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AUTOANTIBODIES DIABETES AND MICRONUTRIENTS IN TYPE 1 DIABETICS AND SIBLINGS OF ABIDJAN DISTRICT, IVORY COAST.

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ABSTRACT

Context: the presence of diabetes autoantibodies has been reported as a factor of predisposition of diabetes in siblings of type 1 diabetics. **Objective**: Search autoantibodies of diabetes anti-ICA, anti-GAD, anti-IA2 in type 1 diabetics and their siblings of district of Abidjan, Ivory Coast, associated with the dosage of micronutrients such as phosphorus, magnesium and calcium. **Materials and methods**: The study population consisted of 49 people, including 19 type 1 diabetics (T1D) and 30 siblings whose first degree of the blood was taken. T1D were recruited to University Hospital center (U.H.C) of Yopougon and Treichville and two NGOs. Serum obtained allowed the determination of anti-islet cell autoantibodies (anti-ICA) detected by the immunofluorescence method on monkey pancreas, anti-glutamic acid decarboxylase autoantibodies (anti-GAD) and anti- phosphatase IA2 detected by ELISA. The dosage of phosphorus, magnesium and calcium was made by the cobas 6000 Roche/Hitachi. **Results:** Anti-ICA was positive to 5.26% in T1D and absent in siblings, anti-GAD were positive in the T1D to 57.89% and 3.33% for the siblings. The anti-IA2 was positive in the T1D to 47.36% and 16.66% for siblings. The anti-ICA-GAD-IA2 combination was present in 5.26% of T1D and absent in the siblings. The GAD-IA2 combination was present in 31.57% of T1D and 3.33% among siblings. The average phosphorus in T1D was 1.86±1.06 mmol/L versus 2.39±1.51mmol/L in the siblings; twice higher than normal values. **Conclusion:** Autoantibodies diabetes in siblings is a marker for the imminence of autoimmune diabetes. Phosphorus is an important marker for the detection and follow up T1D.

KEYWORDS: diabetes autoantibodies, phosphorus, magnesium, calcium, Type 1 diabetes.

INTRODUCTION

The number of type 1 diabetics in the world was 171 million in 2000 and will be 336 million in 2030[1]. Furthermore, other estimates of the number of diabetics in the world, gave 382 million in 2013[2] and 422 million in 2014[3]. Approximately 497.100 children and adolescents suffering from type1 diabetes with 79.100 new cases each year in children under 15 years[4]. In Africa, 39.1 thousand children of 0-14 years with type 1 diabetes in 2013[5]. In Ivory Coast, 2% of children and adolescents with type 1 diabetes[6], against 1% in 1995[7]. In recent years, type 1 diabetes mortality rate has grown exponentially. These are 138.000 people aged 19 to 79 years who died of type 1 diabetes in 2011[5] against 1.2 million deaths due to diabetes in 2012[3]. It is an incurable disease that continues to wreak havoc in Africa and particularly in Ivory Coast. However, little attention is paid to this disease despite the many complications and the enormous costs it generates. Several studies worldwide to eradicate type 1 diabetes, were based on research autoantibodies diabetes and environmental factors among siblings of type 1 diabetes[8-10].The research showed that the presence of these autoantibodies of diabetes in the siblings of type 1 diabetes is a marker for the imminence of autoimmune diabetes and this population is a population at risk with a 5% chance[11]. In Ivory Coast, there is very little data on research autoantibodies diabetes and environmental factors[12]. It is in this light we conducted this research with the general objective is the search for autoantibodies correlated diabetes in search of some

micronutrients in type 1 diabetes and their siblings aged 5 to 21 years in district of Abidjan.

MATERIALS AND METHODS

Materials

Study population

The study population consisted of 49 people. It includes type 1 diabetics (T1D) known, ages 5 to 21 years and followed in two care centers for diabetics district of Abidjan. This is the endocrinology department of U.H.C of Yopougon and diabetes clinic in U.H.C of Treichville. Some type 1 diabetics were recruited from two NGOs that are the Association of Diabetics of Ivory Coast (ADIACI) and New Association of Ivory Coast Diabetics (NADCI). The study population is comprised apparently healthy siblings consanguineous selected of type 1 diabetes, also aged 5 to 21 years. There were 19 T1D and 30 siblings, including 23 boys and 26 girls is a sex ratio of 0.88. The average age of T1D was 12.62 ± 2.75 years and the siblings of diabetics was 12.13 ± 4.94 years. This cross-sectional study began in January 2014 and ended in April 2016.

Biological material

It consists of drawn venous blood in T1D and their siblings.

Methods

Sample collection

The project was approved by the ethics committee of Pasteur Institute of Ivory Coast. Written Informed consent was signed by each patient and their parents. Venous blood of each patient was collected in dry tubes. Serum obtained after centrifugation was stored at - 20°C for later determination of diabetes autoantibodies (anti-GAD, anti-IA2 and anti-ICA) and micronutrients (phosphorus, magnesium and calcium).

Biology of type 1 diabetes

Determination of autoantibodies glutamic acid decarboxylase (GAD) and anti-phosphatase (IA2). The dosage of anti-GAD autoantibodies and anti-IA2 by ELISA was done using commercial kits EUROIMMUN anti-GAD ELISA (IgG) and EUROIMMUN anti-IA2 ELISA (IgG) (Medizinische Labordiagnostika AG, Seekamp 31, D-23560 Lübeck, Germany).

These autoantibodies have been investigated in type 1 diabetes and their siblings. The results are expressed in international units per ml (IU/mL). The test is positive if the titer of anti-GAD and anti-IA2 autoantibodies is greater than or equal to 10 IU/mL [13-16].

Detection of anti-β cell autoantibodies islet (ICA)

Detection of anti-ICA autoantibodies was done by indirect immunofluorescence on monkey pancreas using commercial kits EUROIMMUN monkey pancreas (Medizinische Labordiagnostika AG, Seekamp 31, D-23560 Lübeck, Germany) in T1D and their siblings. With the indirect immunofluorescence method used in this kit, the patient's serum is incubated on monkey pancreas substrates, allowing the binding of the antibodies with the substrate. Wash the blade removes any unbound antibodies. Incubation of the substrate with an anti-human IgG conjugate, labeled with fluorescein, allows the detection of the bound IgG antibody. Reactions are observed under a fluorescence microscope Zeiss AxioLAB, HBO 100W/2, Germany, equipped with appropriate filters. The results are shown directly under a fluorescence microscope. The result is positive if there is presence of a green fluorescence apple cytoplasmic islet showing the presence of antibodies to islet cells[17-21].

Determination of phosphorus, calcium and magnesium[22]

The dosage of the phosphorus was done by the method of Daly using ammonium molybdate reagent in acid medium with the Cobas 6000 Roche/Hitachi, country. In the presence of sulfuric acid, inorganic phosphate is reacted with ammonium molybdate to form phosphomolybdate $(NH_4)_3[PO_4\ (MOO_3)_{12}]$.

$$\text{Phosphate} + \text{Molybdate ammonium} \xrightarrow{H_2SO_4} \text{phosphomolybdate ammonium}$$

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically.

Magnesium assay was done with the cobas 6000 Roche/Hitachi, Germany. The method relies on the reaction of magnesium with blue xylidyle in alkaline medium; the presence of EGTA eliminates the interference of calcium. Magnesium levels in the urine were determined with magnesium depletion tests. Reagent R1 is added to the sample. Addition of R2 and start of reaction: the magnesium ions form a purple complex in alkaline medium with blue xylidyle (diazoniumsalt). The magnesium concentration is measured photometrically by the decrease of the extinction xylidyle blue.

The calcium assay was done with the cobas 6000 Roche/Hitachi, Germany, according to the following principle: the calcium ions react with the 5-nitro-5'-methyl-BAPTA (NMBAPTA) in an alkaline medium to form a complex. Secondly, the complex reacts with EDTA.

alkaline pH

- Ca^{2+} + NM-BAPTA ⟶ complex calcium-NM-BAPTA
- Complex calcium-NM-BAPTA ⟶ calcium complex EDTA

The intensity of the color of the developed complex is directly proportional to the calcium concentration and measured photometrically.

Method of statistical analysis

The GraphPad Prism.V5.01 software was used for statistical analysis of the results and French curves. The

data were analyzed using One-Way ANOVA. T-test nonparametric Tukey was used for the comparison of the variance of diabetes autoantibodies; micronutrients between DT1 and siblings. The difference between two variances was significant if $p < 0.05$.

RESULTS

Immunological markers in type 1 diabetes and their siblings.

The table below presents the results obtained after dosing or detection of different autoantibodies in type 1 diabetics and their siblings. The presence of anti-ICA autoantibodies was 1/19 (5.26%) in type 1 diabetics (F16A) and 0/30 among siblings. The anti-GAD autoantibodies were present in 11/19 type 1 diabetics (F5A, F6A, F7A, F9A, F10A, F13A, F14A, F15A, F16A, F18A, F19A) is a frequency of 57.89% and 1/30 among siblings (F13B) a frequency of 3.33%. The anti-IA2 autoantibodies were present in 9/19 of type 1 diabetics (F2A, F6A, F7A, F11A, F13A, F14A, F15A, F16A, F19A) is a frequency of 47.36% and in 5/30 siblings (F2B, F3C, F7C, F12B, F13B) a frequency of 16.66%. The anti-ICA-GAD-IA2 combination was present in 1/19 of type 1 diabetics (F16A) a frequency of 5.26% and absent in the siblings. The GAD-IA2 combination was present in 6/19 of type 1 diabetics (F6A, F7A, F13A, F14A, F15A, F16A, F19A) is a frequency of 31.57% and 1/30 among siblings (F13B) a frequency of 3.33%.

The average value of anti-GAD autoantibodies in diabetics was 452.70 ± 167.8 IU/mL, while in siblings the average value was 120 ± 0.00 IU/mL. The average value of IA2 autoantibodies in diabetics was 97.06 ± 47.70 IU/mL while that of the siblings was 36.46 ± 18.42 IU/mL (Figure 1). There is no significant difference in anti-GAD antibody and IA2 between T1D and siblings because the levels of significance are respectively $p = 1.464$ and $p = 0.997 > 0.05$.

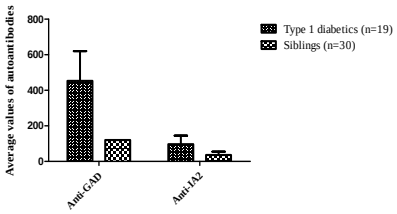

Figure 1: **Comparison of averages between anti-GAD and anti-IA2 autoantibodies in type 1 diabetics and their siblings**

anti-GAD ($p = 1.464 > 0.05$) and anti-IA 2 ($p = 0.997 > 0.05$), there is no significant difference

Dosage of micronutrients in type 1 diabetes and their siblings.

The dosage of micronutrients such as phosphorus, magnesium and calcium in type 1 diabetic patients (19 children) and siblings (30 children) is shown in the table below. Thus, 3 type 1 diabetics (F3A, F4A, F9A) have a phosphorus value three times higher than normal value (0.87-1.45mmol/L); or a frequency of 15.78%. Furthermore, 5 with type 1 diabetes (F1A, F8A, F10A, F11A, F12A) have a value higher than the normal value; or a frequency 26, 31%. At siblings, 8 children (F3B, F3C, F4B, F9B, F9C, F16B, F18B, F18C) have a phosphorus value three higher than normal value, while 13 siblings (F1B, F1C, F8B , F10C, F11B, F11C, F12B, F13B, F14B, F15B, F17B, F19C, F19D) have a phosphorus value above the normal value. Among siblings having a phosphorus value three times higher than normal, one (1) developed IA2 autoantibodies (F3C) and two (2) having a phosphor value higher than normal have developed anti-IA2 autoantibodies (F12B) and anti-GAD-IA2 (F13B).

As for magnesium, 17 type 1 diabetics have their magnesium value in the range of standard values (0.66 to 1.07 mmol/L); or a frequency of 89.47%. For siblings, all 30 members have their magnesium value in the range of standard values; is a frequency of 100% (Table).

Regarding calcium, the table below shows that 4 T1D (F4A, F6A, F8A, F9A) have a calcium value above the normal value (2.15 to 2.55 mmol/L); a frequency of 21.05%. Six (6) siblings (F7B, F9C, F16B, F17B, F19B, F19D) have a calcium value above the normal value; a frequency of 20%. Of the six siblings, none developed autoantibodies diabetes (Table).

The average phosphorus values in type 1 diabetics was 1.86 ± 1.06 (0.87 to 1.45) mmol/L, that of magnesium was 0.79 ± 0.07 (0.66 -1.07) mmol/L and that of calcium was 2.49 ± 0.11 (2.15 to 2.55) mmol/L (table). At the siblings of type 1 diabetes, the phosphorus average was 2.39 ± 1.51 (0.87 to 1.45) mmol/L, that of magnesium was 0.86 ± 0.08 (0.66 to 1.07) mmol/L and that of calcium of 2.43 ± 0.18 (2.15 to 2.55) mmol/L (table). However Figure 2 showing the comparison between the average rate of micronutrients between T1D and their siblings, shows no significant difference because the significance levels for phosphate $p = 0.703 > 0.05$, $p = 0.931$ magnesium > 0.05 and $p = 0.696 > 0.05$.

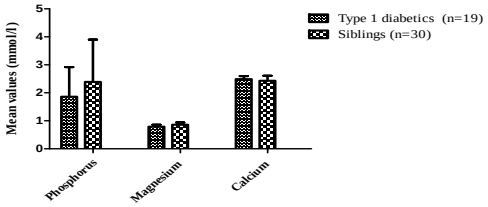

Figure 2: Comparison of different micronutrients phosphorus, magnesium, calcium between type 1 diabetes and their siblings.

Phosphorus ($P = 0.703 > 0.05$); Mg ($p = 0.931 > 0.05$) and Ca ($p = 0.696 > 0.05$) ; there is no significant difference.

Table: Anti-ICA, anti-GAD and anti-IA2 autoantibodies and micronutrients in diabetes type1 and their siblings.

Group	Family	Sex	Age (years)	Discovery of diabetes	Diabetes Autoantibodies			Micronutrients		
					ICA	GAD (UI/ml)	IA2 (UI/ml)	Phosphorus (mmol/l) (0,87-1,45)	Magnesium (mmol/l) (0,66-1,07)	Calcium (mmol/l) (2,15-2,55)
Type 1 diabetics	F1A	M	14	2007	Neg	0	0	1.60	0.71	2.50
	F2A	F	10	2009	Neg	0	24.6	1.38	0.78	2.39
	F3A	F	12	2012	Neg	0	0	3.53	0.88	2.52
	F4A	F	11	2013	Neg	0	0	5	0.92	2.57
	F5A	F	18	2008	Neg	117	0	1.44	0.68	2.35
	F6A	F	12	2014	Neg	52.9	33.2	1.32	0.83	2.64
	F7A	M	9	2011	Neg	82.6	112	1.27	0.71	2.47
	F8A	M	13	2015	Neg	0	0	1,63	0.79	2.61
	F9A	M	15	2015	Neg	101	0	3.43	0.91	2.75
	F10A	M	9	2014	Neg	> 1000	0	1.65	0.71	2.46
	F11A	M	18	2011	Neg	0	119	1.46	0.86	2.30
	F12A	F	13	2016	Neg	0	0	1.67	0.77	2.51
	F13A	F	12	2011	Neg	> 1000	38.4	1.42	0.80	2.55
	F14A	M	18	2007	Neg	10.4	55.1	1.31	0.78	2.53
	F15A	M	10	2010	Neg	36.7	10.9	1.29	0.84	2.39
	F16A	F	10	2014	**Pos**	> 1000	464	-	-	-
	F17A	M	15	2013	Neg	0	0	-	-	-
	F18A	M	17	2014	Neg	97.5	0	1.15	0.73	2.39
	F19A	F	14	2009	Neg	785	16.4	1.30	0.87	2.43
	Average		*12.62 ± 2.75*			*452.70 ± 167.8*	*97.06 ± 47.70*	*1.86± 1.06*	*0.79 ± 0.07*	*2.49±0.11*
Sibling of type 1 diabetics	F1B	F	9		Neg	0	0	1.71	0.81	2.52
	F1C	M	10		Neg	0	0	1.91	0.93	2.47
	F1D	F	21		Neg	0	0	1.27	0.85	2.35
	F2B	F	15		Neg	0	10.7	1.40	0.83	2.45
	F3B	M	8		Neg	0	0	4.80	0.96	1.81
	F3C	M	13		Neg	0	42.6	4.99	1.03	2.33
	F4B	M	8		Neg	0	0	4.84	1.01	2.40
	F5B	F	11		Neg	0	0	1.35	0.75	2.52
	F6B	F	17		Neg	0	0	1.25	0.74	2.36
	F7B	M	21		Neg	0	0	0.95	0.97	2.57
	F7C	F	15		Neg	0	10.8	1.15	0.79	2.50
	F8B	M	5		Neg	0	0	1.87	0.87	2.43
	F8C	F	10		Neg	0	0	1.58	0.79	2.49
	F9B	M	11		Neg	0	0	4.77	0.89	2.36
	F9C	F	21		Neg	0	0	5.03	0.80	2.97
	F10B	F	21		Neg	0	0	1.32	0.78	2.35
	F10C	F	12		Neg	0	0	1.63	0.77	2.43
	F11B	F	11		Neg	0	0	1.79	0.80	2.32
	F11C	M	16		Neg	0	0	1.72	0.95	2.39
	F12B	M	10		Neg	0	106	1.64	0.86	2.47
	F13B	M	5		Neg	120	12.2	1.51	0.69	2.52
	F14B	M	14		Neg	0	0	1.54	0.88	2.33
	F15B	M	9		Neg	0	0	1.54	0.87	2.36
	F16B	F	8		Neg	0	0	5.06	1	2.65
	F17B	F	12		Neg	0	0	1.58	0.89	2.72
	F18B	F	8		Neg	0	0	3.93	0.87	2.25
	F18C	F	12		Neg	0	0	5.08	0.88	2.28
	F19B	F	18		Neg	0	0	1.20	0.84	2.57
	F19C	F	11		Neg	0	0	1.70	0.93	2.46
	F19D	F	5		Neg	0	0	1.69	0.95	2.56
	Average		*12.13 ± 4.94*			*120 ± 0.00*	*36.46 ± 18.42*	*2.39± 1.51*	*0.86± 0.08*	*2.43± 0.18*

DISCUSSION

The results of our studies showed the presence of three autoantibodies anti-pancreatic islet diabetes (anti-ICA), anti-glutamic acid decarboxylase (anti-GAD) and anti-phosphatase IA2 (anti-IA2) in type 1 diabetics. These results corroborate those of Laadhar *et al.*[23] have shown in prospective studies on type 1 diabetics the occurrence of one or more antibodies is a brand development of T1D[23]. The search for these autoantibodies diabetes and especially the anti-GAD in diabetic screening is the best way of autoimmune diabetes or type 1 diabetes[24,25]. According to Guidicelli[26], the search for one of autoantibodies directed against one of the four major antigens do not present a sufficient specificity and sensitivity for identifying individuals at risk[26]. As demonstrated by several authors, research autoantibodies diabetes in diabetics confirms the autoimmune origin of the disease[13,14,27]. This is an important criterion for differentiation of type 1 diabetes and other forms of non-autoimmune diabetes such as type 2 diabetes[28]. Autoantibodies directed against the β cells islets of Langerhans are the best diagnostic markers for the identification of emerging or existing autoimmune processes and for monitoring the course of disease[29,30,14]. At the detection of autoantibodies anti diabetic, our results (GAD = 57%; anti-ICA = 5.26%; anti-IA 2 = 47.36%) in Type 1 diabetics are lower than those given by Guidicelli[26]. Indeed, according to this author, 70 to 80% of newly diagnosed type 1 diabetics have positive anti-GAD autoantibodies and prevalence does not depend on the patient's age. 50 to 70% of children and adolescents with type 1 diabetics have anti-IA2 autoantibodies and 30 to 50% of adults with type 1 diabetics have anti-IA2 autoantibodies. This implies that some of our T1D are newly diagnosed, because according to Kong *et al*[31]. the rate of positivity to anti diabetes exchange antibody 1 year after contracting diabetes[31]. Furthermore, our results indicated that anti-GAD (57%) is the most found antibodies in T1D children of Côte d'Ivoire as shown by Al-Hassani *et al.*[32] in T1D children in the Emirate Arab East Unit[32]. However, the prevalence does not depend on the patient's age[13-16,33]. The results of our research also showed the presence of two types of autoantibodies in our siblings with type 1 diabetics, anti-GAD and anti-IA2 autoantibodies.Two existing anti-GAD-IA2 combination. Indeed, the search for these anti-GAD and anti-IA2 autoantibodies in siblings of diabetics is early detection marker in patients at risk[34,35].The presence of 2 or 3 of these autoantibodies may increase the risk of developing diabetes in the future[8,36]. Some authors have shown that siblings of type 1 diabetics have a 5% chance of becoming type 1 diabetics[9,37-41].

Search micronutrients in our study population showed that the average calcium and magnesium were in normal range. However, those of phosphorus was above normal values (0.87 to 1.45 mmol/L) both in siblings (2.39 ± 1.51 mmol/L) than in type diabetics (11.86 ± 1.06 mmol/L). The results showed that T1D is not associated with osteoporosis which causes an increase urinary phosphate, calcium and magnesium[42,43] resulting in low blood levels of these metabolites[44]. Among siblings having a phosphorus value three times higher than normal, 1/30 developed IA2 autoantibodies (F3C) and 2/30 having a higher than normal value of phosphorus have developed auto-antibody IA2 (F12B) and anti-GAD-IA2 (F13B). According to the characteristics determined by Tietz[45]; Groot and Noll[46] 88% of phosphorus of the human body is in bones as calcium phosphate in the form of apatite Ca^{2+} [Ca 3 (PO4) 2] 32^{-} [45,46]. The rest is involved in the intermediate metabolism of carbohydrates and is contained in substances important physiologically, such as phospholipids, nucleic acids and ATP. In the blood, phosphorus exists in the form of inorganic phosphate and organic phosphate esters. The small amount of extracellular organic phosphorus is found almost exclusively in the form of phospholipids. The report phosphate /calcium level is about 6/10[45,47]. An increase in phosphorus content results in a reduction of calcium levels. This mechanism is influenced by an interaction between the parathyroid hormone and vitamin D. Hyperparathyroidism, poisoning vitamin D and renal failure with reduced glomerular filtration phosphates result in hyperphosphatemia[48]. In addition, the high value of phosphorus in type 1 diabetics, especially among siblings shows that the organism is in a state of hyperglycemia. In this case, the body produces a large amount of phosphorus to degrade excess glucose by the pathways of glycolysis, the oxidative decarboxylation of pyruvate and the Krebs cycle. The result of our work shows that siblings of type 1 diabetics who developed one or two autoantibodies are more exposed to type 1 diabetes because their bodies is in a hyperglycemic state. Therefore, these people at risk should be monitored to prevent the installation of the clinical diabetes. Our results corroborate those of Salmonowicz[10].

The results of our studies have shown the importance to search the autoantibodies of diabetes in type 1 diabetics on one hand and in the other siblings. Indeed, the presence of these autoantibodies in siblings of type 1 diabetics is a marker for the imminence of autoimmune diabetes in this population at risk. The siblings who developed autoantibodies and who also have higher than normal phosphorus values must be followed to prevent the installation of the clinical diabetes. From our results, we can say that phosphorus is an important marker in screening and follow up type 1 diabetes.

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