Correlation between diabetes autoantibodies and environmental parameters in type 1 diabetics and their siblings in Abidjan District, Ivory Coast

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ABSTRACT

Objective: The aim of this work is to determine the correlation between the presence of diabetes autoantibodies and certain environmental parameters and diet in type 1 diabetes (T1D) and their siblings.

Methods: The study population consisted of 49 people, including 19 with T1D and 30 siblings of first degree whose blood and faeces were collected. T1D were recruited from two University hospital centres in Côte d’Ivoire. Serum obtained allowed the determination of anti-ICA autoantibodies by the immunofluorescence method, anti-GAD and anti-IA2 detected by ELISA. Blood parasites were sought by the drop of thick and blood smears. Intestinal parasites were searched by the direct method, Kato and Ritchie techniques. Yeasts isolation was done on Sabouraud chloranphénicol and identifying by the chromatic Candida medium. Pinworms were sought by the anal scotch test technique. Vaccines and food were mentioned on a survey sheet.

Results: The 3 diabetes autoantibodies were present in T1D and 2 combinations anti-GAD-IA2 among siblings (p<0.0001). Hand pinworms, DT1 and their siblings infected with blood parasites are respectively, intestinal parasites, yeast. The diet of T1D significantly different from that of siblings (p=0.031<0.05).

Conclusion: There is no correlation between the presence of diabetes autoantibodies and blood and intestinal parasites, yeasts, pinworms among siblings of diabetics. Their diet should be balanced to avoid the installation of diabetes.

Keywords: Correlation, diabetes autoantibodies, environmental factors, type 1 diabetes

INTRODUCTION

Today type 1 diabetes has become a public health problem. The number of diabetic people 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 type 1 diabetes with 79,100 new cases each year in children less than 15 years [4]. In Africa, 39.1 thousand children of 0-14 years have type 1 diabetes in 2013 [5]. In Ivory Coast, 2% of children and adolescents have type 1 diabetes [6], against 1% in 1995 [7]. These recent years, the death rate from type 1 diabetes 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 Côte d’Ivoire. However, little attention is paid to this disease despite the many complications and the enormous costs it generates. Many studies around the world to eradicate type 1 diabetes, were based on diabetes autoantibodies research
and environmental factors among siblings of type 1 diabetes [8-10]. The research showed that enteroviruses and cow milk proteins are the triggers or accelerators of the autoimmune process [8,11]. Some authors have also shown that the presence of blood and intestinal parasites in type 1 diabetes was the basis of a blood sugar imbalance [12,13]. In Ivory Coast, there are very little data on the relationship between diabetes auto-antibodies and environmental factors [14] and type 1 diabetics children die at an early age. It is in this light that we have done this work which aims is to search the correlation between auto-antibodies in diabetes and intestinal and blood parasites, yeasts, pinworms firstly, vaccines and diet on the other hand.

**MATERIAL AND METHODS**

**Materials**

**Study population:** The study population consists of 49 people. It includes type 1 diabetics (T1D) known, aged 5 to 21 years and followed in two care centres for diabetics in the district of Abidjan (endocrinology department of C.H.U of Yopougon and diabetes clinic of C.H.U of Treichville). Some type 1 diabetics were recruited from two NGOs that are the Association of Diabetics of Ivory Coast (ADIAIC) and New Association of Ivory Coast Diabetics (NAICD). The study population is comprised consanguineous apparently healthy siblings of selected type 1 diabetics, 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 DT1 was 12.62 ± 2.75 years. DT1 and their siblings were 12.13 ± 4.94 years. This cross-sectional study began in January 2014 and ended in April 2016.

**Biological material:** It consists of venous blood and stool collected from T1D and their siblings.

**Methods**

**Sample collection:** Informed consent was signed by each patient and their parents. Information on diet and vaccination status according to the Ivorian calendar were mentioned on a survey sheet. These sheets were filled by type 1 diabetes and their siblings. Venous blood of each patient was collected in EDTA and dry tubes. Serum obtained after centrifugation (3000 rpm for 5 min) was stored at -20°C for the determination of diabetes auto-antibodies (anti-GAD, IA2 and anti-ICA). Whole blood was used to search for blood parasites. Stools of each patient were collected in sterile boxes for the search of intestinal parasites and yeasts.

**Biology of type 1 Diabetes**

**Determination of auto-antibodies glutamic acid decarboxylase (GAD) and anti-phosphatase (IA2):** The dosage of anti-GAD auto-antibodies 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-23 560 Lübeck, Germany). These auto-antibodies have been investigated in type 1 diabetics 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 auto-antibodies is greater than or equal to 10 IU/ml [15-17].

**Detection of anti β-cell autoantibodies islet (ICA):** Detection of anti-ICA auto-antibodies 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, labelled 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 result is positive if there is a fluorescent green apple of cytoplasmic islet characteristic of the presence of islet cell antibodies [18-20].

**Search of blood, intestinal, anal parasites, and yeasts:** Research of blood parasites is made by the embodiment of the drop of thick and blood smear. The principle of the thick drop and blood smear is based on the technique of micro-concentration of a quantity of blood on slide. Noise occurs optical microscope outside the lysed erythrocytes. Parasite density is the number of parasites counted per microliter of blood. This is to count the number of trophozoites to 200 leukocytes parasite density is determined by the ratio:

\[ D = A \times \frac{B}{C} \]
Where,

D: parasitic density;
A: Number of trophozoïtes counted for 200 leukocytes;
B: Standard leukocyte Number: 8000 leukocytes/µl of blood for children under 5 and 6000 leukocytes for more than 5 years;
C: Number of counted leukocytes.

The smear can identify the species of Plasmodium using identification criteria.

Search of intestinal parasites and yeasts was made following three different methods.

1) The direct method

The principle is based on the physiological saddle-water mixture, read microscopically optique Leica DM 1000, magnification X10 and X40. In the presence of protozoan cysts, one drop is added lugol of 2% solution in the preparation colouring cysts membranes yellow-brown and the cytoplasmic structures and technical nuclears. This technique can search vegetative forms cysts protozoa, helminth eggs and larvae.

2) Kato technique

The principle is based on the lightening power glycerin. A small amount of stool is covered with an adhesive cellophane coverslip. The mixture is returned against a blotter disposed on the flat surface and with the thumb, a steady pressure is applied until the sample covers an area equal to that of cellophane strip. The preparation is read optical microscope magnification X10 and X40.

3) Technical Ritchie implied

The principle is based on a combination of sedimentation and the dissolving power of the ether. An amount saddle is diluted to 10th in water 10% formol. Ether is added to the solution obtained after filtration. The tube is then centrifuged at 3000 rpm for 5 min. The centrifugation gives four phases arranged from top to bottom. An ethereal phase, a cake topper made of various debris, a formalin phase and the pellet containing the potential parasites. The first 3 phases are discarded and a few drops of saline are added to culot. The entire base is examined under an optical microscope Leica DM 1000 objectives X10 and X40. This technique allows the search of eggs and larvae helminth and protozoan cysts.

The isolation of yeasts was done on the Sabouraud chloranphénicol medium. The identification was to take a colony of yeasts grown on the Sabouraud chloranphénicol medium and suspended in 1 ml of sterile saline. Then, one drop of yeast suspension is transferred to the chromatic Candida medium and is incubated at 37°C for 24 h. This medium allows the isolation and differentiation of Candida albicans, Candida tropicalis and Candida krusei based colour and colony morphology. Candida albicans: pale green, Candida tropicalis: blue-green, Candida krusei: pink, other white-pink species [21].

Anal scotch test principle

The technique is preferably done in the morning before the toilet and before defecation. A cellophane adhesive fragment or transparent tape is applied using a plastic tube bottom of the radial folds of the previously unfolded anus. The cellophane is then adhered on a slide to be examined by light microscopy. The purpose of this technique is to look for pinworm eggs.

Method of statistical analysis

The GraphPad.Prism.V5.01 software was used for statistical analysis of the results and the graphic representation. The data were analyzed using One-Way ANOVA. Turkey’s t-test nonparametric was used for the comparison of the variance of autoantibodies diabetes between DT1 and their siblings and other biological parameters. The difference between two variances was significant if p<0.05.

RESULTS

Immunological markers in type 1 diabetics and their siblings

The presence of anti-ICA auto-antibodies was 1/19 (5.26%) in type 1 diabetics and 0/30 among siblings (p<0.0001).
For GAD, 11/19 T1D (57.89%) and 1/30 of the siblings (3.33%) were positive (p>0.05). As for anti-IA2, it was found in 9/19 T1D (47.36%) and 5/30 (16.66%) siblings (p=0.0001). The GAD-IA2 combination was detected in 7/19 T1D (36.84%) and only one of the siblings (3.33%). However, the entire study population, only T1D had three anti-ICA-GAD-IA2 auto-antibodies (2.04%). The average concentration of anti-GAD and anti-IA2 auto-antibodies were respectively at T1D of 452.70 ± 167.8 and 97.06 ± 47.70 IU/ml and in siblings of 120 ± 0.00 and 36.46 ± 18.42 IU/ml (Figure 1).

**Dietary factors in type 1 Diabetics and their siblings**

Table 1 shows that the diet of type 1 diabetes was significantly different from their siblings at all food groups (carbohydrates, animal and vegetable proteins, lipids). The significant difference ranged from p<0.0001; p=0.031<0.05 (Table 1). However, among the 5 siblings who developed autoantibodies, only one has developed two auto-antibodies (anti-GAD-IA2). The rest has developed anti-IA2 autoantibody. The patient with the anti-GAD-IA2 combination had a high carbohydrate and lipid diet, medium rich in animal and plant proteins. The four other brothers and type 1 diabetics sisters consumed moderately animal protein. Among these four siblings, three had high lipid consumption and two (50% average) have high carbohydrate consumption. The outbreak of diabetes auto-antibodies may be related to diet in the siblings of type 1 diabetics.

**Infectious factors in type 1 diabetics and their siblings: Blood parasites, intestinal, anal and yeasts**

**Blood parasites:** Blood parasite plasmodium strain malaria were detected in 1/19 of type 1 diabetes (5.26%) and *Plasmodium falciparum* in a (1/30) sibling (3.33%). Their titles were respectively 320 and 120 trophozoites/microliter blood (Figure 2). There is no significant difference between T1D and their siblings in blood parasites, as there are as many as DT1 member siblings infected with blood parasites. Therefore, there is no correlation between the occurrence of type 1 diabetics and blood parasite infection. However, siblings of type 1 diabetics carrying blood parasites did not

![Figure 1](image.png)

**Figure 1** Comparison of frequency between anti-ICA auto-antibodies, anti-GAD and anti-IA2 in type 1 diabetics and their siblings. *** indicates a statistically significant mean difference at p<0.0001 when diabetics value is compared to siblings’ value for each antibody.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Type 1 Diabetics</th>
<th>Sibling</th>
<th>p-value and significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (%)</td>
<td>Medium (%)</td>
<td>Low (%)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>26.31</td>
<td>52.63</td>
<td>21.05</td>
</tr>
<tr>
<td>Animal protein</td>
<td>36.84</td>
<td>42.1</td>
<td>21.05</td>
</tr>
<tr>
<td>Protein</td>
<td>5.26</td>
<td>21.05</td>
<td>73.68</td>
</tr>
<tr>
<td>vegetable</td>
<td>78.94</td>
<td>5.26</td>
<td>15.78</td>
</tr>
</tbody>
</table>

Carbohydrate=rice, attieke, placali, banana foutou, cassava foutou, Foutou yam, fufu, spaghetti, couscous, folio, bread, boiled rice, millet, maize, fonio, cassava, frit. Animal protein=milk products, meat, fish. Vegetable protein=raw peanuts, roasted, boiled, sauce, spread, beans: red, green, white. Lipid=seed sauce, oil: dinor, sunflower, olive, aya. High consumption: 7D/7, Average consumption: 2D/7-5D/7, Low power consumption: 1D/7. The significance values relate to increased workload. p<0.05 is the value of significance.
develop any autoantibodies diabetes. No correlation between the onset of diabetes and blood parasites.  

**Intestinal parasites, anal and yeast:** Intestinal parasites were detected in 3/19 of type 1 diabetics (15.78%) and 7/30 sibling (23.33%) (Figure 3). There was a significant difference of \( p<0.0001 \) between T1D number and siblings infected with intestinal parasites. Among the members of the infected siblings, only one (1) developed anti-IA2 autoantibodies (3.33%, \( p>0.05 \)) with a value of 106 IU/ml. It has no association between the occurrence of anti IA2 autoantibodies and infection with intestinal parasites.  

Pinworms have not been isolated in the entire study population (Figure 3). Yeast meanwhile, were present in 3/19 (15.78%) of type 1 diabetes, and 3/30 (10%) of siblings (Figure 3). There is a significant difference \( p=0.007<0.05 \) between yeast in T1D and their siblings. No member of the yeast carrier siblings has developed auto-antibodies diabetes. There is no association between the appearance of auto-antibodies diabetes and infection by the yeast.  

**DISCUSSION**  
The results of our studies showed the presence of 3 autoantibodies anti-pancreatic islet diabetes (Anti-ICA), anti-glutamic acid decarboxylase (GAD) and anti-phosphatase IA2 in type 1 diabetics. These results corroborate those of Laadhar, et al. [22] who have shown in prospective studies of type 1 diabetes that the occurrence of one or more antibodies is a brand development of T1D. The search for these autoantibodies diabetes in diabetics is the best way of screening for autoimmune diabetes or type 1 diabetes [23,24]. According Guidicelli [25], the search for one of
auto-antibodies directed against one of the four major antigens do not present a sufficient specificity and sensitivity for identifying individuals at risk. As demonstrated by several authors, diabetes autoantibodies research in diabetic confirms the autoimmune origin of the disease [15,26]. This is an important criterion for differentiation of type 1 diabetes and non-autoimmune diabetes such as type 2 diabetes [27]. Autoantibodies directed against the β cells of islets of Langerhans are the best diagnostic markers for the identification of emerging or existing autoimmune processes and for monitoring the course of disease [28,29]. The results of our research also showed the presence of two types of auto-antibodies in our siblings with type 1 diabetes, anti-GAD and anti-IA2 auto-antibodies as well as two existing anti-GAD-IA2 combination. Indeed, the search for these anti-GAD and anti-IA2 auto-antibodies in siblings of diabetics is early detection marker in patients at risk [30,31]. The presence of 2 or 3 of these auto-antibodies may increase the risk of developing diabetes in the future [8,32]. Some authors have shown that siblings of type 1 diabetics have 5% chance of becoming type 1 diabetic [9,33-37].

Several authors in the world have shown that environmental factors are at the base of triggering or accelerating the autoimmune process. These environmental factors are dietary factors, infectious, chemical [8,11,30,38,39]. The results of our work showed that blood parasites were present in type 1 diabetes and their siblings with a significant difference of \( p=0.0006<0.05 \). However, no sibling with blood parasites has developed diabetes auto-antibodies. These results would show that there was no correlation between the appearance of auto-antibodies diabetes and blood parasites. Blood parasites are not an infectious factor in the onset of type 1 diabetes in Ivory Coast.

As for intestinal parasites, they were present in T1D and their siblings with a significant difference of \( p=0.0001 \). These results corroborate those of Yasar [40] who found intestinal parasites such as Giardia and Entamoeba histolytica, tapeworm in type 1 diabetics. Among the siblings, only one has developed the anti-IA2 auto-antibody (3.33%; \( p>0.05 \)) with a value of 106 IU/ml. There is no association between the occurrence of the anti IA2 auto-antibody and infection with intestinal parasites. Therefore, they would not be an environmental factor prediction of autoimmune diabetes.

Yeasts were present in the DT1 and their siblings with a significant difference \( p=0.007<0.05 \). These results corroborate those of Schiefer [41] and Atkinson, et al. [12]. However, no member of the yeast carrier siblings has developed diabetes auto-antibodies. There is no association between the appearance of diabetes auto-antibodies and yeast infection. Yeasts are not an environmental risk factor for prediction of type 1 diabetes.

Pinworms were absent throughout the study population. These results show that pinworms would not be type 1 diabetes environmental risk factors. The results of our work have shown that siblings, who have GAD-IA2 combination, had a high carbohydrate and lipid diet. Also, the 3 siblings who have anti-IA2, had high fat intake. These results show that diet plays an important role in triggering the auto-antibodies of diabetes in the siblings of type 1 diabetes Therefore this population at risk should have a balanced diet.

CONCLUSION

The results of our studies have shown the importance of seeking the blood and intestinal parasites, yeasts and pinworms in type 1 diabetes and their siblings. These settings are not environmental risk factors for type 1 diabetes in Ivory Coast. However, siblings of T1D should have a balanced diet to prevent the installation of the clinical diabetes.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

Informed consent was obtained from T1D patients and their siblings before taking blood samples for testing.

ETHICAL APPROVAL

This study has been examined and approved by the ethics committee of Ivory Coast through Institute Pasteur and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This
study follows the charter of Institute Pasteur based on the law established on article L.1121-2 of the Code of Public Health which states that the interest of people that are amenable to biomedical research always takes precedence over only the interests of science and society. This study is based on results of medically prescribed tests of the clinical practice, from an anonymous database retrospectively evaluated, with no risks to patients, protecting the integrity and anonymity of participants and with no need of informed consents. The approval of an ethics committee of a specific institution is not needed because it was accessed only numerical values of the database (without access to the names, data source or clinic history of patients), collected specifically for research purposes by one of the authors.

AUTHORS’ CONTRIBUTIONS

The first three authors participate in manipulations, analysis of results and writing of the article. The three following authors have contributed to the epidemiological study and attended the microbiological testing; the corresponding author is the team leader and suggested the research goal.

REFERENCES


