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Research Article

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

Bot DY[1*], Ahmed KM[2], Shindang J[1], Ekwempu A[1], Olaniru OB[3], Chundusu D[4], Bot SY[5], Mwantok EA[6], Pwajok PG[7], Ojo EO[1], Igwe O[1], Muhammad AA[1], Afolabi TA[1], Haladu A[8], Ezra K[1]

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

[*]**Correspondence to:** David Bot, Department of Medical Laboratory Science, University of Jos, P. M. B 2084, Jos, Plateau State, Nigeria, Tel: +2348163197222; E-mail: davidbot99@gmail.com

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cells and they include antibodies to insulin and pro-insulin [4]; glutamic acid decarboxylase (GAD) antibodies [5], protein tyrosine phosphatase also called Insulinoma Antigen-2 (IA-2) antibodies [6] and Zinc transporter 8 (ZnT8A) antibodies (recently discovered) [7].

Insulinoma Antigen-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 peptide (a.a. 1-24), extracellular (a.a. 25-576), transmembrane (a.a. 577-600), and intracellular (a.a. 601-979) domains [8]. Insulinoma antigen-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 monozygotic 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), Insulinoma antigen-2 beta antibodies (IA-2 β A) and Zinc transporter-8 (ZnT8A).

Seroposivity 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 Insulinoma antigen-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 9.8965oN 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) apparently 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 MEDIPAN 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-IA2 Enzyme-Linked Immunosorbent Assay (ELISA) kit was used to determine the frequency (presence or otherwise) of insulinoma antigen-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 divalently 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 correlates with the amount of IA-2 antibody in the patient's serum and the unbound IA-2-Biotin are removed by washing the plate with a wash buffer. 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 flicked out on an absorbent paper.
- 100 µl of diluted streptavidin-peroxidase was added to each well. 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 flicked out on an absorbent paper. 100 µl of substrate solution (3, 3', 5, 5'-Tetramethylbenzidin) was added to each well 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 rocked gently 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 caliberation 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.

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