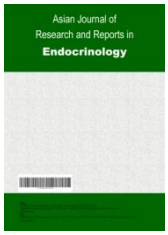

Asian Journal of Research and Reports in Endocrinology

4(2): 62-68, 2021; Article no.AJRRE.71063

Autoantibodies Associated with Diabetes Mellitus in Nigerian Subjects Resident in Port-Harcourt Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DO and DGT designed the study and wrote the protocol. Author TNS wrote the first draft of the manuscript and managed the biochemical and statistical analyses and the literature searches of the study. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Arun Kumar Kapoor, Rohilkhand Medical College & Hospital, M.L.N. Medical College, India.
Reviewers:
(1) Aaron Lucander, University of Alabama, USA.
(2) Guangzhao Li, George Washington University, USA.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71063

Original Research Article

Received 14 May 2021
Accepted 19 July 2021
Published 28 July 2021

ABSTRACT

Aim: The aim of this study was to determine the autoantibodies associated with diabetes mellitus among diabetics in Port- Harcourt of Rivers State, Nigeria
Study Design: This study is a cross-sectional study.
Place and Duration of Study: This study was conducted at Chemical Pathology Department, Rivers State University Teaching Hospital, Port Harcourt, Nigeria, between December, 2019 and February, 2020.
Methodology: A total of 244 subjects, both male and females, 132 subjects 112 controls, aged within 30-70 years. Five (5) ml of blood sample for Islet cell cytoplasm autoantibodies (ICA), Glutamic acid decarboxylase autoantibodies (GADA), Insulinoma-associated-2 autoantibodies (1A-2A) and zinc transporter 8 autoantibodies (ZnT8A), were collected serum obtained was analyzed using Enzyme-linked immunoasorbent assay. Data were analyzed statistically with SPSS version 22.0 and value considered significant at p≤ 0.05.
Results: The mean ± S.D of serum ICA were 4.48± 2.18u/ml (control) and 14.91±11.11 u/ml (subject), GADA value were 0.99 ± 0.22u/ml(control) and 1.78 ±0.77u/ml (subjects), IA-2A values

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were 3.83±1.56 u/ml (control) and 4.20±3.26u/ml and ZnT8A values were 5.61±4.29u/ml (control) and 6.02±3.80u/ml (subjects). The comparison of mean showed significant difference at p=0.0001 for ICA but no significant differences observed GADA, IA-2A and ZnT8A at p=0.152, p=0.595 and p=0.686 respectively.

Conclusion: Diabetes mellitus patient with positive ICA, GADA and IA-2A autoantibodies, higher HbA1c and lower C-peptide suggest an autoimmune or intracellular damage of beta-cells in T2DM and need for insulin dependence or progression to LADA.

Keywords: Autoantibodies; diabetes mellitus; Port Harcourt metropolis; Nigerian.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder and it is characterized by chronic hyperglycemia affecting carbohydrate, fat and protein metabolism, which has defied several medication and its management and treatment is difficult as a result of misclassification of the types. DM is caused by several factors, but for the purpose of this study autoantibodies as a cause of diabetes is the point of interest. Knowledge of these autoantibodies is necessary for diabetes characterization, prognosis and management and treatment.

Autoantibodies are proteins produced by the immune system that has been shown to be associated with TIDM [1]. There are five most common types of autoantibodies associated with type 1 diabetes such as Islet cell cytoplasm autoantibodies (ICA), Glutamic acid decarboxylase autoantibodies (GADA), Insulinoma-associated-2autoantibodies (1A-2A, Insulin autoantibodies (1AA) and zinc transporter 8 autoantibodies (ZnT8A) and each antibody mounted its attack on their specific auto-antigens such as, islet cell (ICA), glutamate decarboxylase-65 (GAD), islet antigen-2 (IA-2), insulin (IA) and zinc transporter 8 (ZnT8) respectively [2]. The presence of these autoantibodies suggest beta-cell damage. Since the continuous attack by autoantibodies mounted against beta cells of the pancreas causes a destruction and inability of the cells of the pancreas to produce insulin [3]. Most of the destruction is caused by autoantibodies such as Islet cell cytoplasm autoantibodies (ICA), Glutamic acid decarboxylase autoantibodies (GADA), Insulinoma-associated-2autoantibodies (1A-2A, Insulin autoantibodies (1AA) and zinc transporter 8 autoantibodies (ZnT8A) [1].

Islet autoantibodies appear early in life in parents and offspring at high genetic risk because the presence of Islet autoantibodies trigger beta-cell damage [4]. The presence of autoantibodies in a diabetic individual suggest the site of destruction by the specific antibody identified. Each antibody have a specific target site. Therefore, the aim of this study was to determine the autoantibodies associated with diabetes mellitus among diabetics in Port- Harcourt of Rivers State, Nigeria

2. MATERIALS AND METHODS

2.1 Study Design/ Area/Subject Characteristics

This was a cross-sectional study carried out in Rivers State University Teaching Hospital (RSUTH) on diabetic subjects and healthy individual in Port Harcourt.

The subjects recruited for the study were diabetic and non-diabetic subjects that visited Rivers State University Teaching Hospital (RSUTH) from Endocrinology, Obstetrics and Gynaecology units of the hospital. Also, offspring of diabetics and non-diabetic subjects were recruited for the study. A total of 244 subjects, both male and females, 132 subjects 112 controls, aged within 30-70 years.

2.1.1 Inclusion Criteria

All diabetic subjects and Non diabetic subjects within age ranges 30 -70years.

2.1.2 Exclusion Criteria

Individual with thyroiditis, diabetes subjects that were smokers, as those could affect the measured parameters.

2.2 Sample Size Calculation

The sample size was determined using

Lesli Kish's Formula: $N = \frac{Z^2.d.q}{D^2}$

Where N – minimum number of subjects

Z - 1.96

p- prevalence of Diabetes Mellitus in Nigeria (2018)

q – 1- p

D – confidence level = 0.05

The prevalence of type 2 diabetes mellitus and type 1 diabetes mellitus diagnosed were 7% and 4% respectively in Nigeria. So this was used to calculate the sample size [5].

Total sample size =74

2.3 Sample Collection and Laboratory Analysis

2.3.1 Collection of Blood Sample

Prior to sample collection, participant consent was obtained after thorough explanation of research purpose and its importance at the endocrinology, obstetrics and gynecology out subjects waiting hall. After this sample bottles were labelled systematically and carefully. Confidence was then built in subjects while seated to prevent panic or fear. Prominent vein selected, a tourniquet tied around the arm and skin cleaned with cotton wool soaked in methylated spirit with cotton wool to disinfect the area. Patient made a fist. Five (5) ml syringe with 21 or 18 Gauge needle was inserted in the ante-cubital fossa vein and blood drawn. Punctured area was wiped with dried cotton wool and pressure exerted over site to avoid thrombosis and further bleeding. Blood withdrawn was dispensed into K_2 EDTA and plain bottles in volumes of two (2) ml and three (3)ml respectively.

K_2EDTA samples were taken immediately for HbA1c analysis while the plain blood samples were allowed to clot and then centrifuged at 12,000 rpm for 5 minutes. Serum was obtained and stored at 4^0c temperature in a refrigerator until the day laboratory analysis was performed. Prior to sample analysis, serum samples were allowed to thaw and mixed properly for homogeneity.

2.3.2 Determination of Fasting Blood Sugar (FBS)

Fasting blood sugar was determined by glucose oxidase method using URIT auto-analyzer Laboratories Inc Company, USA. Glucose in plasma is catalysis by glucose oxidase, oxidation of glucose produces hydrogen peroxide and gluconic acid, hydrogen peroxide in present of peroxidase with 4-aminophenazone and phenol produce a pink colour.

2.3.3 Determination of Serum 3-screen Cell Cytoplasmic Autoantibodies (ICA)

3 - Screen ICA was determined using EMP-M201 microplate Reader USA and 3 screen ELISA (IA-2, GAD and ZnT8) kit manufactured by RSR Limited Parctyglas, Cardiff,UK [6].

2.3.4 Determination of GADA

GADA was determined using EMP-M201 microplate Reader USA and GADA ELISA kit manufactured by RSR limited parctyglas ,Cardiff ,UK [6].

2.3.5 Determination of 1A-2A

IA-2A was determined using EMP-M201 microplate Reader USA and IA-2A ELISA kit manufactured by RSR limited parctyglas, Cardiff, UK. [6]

2.3.6 Determination of ZnT8A

ZnT8A was determined using EMP-M201 microplate Reader USA and ZnT8A ELISA kit manufactured by RSR limited parctyglas Cardiff ,UK [6]

2.3.7 Determination of C-peptide

The analysis was done using Mindray Biochemistry autoanalyzer model B S-120, Shenzhen China [8].

2.3.8 Determination of Glycated haemoglobin (HbA1c)

Ion exchange resin method using Sysmex auto analyzer model KV -21n Kobe, Japan [7] was used to determine **glycated haemoglobin (HbA1c).**

2.4 Statistical Analysis

Data obtained from subjects via the questionnaire and results were analyzed using, Excel program, Statistical Package for Social Sciences (SPSS) version 22.0 and expressed as mean ± standard deviation. Differences between two means were analyzed using independent sample t-test with p-values less than 0.05 being considered significant. Pearson correlation was also used to compare autoantibodies with anthropometric parameter glycemic parameters.

3. RESULTS AND DISCUSSION

Autoantibodies associated with diabetes are proteins that cause diabetes mellitus and specifically associated with autoimmune diabetes and sometimes found in uncontrolled and non-managed type 2 diabetes leading to Latent Autoimmune Diabetes in Adult (LADA). The various autoantibodies have target antigen revealing the attacked sites, therefore knowledge of these autoantibodies is very important and better tool for proper classification , management, long -term prognosis, diagnosis and choice of treatment of diabetes.

There was a high significant level of ICA observed in diabetic than non-diabetic subjects (Table 1). This may be an indication of TIDM because ICA targeted against tyrosine phosphatase, glutamic acid decarboxylase and zinc transporter-8 pancreatic beta-cell enzymes leading to beta-cell damage and this was consistent with the work of Ake [2] which revealed that ICA in diabetes patient was an indication of autoimmune type 1 diabetes and it served as an indication of the risk factor of TIDM. High significant level of ICA was consistent with the study of Shu-ling, [9] in which it was reported that ICA comprises of all antibodies directed against the endocrine cells of the pancreas and the target antigens of ICA are predominantly tyrosine phosphatase, glutamic acid decarboxylase and zinc transporter 8 ICA is identified in TIDM and is an indication of LADA in T2DM.

This agreed with the work of Trabucchi et al. [10] which revealed that ICA detected in subjects previously diagnosed with type 2 diabetes is an indication of latent autoimmune diabetes of adulthood (LADA) and about 10% to 15% of people diagnosed with type 2 diabetes have ICA. There was a significant high level of FBS in diabetes subjects than in non-diabetes. Reason being that diabetic individual compensating feedback mechanism that maintain normal blood glucose level fails thus hyperglycemia which causes insufficient production of insulin in the pancreas, or insulin resistance. This was consistence with the work of blood sugar levels are high and insulin levels are low.

Insulin and C-peptide are secreted in equimolar amounts by the beta-cells of the pancreas but the metabolic clearance of insulin is much more rapid than that of C-peptide [11]. The measurement of plasma C-peptide concentrations may be more reliable as an indication of endogenous insulin production. Since C-peptide has a longer half-life and is present in peripheral blood in higher molar concentrations than insulin, making it less prone to marked fluctuations. However, as C-peptide is cleared by the kidneys, raised concentrations may occur in renal impairment. [12]. C-peptide is a prominent factor in the pathogenesis of micro-vascular complications in TIDM [13].

There was a significantly reduced level of Connecting-peptide (C-peptide) in diabetes mellitus subjects (DMS) than in non DMS. The reason being that pancreas beta-cells may be destroy in DM. The finding observed in this study was consistence with the work of Elias *et al* [14], lower C-peptide level are observed in both type 1 and type 2 diabetes. And individual with type 1 diabetes typically present lower C-peptide levels than individuals with type 2 diabetes since more insulin are produced in response to glucagon. This finding was consistence with work of Ahmad & Reza [15] that observed that DM individuals had decreased production of endogenous insulin and reduced plasma level of C-peptide because of beta cell destruction.

HbA1c provides a reliable measure of chronic hyperglycemia and correlates well with the risk of long-term diabetes complications and it is a reliable biomarker for the diagnosis and prognosis of diabetes [16]. Also from the result significantly higher level of HbA1c were observed among DM than in non DM. the reason for

Table 1. Autoantibodies and glycemic parameter of Diabetic and non-diabetic subjects

Parameters	Diabetes subjects	Control subjects	P-value	Remark
ICA (U/ml)	14.91±11.11	4.48 ± 2.18	0.010	S
GADA(U/ml)	1.78 ± 0.77	0.99 ± 0.22	0.152	NS
IA-2A(U/ml)	4.20 ± 3.27	3.83 ± 1.56	0.595	NS
Zn8TA(U/ml)	6.02 ± 3.80	5.61 ± 4.29	0.686	NS
FBS mmol/l	10.31 ± 6.72	5.55 ± 1.60	0.001	S
C-peptide U/ml	1.56 ± 0.67	1.71 ± 0.88	0.001	S
HbA1c%	6.41 ± 2.20	5.08 ± 0.71	0.004	S

S – Significant different at p< 0.05, NS –Non-significant

Table 2. Glycemic Parameters correlated with autoantibodies of non-diabetic (control) parents

Parameters		FBS (mmol/l)	C peptide (U/ml)	HbA1c (%)
ICA (U/ml)	r value	-0.225	-0.194	-0.011
	p-value	(0.269)	(0.341)	(0.958)
GADA(U/ml)	r value	0.319	0.600**	-0.182
	p-value	(0.112)	(0.001)	(0.384)
IA-2A (U/ml)	r value	0.148	-0.211	-0.282
	p-value	(0.472)	(0.301)	(0.172)
ZnT8A(U/ml)	r-value	0.233	0.125	-0.096
	p-value	(0.252)	(0.543)	(0.649)

significant high level of HbA1c may be because in diabetes excess glucose that cannot be move from cell to store as glycogen binds to heamoglobin of red blood cell as result of pancreas failure to release insulin or insulin resistance. It was also observed that HbA1c is an effective tool in establishing the diagnosis of diabetes, especially in low- and middle-income (developing) countries and hard-to-reach (poor) populations, endorsed for diagnosis of diabetes and HbA1c test should be implemented as part of the diagnostic and prognostic tool, for better patient care and successful clinical outcomes. The work of Li et al [17], on HbA1c and survival in maintenance hemodialysis patients with diabetes in a Chinese population, observed low HbA1c in diabetic patient with moderate hyperglycemia and it was concluded that low HbA1c increases mortality risk in diabetic patients.

Glycemic Parameters correlated with autoantibodies of non-diabetic parents (control) (Table 2) showed that FBS mmol/l has no correlation with ICA, GADA, IA-2A and ZnT8A. C-peptide (u/ml) also had no correlation with ICA, IA-2A and ZnT8A, but there was correlation with GADA. HbA1c % has no correlation with ICA, GADA, IA-2A and ZnT8A. FBS, and HbA1c both had no correlation with ICA, GADA, IA-2A and ZnT8A since the subjects were non-diabetic parents. Though C-peptide has no correlation with ICA, IA-2A and ZnT8A, but there was correlation with C-peptide and GADA. This revealed that some of the non- diabetic subject may be a predictive risk factor for LADA. This was consistent with the following works: GADA is the most sensitive immune antibody used for the diagnosis of LADA, a positive GADA and low C-peptide is an indication of TIDM and a the presence of GADA in T2DM with low C-peptide is a sign of LADA and patients need insulin treatment since the body is not producing insulin or the one produced is not used (insulin resistance) [18].

Glycemic parameters correlated with autoantibodies of diabetic parents (Table 3) showed that FBS (mmol/l) had no correlation with ZnT8A, IA-2A and ICA, but there was correlation with GADA. C-peptide (u/ml) has no correlation with ZnT8A, IA-2A, GADA and ICA HbA1c % also had no correlation with ZnT8A, and ICA, but there was correlation with IA-2A and GADA.

Table 3. Glycemic Parameters correlated with autoantibodies of diabetic parents

Parameters		FBS (mmol/l)	C peptide (U/ml)	HbA1c (%)
ICA (U/ml)	r value	0.172	0.069	0.032
	p-value	(0.253)	(0.649)	(0.834
GADA(U/ml)	r value	0.522**	0.149	0.337**
	p-value	0.000)	(0.323)	(0.022)
IA-2A (U/ml)	r value	0.179	0.261	0.400**
	p-value	(0.235)	(0.080)	(0.006)
ZnT8A(U/ml)	r-value	-0.021	0.243	-0.053
	p-value	(0.888)	(0.104)	(0.729)

Table 4. Glycemic Parameters of Diabetic and non - diabetic subjects

Subject	N	FBS (mmo/l) M±SD	C-peptide (U/ml) M±SD	HbA1c% M±SD
Diabetic Subject	132	10.31 ± 6.72	1.56 ± 0.67	6.41 ± 2.20
Control Subject	112	5.55 ± 1.60	1.71 ± 0.88	5.08 ± 0.71
P-value		0.001	0.001	0.004
Remark		S	S	S

N – Number of subjects, S – Significant

FBS had correlation with GADA; and HbA1c had correlation with GADA and IA-2A. GADA and IA-2A are intracellular auto-antigens present in beta-cells. Autoimmune damage of beta-cells causes the release of these intracellular auto-antigens. All T2DM persons showed an increased FBS and higher levels of HbA1c. So, FBS and HbA1c having correlation with GADA (the most sensitive immune antibody for LADA) is an indication of T2DM in individuals progressing to LADA. Also the presence of IA-2A in diabetic patients shows an increased risk of autoimmune diabetes [19]. This is in agreement with the work of [20]. About 80% of diabetic patients with GADA were an asymptomic person showing an increased risk of TIDM development and about 60% of individual with IA-2A had an increased risk of TIDM [21,22].

4. CONCLUSION

Diabetes mellitus patient with positive ICA, GADA and IA-2A autoantibodies, higher HbA1c and lower C-peptide suggest an autoimmune or intracellular damage of beta-cells in T2DM and need for insulin dependence or progression to LADA. Also presence of IA-2A in T2DM is TIDM individual masquerading as T2DM on the other hand. GADA in non-diabetic subject is a predictive risk factor for LADA. The author recommended that autoantibodies tests should be performed on Chronic Diabetic patient on anti-diabetic medication to monitor patient progression towards insulin dependence or LADA.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Ethical approval was obtained from Ethics and Research Committee of Hospital Management Board, Port- Harcourt of Rivers State. Sample collection approval was obtained from various Heads of Department and finally from the Chief Medical Director (C.M.D) of RSUTH, Port Harcourt, Nigeria.

ACKNOWLEDGEMENTS

Author are grateful to Mr Sordum Neebee for funding, Mr Deeka Torkia Lah Nwikpo for the procurement of reagents, Hannah Jojo, Effective Laboratories and Andy-Nwokocha Mary of Rivers State University Teaching Hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71063