Original Article

Serum Levels of Anti Glutamic Acid Decarboxylase and Insulin Auto Antibodies in First Degree Relatives of Patients with Diabetes Mellitus

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ABSTRACT

Antibodies to Glutamic Acid Decarboxylase (anti-GAD) and Insulin Auto Antibodies (IAA) mediate the autoimmune destruction of pancreatic islet cells and have been used to predict the risk of future development of Diabetes Mellitus (DM). These circulating antibodies are usually detected in body fluids and tissues several years before the onset of hyperglycemia. This helps in identifying individuals at risk of future development of DM thereby providing a good lead time for secondary preventive measures such as Therapeutic Lifestyle Changes to be introduced. This study determined the prevalence of Anti-GAD and IAA as a major risk factor for developing DM among First Degree Relatives (FDR) of DM patients in Kano, Northwestern Nigeria. This was a cross-sectional descriptive study carried out on 100 randomly selected FDR of individuals with DM in the diabetic clinic of Aminu Kano Teaching Hospital (AKTH). Control group recruited were 100 apparently healthy subjects with no family history of DM. Anti-GAD and IAA were measured using Enzyme Linked Immunosorbent Assay (ELISA) based technique. GAD Ab levels >32 ng/mL and IAA levels >39 ng/ml were considered positive for Antibodies. Data obtained was analyzed using statistical package for social sciences (SPSS) version 20.0. Anti-GAD and IAA were present in 81% (O.R= 1.9, 95% CI= 1.0-3.7, p= 0.050) and 66% (O.R= 1.9, 95% CI= 1.7-5.6), p <0.001) of FDR of DM patients compared to controls 69% and 34%. The mean level of Anti-GAD was also higher among the FDR of diabetics compared to controls (68.4±31.9 vs 45.3±22.4, p <0.001). The mean IAA level was also higher among the FDR of diabetics compared to controls (61.0±20.7 vs 51.8±11.7, p <0.001). This study demonstrated a high Anti-GAD and IAA positivity among first degree relatives of diabetics compared to controls underscoring the importance of screening for risk factors. Preventive measures like lifestyle changes can be appropriately applied among to slow the progression to diabetes mellitus.

Keywords: *Anti-GAD, Diabetes Mellitus, First Degree Relatives, IAA.*

INTRODUCTION

Most type 1 DM results from cell-mediated autoimmune destruction of the insulin-secreting cells of pancreatic β-cells.[1,2] The destruction mediated by T cells occurs months or years before the clinical presentation and symptoms evolve when 80% to 90% reduction in the volume of the β-cells occurs.[3] Circulating antibodies regarded as markers of β-cell autoimmunity include Islet cell antibodies(ICAs), Insulin autoantibodies (IAAs) and Antibodies to the 65-KD isoform of glutamic acid decarboxylase (GAD_{65})[1,2] The most prevalent autoantibodies are directed at GAD_{65} , the tyrosine phosphate-like protein autoantibodies and Insulin autoantibodies.[3-8] In a study that was carried out there is some evidence of ethnic differences in the autoimmune response.[9] The prevalence of these antibodies have been studied with variable results. In a study that was carried out in Riyadh Saudi Arabia on prevalence of autoantibodies in children newly diagnosed with type 1 DM , the result showed that 67% were positive for ICA, 36% for IAA and 84.4% for GAD.[4] Two surveys in the Netherlands showed prevalence of ICA of 0.24% and 0.29% respectively.[5,6] The frequency of ICA among first-degree relatives ranged from 2.6% in parents in the United States to 7.8% in Finnish siblings.[7,8] Different data was reported from Africa , with the prevalence of 14% in Nigeria, 13.5% in Ghana and 7.3% in Tanzania.[10] Autoantibodies as predictors of future risk of DM can be used to identify individuals at high risk of DM, providing good lead time for secondary preventive measures. The exact prevalence of the antibodies and their role in Type 1 DM has not been determined in the study population. This study therefore aimed to determine the prevalence of Anti-GAD and IAA among first degree relatives of patients with diabetes mellitus (type I and type II) in AKTH, Kano.

MATERIALS AND METHODS

This cross-sectional study was conducted among 100 FDR of DM patients receiving clinical care at Aminu Kano Teaching Hospital, Kano, Nigeria. Sample size was determined using Fisher's statistical formula[11] and Prevalence[1] from previous studies. Ethical approval and informed consent were obtained from the Ethical Research Committee of AKTH and study subjects respectively. Apparently Healthy FDR (> 18years) accompanying DM patients to the Hospital were consecutively sampled over a period of 2 months from August to September of 2017. FDR for the purpose of this study were siblings and offspring of DM patients. DM was ruled out by fasting plasma glucose screening in subjects while Pregnant and Breast-feeding mothers were excluded. A pre-tested questionnaire was administered and information on bio-data and biophysical parameters of the participants measured. Blood sample was collected from first degree relatives accompanying patients to the hospital after an overnight fast. Transport fare to hospital on the second day was compensated. The blood specimen was processed and serum was stored for subsequent analysis at -20^0c. Quantitative measurement of autoantibodies to glutamic acid decarboxylase and insulin autoantibodies was carried out using high enzyme-linked immunosorbent assay (ELISA).[12,13] All data collected was entered into excel spread sheets. This was subsequently cleaned by filtering and sorting. Cleaned data was exported into Statistical Package for the Social Sciences (SPSS) version 20 for analysis. Mean and Standard deviation was used to summarize quantitative data while proportions and percentages was used for qualitative data Statistical analysis to compare qualitative variables was done using chi-square test, while analysis of quantitative variables was done using students t-test. In all the analysis, 5% alpha level of significance which corresponds to 0.05 was considered as statistically significant.

RESULTS

One hundred subjects, who were first degree relatives of patients with diabetes mellitus (group1) and 100 age-matched apparently healthy individuals with no family history of DM as controls (group 2) participated in this study which was conducted from April 2016 to January 2017. The age range of study subjects was between 13 and 62 years (mean = 27.9 ± 9.2). Majority of the study subjects (64.5%) were within the age group of 18 - 28 years (figure 1). There were slightly more females than males with a ratio of 1.2: 1 among the study subjects. Some of the study subjects were also students. Others were civil servant, housewives, businessmen, tailoring farmers, traders & drivers (See table 1). The mean concentration of fasting plasma glucose was found to be similar among group 1 and group 2 (p= 0.991). The mean serum level of anti-GAD

Table 1: Socio demographic characteristics of the studies subjects

Characteristics		Study subjects		P value
		Group 1	Group 2	
Age (years)*		27.6 ± 9.7	28.2 ± 8.7	0.603
Gender	Male	45	65	0.004
	Female	55	35	0.003
Marital Status	Married	39	29	0.305
	Single	59	67	0.300
	Divorced	2	4	<0.001
Occupation	Student	50	64	? 0.001
	C/Servant	8	23	<0.001
	H/wives	16	5	<0.001
	B/men	15	3	<0.001
	Tailoring	6	2	<0.001
	Others	5	3	<0.001

*Result of age is in mean ± SD while other results are in numbers with the corresponding percentage in brackets.

Table 2: Fasting plasma glucose, anti-GAD antibody and Insulin autoantibodies (mean ± SD) of the study subjects

Parameters	**Study subjects**		**P value**
	Group 1	**Group 2**	
	(n = 100)	(n = 100)	
FPG (mmol/L)	5.0 ± 1.4	5.0 ±1.1	0.991
Anti-GAD (ng/mL)	68.4 ± 31.9	45.3 ± 22.4	0.001
IAA (ng/mL)	61.0 ± 20.7	51.8 ± 11.7	?0.001

Table 3: Prevalence of anti-GAD antibody and insulin autoantibodies among study subjects

Parameters	**Group 1 (%)**	**Group 2 (%)**	**P**
Anti-GAD antibody	81.0	69.0	0.050
Insulin autoantibodies	65.9	34.1	?0.001

was found to be higher among group 1 study subjects compared to group 2 study subjects (p ? 0.001). The mean concentration levels of insulin autoantibodies were also higher in group 1 compared to group 2 study subjects (See table 2). Prevalence of Anti-GAD antibody was found to be higher among the first-degree relatives of patients with diabetes mellitus, (81.0%), compared to the controls (69.0%). The prevalence of IAA positivity was significantly higher (65.9% vs 34.1%) among group 1 study subjects compared to group 2 (See table 3).

DISCUSSION

Diabetes mellitus runs in families, and individuals who have relatives with DM are at higher risk of developing this metabolic disorder compared to general population. In this study, the mean fasting plasma glucose level was found to be similar and within reference range between the first-degree relatives of diabetics and the controls. DM being a disorder with spectrum of changes from Normoglyaemic to hyperglycaemic phase at early stages may be asymptomatic in keeping with these findings.

IAA and anti-GAD have been implicated in the pathogenesis of DM and their presence in plasma is predictive of future development of diabetes. In this cross-sectional study, anti-GAD positivity was twice as common in FDR of DM patients than the controls. Similar observations were made in studies albeit among type 2 DM. [14,15,16,17,18,19,20]

Our study also showed that Glutamic Acid Decarboxylase Antibody (GADA) positivity in FDR of diabetics tended to be of younger age compared to those who were GADA negative. GADA has been shown to be more associated with Type 1 DM which is commoner among the young age groups. Lutale and colleagues in Tanzania reported significant difference between first degree relatives of diabetics who were GADA positive and those who were GADA negative.[21] Similar to other studies we did not find any gender predisposition to GADA positivity.[16,21]

IAA positivity was three times as common among the FDR of diabetic patients in comparison to controls. This finding agrees with a study carried out in the United States which showed that IAA was associated with family history of diabetes.[22] However, the prevalence observed (65.9%) in our study was slightly lower than the prevalence (87.8%) in their study. This difference might be explained by the fact that their study was restricted to relatives of Type 1 DM patients whereas our study involved relatives of both Type 1 and 2 DM patients. The prevalence in this study is also higher than the prevalence of 22.2% found in a study conducted in Poland even though the study subjects in the polish study were only first degree relatives of Type 1 DM.[23] The ELISA used in this study is more sensitive than the commercial radioimmunoassay used in the studies mentioned. Similarly, IAA demonstrated no age, gender, BMI or FPG predilections.[24]

CONCLUSION

Antibodies to glutamic acid decarboxylase are significantly higher among first degree relatives of diabetic. The insulin auto-antibodies positivity is three times commoner among the first-degree relatives of individuals with DM compared to controls.

Recommendation

Screening for GADA and IAA should be routinely carried out among first degree relatives of diabetic patients so that those who have high levels of the antibodies will be counselled and educated on preventive measures to prevent or delay progression to overt diabetes mellitus.

Conflict of Interest

The authors have no conflict of interest to declare.

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