Predicting Diabetes in Relatives of Diabetic Patients Using Insulinoma Antigen-2 Antibody-A Biomarker for Type 1 Diabetes in Jos, Nigeria

Bot DY1, Ahmed KM2, Shindang J1, Ekwempu A1, Olaniru OB3, Chundusu D4, Pwajok PG5, Ojo EO1, Igwe O1, Muhammad AA1, Afolabi TA1, Haladu A8, Ezra K1

1Department of Medical Laboratory Science, University of Jos, Nigeria; 2Chemical Pathology Laboratory, Gombe State Specialist Hospital, Gombe; 3Chemical Pathology Department, Jos University Teaching Hospital, Jos, Nigeria; 4NANEL Medical Laboratory, Murtala Mohammed Way, Jos, Nigeria; 5Chemical Pathology Department, Federal School of Medical Laboratory Science, Jos University Teaching Hospital, Jos, Nigeria; 6Chemical Pathology Section, Medical Laboratory Services Department, Plateau State Specialist Hospital, Jos, Nigeria; 7Chemical Pathology Department, College of Health Technology Pankshin, Plateau State, Nigeria; 8Chemical Pathology Laboratory, Bauchi State Specialist Hospital, Bauchi, Nigeria

ABSTRACT

Background: Type 1 diabetes is a chronic autoimmune disease caused by the destruction of insulin-secreting islet cells of the pancreas by several islet cell-specific autoantibodies that can be detected many months or years before the onset of diabetes. The presence of these antibodies can be attributed largely to environmental agents and also genetic factors. Knowing the frequency of these autoantibodies in a population is an important step for a better understanding, diagnosis and management of Type 1 diabetes. The aim of the research was to screen and identify those at greatest risk of diabetes (relatives of diabetic patients) early in life, as a precautionary step with the hope to deliver care in order to avoid the disease and its complications later in life.

Method: The study was conducted on eighty-eight apparently healthy young and adolescent first-degree relatives of diabetic patients in Jos metropolis. Blood samples were collected, centrifuged and serum was aseptically separated within two hours. A commercial ELISA test kit - Medizym® anti-IA2 was used to determine the presence of anti-IA-2 autoantibodies in serum obtained from participants enrolled in the study.

Results: The results obtained showed twelve participants of both sexes (13.64%) having positive titers of the IA-2 antibodies which were statistically significant.

Conclusion: From the results, we conclude that with significant titers of the IA-2 antibodies among young adolescents, there is the likelihood of them developing diabetes later in life depending on the period of exposure to the factors responsible for triggering the autoimmune process. The results are hereby discussed and recommendations made.

Keywords: Type 1 Diabetes; Autoantibodies; Insulin; Insulinoma Antigen-2; Autoimmune; Hormone; Chromosome; Adolescents

BACKGROUND

Type 1 Diabetes, also known as Insulin-Dependent Diabetes Mellitus (IDDM), results from a chronic autoimmune destruction of the insulin-secreting pancreatic beta cells, probably initiated by exposure of genetically susceptible host to an environmental agent [1]. It remains a major cause of mortality and morbidity worldwide [2] with an increase incidence in developing countries [3]. Several auto antibodies have been implicated in the autoimmune destruction of the beta
cells and they include antibodies to insulin and pro-insulin [4];
glutamic acid decarboxylase (GAD) antibodies [5], protein
tyrosine phosphatase also called Insulinoantigen-2 (IA-2)
antibodies [6] and Zinc transporter 8 (ZnT8A) antibodies
(recently discovered) [7].

Insulinoantigen-2 or protein tyrosine phosphatase is a
105,847 Da transmembrane protein that belongs to the protein
tyrosine phosphatase family anchored in the membrane of the
dense core (insulin) secretory granules and is processed by furin-
like convertases during granule maturation. It has 979 amino
acids with four domains consisting of a signal peptid (a.a. 1-24),
extracellular (a.a. 25-576), transmembrane (a.a. 577-600), and
intracellular (a.a. 601-979) domains [8]. Insulinoantigen-2
autoantibody (IA-2A) is directed exclusively to the intracellular
domain, primarily to the carboxylic (COOH)-terminus and, to a
somewhat lesser extent, to the juxtamembrane region [9-11].

An earlier study reported autoantibodies to IA-2 present in up
to 80% of children and adolescents at diagnosis of Type 1
Diabetes and in individuals in the pre-diabetic phase that
 correlated with the rapid progression to overt Type 1 Diabetes
[12]. Many studies support this generally held view but a few
indicate that the presence of IA-2A in particular, which is often
associated with other antibodies, confers a higher risk of rapid
progression toward clinical onset than multiple antibodies per se
[13]. It is suggested that the production of IA-2 autoantibodies
coincides with a critical switch in disease progression, where the
intracellular domain of IA-2 may only become visible to the
immune system at the outer cell surface in the case of beta cell
damage or dysfunction. Several studies on the predictive power
of IA-2A have shown a high specificity, sensitivity and Positive
Predictive Value (PPV) for developing diabetes in monogygotic
twins (all>90%) and in first-degree relatives where the 5 years
PPV ranged from 65% to 85% [14,15]. The prevalence of IA-2
antibody varies not only with age, but also with Human
Leukocyte Antigen (HLA). The HLA class II gene alleles on
chromosome 6p21 is the strongest genetic risk determinant for
Type 1 Diabetes [16], conferring up to 40%-50% of the
inheritable diabetes risk [17] and is greatest in patients carrying
HLA DR4 and the HLA DQA1 0301-DQB1 0302 (DQ8)
genotype [18,19]. The autoantibodies are said to activate
complement leading to lysis of the pancreatic islets cells [20].

Attempts to predict Type 1 Diabetes with the aim of preventing
the disease have focused on autoantibodies as disease markers;
studying T-cell changes is technically difficult and less specific.
Several studies have demonstrated the predictive value of Islet
Cell Antibodies (ICA). Testing for IA-2A complements
Glutamic Acid Decarboxylase Antibody (GADA), as more than
90% children have antibodies to at least one of these proteins at
onset of diabetes. IA-2A and GADA make the predominant, but
not exclusive contribution to ICA-reactivity 3 [19,20]. Since they
are in consequence highly prevalent in pre-diabetic people and
can be identified with sensitive recombinant assays, IA-2A
and GADA are now widely used for population screening and
diabetes prediction together with other antibodies such as
Insulin Autoantibody (IAA), Insulinoantigen-2 beta
antibodies (IA-2B A) and Zinc transporter-8 (ZnT8A). Seropositivity
for IA-2 autoantibody (> 0.02 nmol/L) is
supportive of a diagnosis of Type 1 Diabetes, a high risk for
future development of diabetes; and a current or future need for
insulin therapy in patients with diabetes.

The main aim of this study was to assay for the frequency of
Insulinoantigen-2 antibody as a predictive biomarker of Type
1 Diabetes in young and adolescent non diabetic relatives of
diabetic patients in Jos Metropolis. The objectives were; to
screen for the presence of autoantibodies in first degree relatives
of diabetes mellitus patients and check for sex distribution of
the autoantibodies.

MATERIALS AND METHODS

Study area

The study was conducted at Plateau State Specialist Hospital Jos,
located on latitude 9.8965°N and longitude 8.8583°E. The hospital has a Diabetes unit which made it easier to have access
to the patients and their first degree relatives. Eighty eight (88)
appearently healthy young and adolescent first degree relatives of
diabetic patients comprising fifty four (54) males and thirty four
(34) female subjects of age ranging between 5-16 years were
recruited for the study. The test was carried out in the Chemical
Pathology Laboratory of the Jos University Teaching Hospital Jos, Nigeria.

Participants in the study

All the eighty eight (88) apparently healthy young and
adolescent subjects (participants) were first degree relatives
of diabetic patients who attend the diabetic clinic in the diabetic
unit of Plateau State Specialist Hospital Jos.

Ethical consideration

Ethical clearance for the research was obtained from the Plateau
State Specialist Hospital Ethical Committee, while oral and
written consents were obtained from parents and subjects
(participants) before collection of their blood specimens.

Blood specimen collection and preparation

Two milliliter (2 ml) of venous blood specimen from the
subjects/participants was obtained, placed in a plain tube and
allowed to clot and centrifuged; and the serum was obtained
and stored at -20°C. The entire eighty eight specimens were
collected within a period of four weeks (4 wks). The kit was
manufactured by MEDIPIAN GMBH Germany, on 26th
September, 2011, Lot No: AL 3803-E-10-11-09-26.

Method of assay

Enzyme-Linked Immunosorbent Assay (ELISA) was used to
carry out the both qualitative and quantitative estimation of the
protein tyrosine phosphatase antibody (IA-2A). Commercial
Medizym® anti-I2A Enzyme-Linked Immunosorbent Assay
(ELISA) kit was used to determine the frequency (presence or
otherwise) of insulinoantigen-2 antibody in the serum of the
subjects/participants. The manufacturer’s instructions were duly followed strictly in running the test.

**Principle of the test assay**

Based on the ability of IA-2 antibodies in patient's serum binding divalenty and forming a bridge between immobilized IA-2 coated on the microtiter plate and liquid phase IA-2-Biotin complex. The bound IA-2-Biotin could be quantified by addition of streptavidin-peroxidase and a colorogenic substarte (3, 3', 5, 5'-Tetramethylbenzidin) and reading the optical density (O.D) at 450 nm.

**Assay procedure**

All materials and reagent were brought to room temperature before the assay is to be carried out and the steps involved in the procedure are:

- A micropipette was used to aspirate 50 µl of calibrators (1-5), controls (C1 (negative) and C11 (positive)) and dispense into microtiter plate wells B1-H1.
- 50 µl of the serum specimens were aspirated and dispense into the remaining microtiter plate wells coated with IA-2 antigen.
- 25 µl of an Enhancer (K) was added to all the wells on the microtiter plate and gently rocked for 10 seconds. The plate was covered with an aluminum foil and incubated for eighteen hours (18 hrs) at 2-8°C.
- A microplate washer was used to aspirate and wash the microplate 3 times with 300 µl of the washing solution with 5 seconds soaking time each. The microplate was "flick out" by striking the wells sharply onto an absorbent paper to remove residual droplets.
- 100 µl of the reconstituted IA-2-Biotin solution was added to each well. The plate was covered and incubated for 60 minutes at room temperature with intermittent shaking.
- The plate was aspirated and washed 3 times with 300 µl of wash buffer in a microplate washer and gently rocked for 10 seconds. The plate was covered and incubated at room temperature for 20 minutes with intermittent shaking.
- The plate was aspirated and washed 3 times with 300 µl of wash buffer in a microplate washer and gently rocked for 10 seconds. The plate was incubated for 20 minutes in the dark at room temperature.
- 100 µl of stop solution (0.25 M sulfuric acid) was added to each well and gently rocked for 10 seconds.
- The optical density was read at 450 nm within five (5) minutes after adding the stop solution.
- The readings were calculated from a calibration curve which was prepared.
- Normal value for IA-2 is ≤ 0.02 nmol/L for all ages.

**Statistical analysis**

Statistical analysis was done using Chi-square test to establish the relationship between the presence of the antibody with gender and also with age groups of the participants.

**RESULTS**

This study was conducted using commercial Medizym® anti-IA2 ELISA kit to determine the frequency of anti-IA-2 autoantibodies also known as protein tyrosine phosphatase antibody in serum of eighty-eight apparently healthy young adolescent first-degree relatives of diabetic patients with a mean age of 10.6 years. The results indicated that twelve of the participants (13.64%) were found to have reasonably high titers of the IA-2 antibody 0.058 ± 0.007 nmol/L with 2 (5.88%) of them females, while 10 (18.52%) males (Table 1).

**DISCUSSION**

Twelve participants representing 13.64% were found to have reasonably high titers of the IA-2 antibody, suggesting or indicating an autoimmune process against the pancreatic beta cells of Langerhans. As shown in Table 1, the frequency of IA-2 in relation to participants’ gender of which 34 were female subjects, with 2 (5.88%) of them were positive. Also, 54 male participants were recruited out of which 10 (18.52%) were positive with high autoantibody titers of IA-2. These finding agree with an earlier study, primarily of first-degree relatives followed over time which demonstrated that islet cells autoantibodies may predict Type 1 Diabetes [21].

**Table 1:** Frequency of IA-2 antibody in relation to participants’ gender.

| Gender | Female | Male | Total |
|--------|--------|------|-------|
| Positive | 2 (5.88%) | 10 (18.52%) | 12 (13.6%) |
| Negative | 32 (94.12%) | 44 (81.48%) | 76 (86.4%) |
| **TOTAL** | 34 (100%) | 54 (100%) | 88 (100%) |

p-value=0.097

We observed that a higher percentage of boys were recorded with the Protein Tyrosine Phosphatase Antibody (IA-2 antibody) 0.062 ± 0.005 nmol/L, with 6 positive male subjects having diabetic mothers and 4 positive male subjects have diabetic fathers, which is in accordance with study in Nigeria where autoimmune Type 1 Diabetes was found to be more prevalent in boys than girls [22]. It is thought that some of the mother’s chromosomal material or DNA, gets inactivated when passed on to the child, thereby accounting for the difference in the children’s diabetes risk, but maternal hyperglycemia can perturb fetal islet development [23], which could affect tolerance to the islet cells.

The frequency of IA-2 in relation to age groups of the participants in Table 2, shows that one out of 26 subject in the age group 5-8 years was positive with a titer of 0.035 ± 0.0 nmol/L representing 3.85%. In the age group 9-12 years, five
were positive out of 36 subjects 5 (13.89%) with a titer of 0.055 ± 0.004 nmol/L while 6 (23.08%) were positive out of the 26 subjects in the age group 13-16 years, with a titer 0.061 ± 0.002 nmol/L. The frequency of IA-2 increases with age of the participants, only one subject was positive in the age group 5-8 years while 5 and 6 subjects were positive in the age group 9-12 and 13-16 years respectively. This may suggest that the onset of the autoimmune process may be dependent not only on genetic susceptibility, but also on the duration of exposure to the autoimmune triggers, agreeing with the findings of WHO Diamond Project Group on Epidemics and Tuomilehto which said that the disease eventually develops as one grows older.

### Table 2: IA-2 Antibody in relation to participants’ age group.

| Age group (Years) | Positive | Negative | Total |
|-------------------|----------|----------|-------|
| 5-8               | 1 (3.85%)| 25 (96.15%)| 26 (100%) |
|                   | 0.035 ± 0.0 | 0.013 ± 0.002 | 0.024 ± 0.08 |
| 9-12              | 5 (13.89%)| 31 (86.11%)| 36 (100%) |
|                   | 0.055 ± 0.004 | 0.012 ± 0.003 | 0.033 ± 0.08 |
| 13-16             | 6 (23.08%)| 20 (76.92%)| 26 (100%) |
|                   | 0.061 ± 0.002 | 0.013 ± 0.005 | 0.043 ± 0.05 |

These autoantibodies are valuable markers to predict Type 1 Diabetes and can be detected many months or years before the onset of diabetes [24]. Therefore, knowing the frequency of these autoantibodies in a population is an important step for a better understanding, diagnosis, awareness and management of Type 1 Diabetes. In particular, the presence of IA-2 antibodies could possibly be attributed largely to environmental agents associated with lifetime risk of Type 1 Diabetes and also genetic factors [25,26]. Environmental agents that could trigger the production of these autoantibodies include viral infections, immunotoxicants and some foods that are consumed [27,28].

### CONCLUSION AND RECOMMENDATIONS

From the results obtained, it can be concluded that the twelve subjects with significant titer of the IA-2 antibodies are likely to have diabetes later in life depending on period of exposure to the factors responsible for triggering the autoimmune process. We recommend that further work should be carried out to ascertain the specific agents/triggers (including genetic factors) to Type 1 Diabetes mellitus. We also recommend that the use of this method of diagnosis should be used for screening in our Medical Laboratories to enhance the prediction of diabetes. Awareness campaigns should be mounted up to enlighten the public about the dangers of exposure to the disease triggers/agents, thereby preventing the onset of the disease which is far better than managing the condition when it has developed. Relatives of diabetic patients should be encouraged to do regular checks of their health status and avoid exposure to any agents that can trigger Type 1 Diabetes.

### CONFLICT OF INTEREST

The Authors declare that they had no conflict of interest on this work and its subsequent publication.

### AUTHORS’ CONTRIBUTION

All the authors contributed in no small measure from the conceptualization to the actualization of the project by active participation in sample collection, laboratory assay, statistical analysis and the preparation of the manuscript for publication.

### REFERENCES

1. Cahill GF, McDevitt HO. Insulin-Dependent Diabetes Mellitus: The Initial Lesion. N Engl J Med. 1981;304:1454-1465.
2. Gerstein HC, Santaguida P, Raina P, Morrison KM, Balion C, Hunt D, et al. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: A systemic overview and meta-analysis of prospective studies. Diabetes Res Clin Pract. 2007;78:305-312.
3. Ismael-Beigi F. Glycaemic Management of Type 2 Diabetes Mellitus. N Engl J Med. 2012;366:1319-1327.
4. Palmer JP, Asplin CM, Clemons P, Lyen K, Tarpati O, Raghu PK, et al. Insulin antibodies in insulin-dependent diabetes before insulin treatment. Science. 1983;222:1337-1339.
5. Baekkeskov S, Annoost HJ, Christgau S, Reertz, A, Solimena M, Cascalho M., et al. Identification of the 64k auto antigen in insulin-dependent diabetes mellitus as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature. 1990;347:151-156.
6. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, et al. Ilet cell antigen 512 is a specific islet autoantigen related to protein tyrosine phosphatases. J Immunol. 1994;152:3183-3188.
7. Lampasona V, Petrone A, Tiberti C, Capizzi M, Spoletini M, Pietro S, et al. Zinc Transporter 8 Antibodies Complement GAD and IA-2 Antibodies in the Identification and Characterization of Adult-Onset Autoimmune Diabetes. Non-Insulin Requiring Autoimmune Diabetes (NIRAD) 4. Diabetes Care. 2010;33:104-108.
8. Mzait H, Trajkovski M, Kersting S, Ehninger A, Altkruger A, Lemaire RP, et al. Synergy of glucose and growth hormone signalling in islet cells through ICA512 and STAT5. Nat Cell Biol. 2006;8:435-445.
9. Lampasona V, Bearutto M, Genovese S, Bosi E, Ferrari M, Bonifacio E. Autoantibodies in insulin-dependent diabetes recognize distinct cytoplasmic domains of the protein tyrosine phosphatase-like IA-2 autoantigen. J Immunol. 1996;157:2707-2711.
10. Zhang B, Lan MS, Notkins AL. Autoantibodies to IA-2 in IDDm: location of major antigenic determinants. Diabetes. 1997;46:40-43.
11. Xie H, Zhang B, Matsumoto Y, Li Q, Notkins AL, Lan MS. Autoantibodies to IA-2 and IA-2 beta in insulin-dependent diabetes mellitus recognize conformational epitopes: location of the 37- and 40-kDa fragments determined. J Immunol. 1997;159:3662-3667.
12. Pozzilli P, Manfrini S, Monetini L. Biochemical markers of type 1 Diabetes: clinical use. Scand J Clin Lab Invest. 2001;61:38-44.
13. Decochez K, De Leeuw I, Kymeenu L, Mathieu C, Rottiers R, Weerts I, et al. IA-2 autoantibodies predict impending Type 1 diabetes in siblings of patients. Diabetologia. 2002;45:1658-1666.
14. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, et al. Prediction of Type 1 Diabetes in First-Degree Relatives
Using a Combination of Insulin, GAD, and ICA512/aCA-2 Autoantibodies. Diabetes. 1996;45:926-933.

15. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: First Assay Proficiency Evaluation. Diabetes. 2003;52:1128-1136.

16. Lambert AP, Gillespie KM, Thomson G, Cordel HJ, Todd JA, Gale EAM, et al. Absolute Risk of Childhood-Onset Type 1 Diabetes Defined by Human Leukocyte Antigen Class II Genotype: A Population-Based Study in the United Kingdom. J Clin Endocrinol Metab. 2004;89:4037-4043.

17. Pugliese A. Genetics of Type 1 Diabetes. Endocrinol Metab Clin North Am. 2004;33:1-16.

18. Hawa M, Rowe R, Lan MS, Notkins AL, Pozzilli P, Christie MR, et al. Value of antibodies to islet protein tyrosine phosphatase-like molecule in predicting Type 1 Diabetes. Diabetes. 1997;48:1270-1275.

19. Gorus FK, Goubert P, Semakula C, Vandewalle CL, De Schepper J, Scheen A, et al. IA-2-autoantibodies complement GAD65-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. Diabetologia. 1997;40:95-99.

20. Tjernberg J, Ek Dahl KN, Lambris JD, Korsgren O, Nilsson B. Acute Antibody-Mediated Complement Activation Mediates Lysis of Pancreatic Islets Cells and May Cause Tissue Loss in Clinical Islet Transplantation. Transplantation. 2008;85:1193-1199.

21. Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, et al. A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. N Engl J Med. 1990;323:1167-1172.

22. Akanji AO. Causes of diabetes mellitus in Africans. Nigerian Med Practit. 1996;17:30-34.

23. Ryan EA, Liu D, Bell RC, Finegood DT, Crawford J. Long-term consequences in offspring of diabetes in pregnancy; studies with syngeneic islet-transplanted streptozotocin-diabetic rats. Endocrinology. 1995;136:5587-5592.

24. Inadera H. The immune system as a target for environmental chemicals: Xenoestrogens and other compounds. Toxicol Lett. 2006;164:191-206.

25. WHO DIAMOND Project Group on Epidemics, Tuomilehto J. Childhood diabetes, epidemics, and epidemiology: an approach for controlling diabetes. Am J Epidemiol. 1992;135:803-816.

26. Kumar D, Geemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, et al. North American twins with IDDM: Genetic, Etiological, and Clinical Significance of Disease Concordance According to Age, Zygosity, and the Interval after Diagnosis in First Twin. Diabetes. 1993;42:1351-1363.

27. Bingley JP. Clinical applications of diabetes antibody testing. J Clin Endocr Metabol. 2010;95:25-33.

28. Kulmala P, Savola K, Petersen PS, Vahasalo P, Karjalainen J, Lopponen T, et al. Prediction of Insulin-Dependent Diabetes Mellitus in siblings of children with diabetes. A population-based study. The Childhood Diabetes in Finland Study Group. J Clin Invest. 1998;101:327-336.