ethics committee of Pasteur Institute of Côte d'Ivoire. It consisted in selecting T1D in Yopougon and Treichville U.H.C as well as NOGs, DIACI and NDIACI. Folders of T1D aged from 5 to 21 years were selected. The year of screening for diabetes was between 2007 and 2016. The contacts of the parents of each patient (telephone, PO BOX and email) were identified and each parent of the patients was then contacted by telephone. During the conversations, they were told about the benefit of the study, which was to make early detection of type 1A diabetes in their families, to prevent other coming years cases in their family. It was asked after a good understanding to each parent, the existence or not of siblings of the diabetic child aged from 5 to 21 years. T1D who met these conditions were selected. The parents were invited with their children in the different U.H.C for sampling. On the site, parents were invited to sign informed consent cards. They were then offered a consent form for the child's consent and a survey containing clinical information for diabetics on the one hand and for siblings on the other hand. For the illiterate parents, the consent has been materialized by a borrowing of the right index finger in the presence of a witness who also has given this agreement by signature of the same form.

Search of Anti-islet Cells Autoantibodies

This research is done by indirect immunofluorescence on monkey pancreas using a commercial kit (Euroimmun®, Germany). Sections of monkey pancreas are incubated with the patient's diluted serum. The reaction is positive if the IgA, IgG and IgM class-specific antibodies bind to the β -cells of the Langerhans islets of the monkey pancreas. In the second step, fluorescein-labeled anti-human IgG antibodies bind to the antibody-antigen complex for fluorescence microscopy detection [14-16].

Search of Anti-glutamic Acid Decarboxylase Autoantibodies and Anti-tyrosine Phosphatase IA2 Autoantibodies

The dosage of these two autoantibodies is done by ELISA method. The anti-GAD ELISA is a quantitative in vitro assay for GADA. Patient serum is incubated with GAD coated onto a microplate. If sample is positive, specific autoantibodies bind to the GAD. Bound antibodies are able to act divalently and form a bridge between GAD on microplate well and biotin-labeled GAD, which is added in a second incubation step. To detect the bound biotin, a third incubation is carried out using enzyme-labeled avidin, which is capable of promoting a color reaction. The intensity of the color formed is proportional to the antibody against GAD.

The anti-IA2 ELISA is a quantitative in vitro assay for IA2A. Patient serum is incubated with IA2 coated onto a microplate. If sample is positive, specific autoantibodies bind to the IA2. Bound antibodies are able to act divalently and form a bridge between IA2 on microplate well and biotin-labeled IA2, which is added in a second incubation step. To detect the bound biotin, a third incubation is carried out using enzyme-labeled avidin, which is capable of promoting a color reaction. The intensity of the color formed is proportional to the antibody against IA2 [8, 17-19].

Statistical Analysis

Statistical analysis of the data was done with the GraphPad Prism.V5.01 software. The Student's t-test allowed statistical analysis of the means between T1D and their siblings. The difference between two variances was

significant if $p < 0.05$.

3. Results

The anti-islet cells antibodies (anti-ICA) were present in 1/28 T1D (F16A) and absent in siblings, diagnosed in 2014. Anti-glutamic acid decarboxylase autoantibodies (anti-GAD) were present in 16/28 T1D patients (57.14%) and 2/59 in siblings (F13B and F25C) (3.39%) (Table 1). The mean value of anti-GAD autoantibodies in T1D was 448.3 ± 126.5 IU / ml and 128.5 ± 8.50 IU / ml in siblings ($p < 0.0001$). It should be noted that anti-phosphatase autoantibodies (anti-IA2) were present in 13/28 T1D (46.43%) and 10/59 in siblings (16.95%) (Table 1). The mean value of IA2 autoantibodies in T1D was 114.2 ± 37.42 IU / ml and 33.17 ± 9.6 IU / ml in siblings ($p = 0.0021 < 0.05$).

Table 1. Age, autoantibodies anti-islet cells, anti-GAD and anti-IA2 in type 1A diabetics and their siblings

Type 1A Diabetics	Identification	Age and sex of patient	Anti-Islet Cells (indirect immunofluorescence)	Anti-GAD (ELISA) (UI/ml)	Anti-IA2 (ELISA) (UI/ml)
1	F1A	14 ans / M	Négative	0	0
2	F2A	10 ans / F	Négative	0	24.6
3	F3A	12 ans / F	Négative	0	0
4	F4A	15 ans / M	Négative	0	0
5	F5A	18 ans / F	Négative	117	0
6	F6A	12 ans / F	Négative	52.9	33.2
7	F7A	9 ans / M	Négative	82.6	112
8	F8A	13 ans / M	Négative	0	0
9	F9A	15 ans / M	Négative	101	0
10	F10A	9 ans / M	Négative	1126	0
11	F11A	18 ans / M	Négative	0	119
12	F12A	13 ans / F	Négative	0	0
13	F13A	12 ans / F	Négative	1139	38.4
14	F14A	18 ans / M	Négative	10.4	55.1
15	F15A	10 ans / M	Négative	36.7	11.9
16	F16A	10 ans / F	Positive	1432	464
17	F17A	11 ans / F	Négative	0	0
18	F18A	17 ans / M	Négative	97,5	0
19	F19A	14 ans / F	Négative	785	16.4
20	F20A	11 ans / M	Négative	0	321
21	F21A	14 ans / F	Négative	113.2	164.5
22	F22A	10 ans / F	Négative	541,2	0
23	F23A	13 ans / M	Négative	0	0
24	F24A	13 ans / F	Négative	0	0
25	F25A	12 ans / F	Négative	1128	36.2
26	F26A	11 ans / F	Négative	0	0
27	F27A	12 ans / F	Négative	458	89
28	F28A	15 ans / M	Négative	25.6	0
Siblings					
1	F1B	10 ans / M	Négative	0	0
	F1C	9 ans / F	Négative	0	0
	F1D	21 ans / F	Négative	0	0
2	F2B	15 ans / F	Négative	0	10.7
	F2C	9 ans / M	Négative	0	0
3	F3B	12 ans / F	Négative	0	0
	F3C	13 ans / M	Négative	0	42.6
4	F4B	12 ans / F	Négative	0	0
	F4C	9 ans / F	Négative	0	0
5	F5B	11 ans / F	Négative	0	0
6	F6B	17 ans / F	Négative	0	0
	F6C	12 ans / F	Négative	0	0
7	F7B	21 ans / M	Négative	0	0
	F7C	15 ans / F	Négative	0	10.8

F1A= type 1A diabetic of family 1; F1B, C, D= siblings of type 1A diabetics; <10 IU/ml= négative, ≥10 IU/ml= positive

Table 1 (after)

Siblings	Identification	Age and sex of patient	Anti-Islet Cells (indirect immunofluorescence)	Anti-GAD (ELISA) (UI/ml)	Anti-IA2 (ELISA) (UI/ml)
8	F8B	11 ans /F	Négative	0	0
	F8C	9 ans /M	Négative	0	0
9	F9B	21 ans / F	Négative	0	0
	F9C	11 ans / M	Négative	0	0
10	F10B	12 ans / F	Négative	0	0
	F10C	21 ans /F	Négative	0	0
11	F11B	16 ans / M	Négative	0	0
	F11C	11 ans / F	Négative	0	0
12	F12B	10 ans /M	Négative	0	106
	F12C	12 ans / F	Négative	0	0
13	F13B	5 ans / M	Négative	120	12.4
	F13C	11 ans / F	Négative	0	0
14	F14B	14 ans / M	Négative	0	0
	F14C	13 ans / F	Négative	0	0
15	F15B	9 ans /M	Négative	0	0
	F15C	14 ans / M	Négative	0	0
16	F16B	8 ans /F	Négative	0	0
	F16C	13 ans /F	Négative	0	0
17	F17B	8 ans / M	Négative	0	0
	F17C	14 ans / F	Négative	0	0
18	F18B	12 ans / F	Négative	0	0
	F18C	8 ans / F	Négative	0	0
19	F19B	18 ans /F	Négative	0	0
	F19C	11 ans / M	Négative	0	0
20	F19D	5 ans / F	Négative	0	0
	F20B	12 ans /M	Négative	0	0
21	F20C	13 ans / F	Négative	0	32.8
	F21B	15 ans /M	Négative	0	18.7
22	F21C	8 ans /F	Négative	0	0
	F22B	16 ans /M	Négative	0	0
23	F22C	13 ans/M	Négative	0	0
	F23B	7 ans / F	Négative	0	0
24	F23C	17 ans/M	Négative	0	0
	F23D	18 ans / M	Négative	0	0
25	F24B	8 ans / M	Négative	0	0
	F24C	11 ans /M	Négative	0	0
26	F25B	15 ans /M	Négative	0	0
	F25C	13 ans / F	Négative	137	61
27	F26B	14 ans /M	Négative	0	0
	F26C	21 ans /M	Négative	0	0
28	F26D	5 ans / F	Négative	0	13.2
	F27B	11 ans / M	Négative	0	0
29	F27C	14 ans /M	Négative	0	0
	F28B	13 ans / F	Négative	0	23.7
30	F28C	17 ans / M	Négative	0	0

F1B, C, D= siblings of type 1A diabetics; <10 IU/ml= négative, ≥10 IU/ml= positive

Fig.1A showed the titer of anti-GAD autoantibodies according to T1D age. Indeed, the peak of anti-GAD autoantibodies in T1D in Côte d'Ivoire was observed at the age of 9 years with a maximum value of 604.30 ± 521.70 IU / ml. Then, a decrease in the values of the anti-GAD autoantibodies was observed in T1D at the age of 10 years, 12 years and 14 years (502.50 ± 333.50 , 352.60 ± 212.20 , 301.00 ± 245) IU / ml, followed a fall in these values at 15 years, 17 years and 18 years (42.20 ± 30.31 , 97.50 ± 00 , 10.40 ± 00) IU / ml (Figure 1A).

Fig. 1B showed the titer of anti-IA2 autoantibodies according to T1D age. At the age of 9 years, the titer of anti-IA2 autoantibodies in T1D was 56 ± 50 IU / ml, then a peak of these anti-IA2 autoantibodies was observed at the age of 10 years with a maximum value of 125.10 ± 90.10 IU / ml. At 12, 14 and 18 years old, a decrease of the values of anti-IA2 autoantibodies was observed with respective titers of 49.20 ± 13.31 IU / ml, 60.30 ± 52.31 IU / ml and 58.03 ± 34.38 IU / ml (Figure 1B).

Figure 1. (Part A and Part B): Mean value of anti-GAD and anti-IA2 autoantibodies according to the age of type 1A diabetes patients

In siblings of T1D, the titer of anti-GAD autoantibodies at 5 years old was 120 IU / ml. The peak was observed at 13 years old with a maximum value of 137 IU / ml (Figure 2A).

At 5 years old, the titer of anti-IA2 autoantibodies in

siblings of T1D was 11.60 ± 4.27 IU / ml, then a peak of these autoantibodies was observed at the age of 10 years with a maximum value of 53 ± 40 IU / ml (fig. 2b). Then, a decrease of the values of anti-IA2 autoantibodies was observed at the age of 13 years and 15 years with the respective titers of 40.03 ± 7.98 IU / ml and 13.40 ± 2.65 IU / ml (Figure 2B).

Figure 2. (Part A and Part B): Mean value of anti-GAD and anti-IA2 autoantibodies according to the age of type 1A diabetic siblings

4. Discussion

The results showed that glutamic acid decarboxylase autoantibodies (anti-GAD) and phosphatase autoantibodies IA2 (anti-IA2) were present in T1D and their siblings with a significant difference ($p < 0.0001$; $p = 0.0021 < 0.05$). Islet cells autoantibodies were present in a single T1D and absent in siblings. These results show that T1D who developed these autoantibodies are actually T1D. Indeed, type 1A diabetes is an autoimmune disease due to the destruction of pancreatic islet β -cells by T cells. These results corroborate those of Delic-Sarac et al. [10], who found anti-GAD and anti-IA2 autoantibodies in the diabetic patient's serum. The presence of these autoantibodies in siblings of T1D shows that they constitute a population at risk for diabetes, as Siljander et al.

[12], De Grijse et al. [13] and Simonen-Tikka et al. [20], showed in their study. Anti-ICA, anti-GAD and anti-IA2 auto-antibodies are very important markers for screening for type 1A diabetes.

The peak of anti-GAD autoantibodies observed at 9 years old (604.30 ± 521.70 IU / ml) and the peak of anti-IA2 autoantibodies observed at the age of 10 years (125.10 ± 90.10 IU / ml) in T1D show that the autoimmune reaction in these patients is maximal. Thereby, the body produces a large amount of anti-GAD and anti-IA2 autoantibodies directed against glutamic acid decarboxylase and tyrosine phosphatase IA2 located in pancreatic β -cells. The age of 9 years old would be the favorable age for the production of anti-GAD autoantibodies and the age of 10 years old would be the favorable age for the production of anti-IA2 autoantibodies in T1D in Côte d'Ivoire. These results corroborate those of Boitard and Mallone [7].

In siblings, the peak of anti-GAD autoantibodies observed at 13 years old (137 IU / ml) and the peak of anti-IA2 autoantibodies observed at 10 years old (53 ± 40 IU / ml) show that these patients constitute a population at risk for diabetes. These results show that the autoimmune reaction in siblings of TD1 has started. Thereby, at 13 and 10 years old their organs produce a large amount of anti-GAD and anti-IA2 autoantibodies directed against glutamic acid decarboxylase and tyrosine phosphatase IA2 located in pancreatic β -cells. These patients should be followed to avoid the installation of clinical diabetes. 13 years old and 10 years old would be favorable for the production of anti-GAD and anti-IA2 in siblings of T1D in Côte d'Ivoire. These results corroborate those of Andrea et al. [21], in a cohort study shown that the age of appearance of autoantibody was a major determinant of the age at diabetes diagnosis, accounting for 47% of the variance ($r = 0.69$, $P < 0.0001$). The mean age of appearance of first autoantibody varied by group: 6.1, 5.5, and 3.8 years for one, two, or three antibodies, respectively ($P = 0.0007$).

5. Conclusions

This study showed the importance of searching diabetes autoantibodies in T1D and their siblings in Côte d'Ivoire. In fact, these autoantibodies constitute an important classification criterion for diabetes and a marker for early detection of diabetes in siblings of T1D. Indeed, anti-GAD and anti-IA2 autoantibodies are produced in large quantities in T1D at the respective ages of 9 years old and 10 years old. In siblings, anti-GAD and anti-IA2 autoantibodies are produced in large quantities at the respective ages of 13 years old and 10 years old. This could explain the high mortality rate among young T1Ds. This research will allow a biological application of diabetes autoantibodies to improve the diabetes care in Côte d'Ivoire.

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