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The Prevalence of Autoimmune Diabetes Among Diabetes Mellitus Patients in Kumasi, Ghana

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Abstract: This study investigate the occurrence and the prevalence of autoantibodies and the metabolic characteristics of autoimmune and antibody-negative type 2, diabetes in recently diagnosed diabetes mellitus patients in Kumasi, Ghana. This study involved a total of 120 recently diagnosed (< 1 year) Ghanaian diabetes mellitus patients (17 insulin-requiring and 103 non insulin-requiring) and 60 controls. A standardized questionnaire was used. Blood pressure and anthropometric measurements were taken. Fasting glucose, lipid and lipoprotein concentrations were measured by enzymatic methods and HbA_{1c} levels by agglutination test. Serum insulin level and autoantibodies (ICA, GAD ab and IAA) were analyzed by enzyme-linked immunosorbent assay (ELISA). Out of the 17 insulin-requiring, six were positive for either GAD ab or ICA or both. Out of the 103 non insulin-requiring, 16.5% were positive for ICA and /or GAD ab and/or IAA. The prevalence of Latent Auto-immune Diabetes of Adults (LADA) in the non-insulin requiring and in the total diabetic patients, were 13.5 and 11.7%, respectively. The prevalence of autoimmune type 1 diabetes in the studied population was 7.5% and that of autoimmune diabetes in the total diabetic population was 19.2%. Autoimmune and antibody-negative type 2, diabetes did not differ ($p = ns$) in the mean values of clinical and metabolic parameters, except hypertension, central obesity and HbA_{1c} values. Autoimmune diabetes occurs in recently diagnosed diabetic patients in Ghana at prevalence comparable to that in developed countries. Both ICA and GAD ab tests are required to identify autoimmune diabetes.

Key words: Autoantibody, obesity, hypertension, HbA_{1c}, LADA

INTRODUCTION

The characteristic lymphocytic infiltration of the islet cells and associated autoantibodies, including Islet Cell Autoantibodies (ICA), autoantibodies to Glutamic Acid Decarboxylase (GAD ab), insulin autoantibodies (IAA) and autoantibodies to tyrosine-phosphatase protein (insulinoma associated antigen 2-IA-2 and IA-2 β) (Verge *et al.*, 1996) formed against a variety of islet cell antigens, provide early markers of autoimmune disease activity. The measurement of these autoantibodies had been shown to be extremely useful in assisting the physician with the prediction, diagnosis and management of patients with diabetes mellitus (Verge *et al.*, 1996). Shortly after the original description of islet cell antibodies (ICAs) as a marker

for childhood type 1 diabetes, it was realized that some adult-onset patients are also ICA positive (Irvine *et al.*, 1977). Subsequently (GAD ab) were also discovered as another marker of type 1 diabetes. (Baekkeskov *et al.*, 1982). On one hand, studies indicate that as many as 10% of adult patients, initially diagnosed as type 2, eventually become insulin dependent (Niskanen *et al.*, 1995). These diabetic patients, initially masquerading as type 2, are in fact late-onset, or slow developing, type 1 diabetic patients. Like classical type 1, late onset type 1 diabetes results from the autoimmune destruction of the beta cells. Zimmet (1995) introduced the term latent autoimmune diabetes of adults (LADA) to describe autoimmune late onset type 1 diabetes. Earlier reports however, had found ICA and GAD ab to be rare among Nigerians, Filipinos or patients

of African origin (Medici *et al.*, 1999; Oli *et al.*, 1980). To our knowledge, it is not known whether autoantibodies or autoimmune diabetes is present among Ghanaian diabetic patients. The objective of this study was to investigate the occurrence and prevalence of autoantibody patterns and the clinical and metabolic characteristics of autoimmune diabetes and antibody-negative type 2 diabetes among recently diagnosed diabetic patients in Ghana.

MATERIALS AND METHODS

Patients: Newly and recently diagnosed (< 1 year) diabetes mellitus patients on insulin or diet/oral hypoglycaemic drugs, but not both, were consecutively selected until a sample size of 120 (including 31 males and 89 females) was achieved. For convenience, those who were on insulin would be referred to as insulin requiring and those who were not on insulin as non-insulin requiring. Diabetes was defined according to the World Health Organization (1999) criteria. Healthy controls included apparently healthy subjects, who were additionally assessed as normal by blood haemoglobin, total and differential white blood cell count, Body Mass Index (BMI), waist circumference, blood pressure, with normal glucose tolerance, as assessed by a 75 g oral glucose tolerance test using World Health Organization (1999) criteria, with an absence of diabetes mellitus within first-degree relatives. These patients had no obvious disease, physiological state or intake of pharmacologically active agents. Consecutive age and sex-matched healthy subjects who met the inclusion criteria were selected until a sample size of 60 healthy subjects (25 males and 35 females) was achieved.

Methods: The study was conducted at the Komfo Anokye Teaching Hospital/School of Medical Sciences, Kumasi in the Ashanti region of Ghana. All study protocols were approved by the Committee for Human Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology. All participants consented to participate in the research.

A standardized questionnaire and patients' medical history folders were used to collect information on demographic and clinical characteristics such as age, sex, ethnic group (tribe), duration of diabetes, age of onset, medication history, family history of diabetes,

hypertension, other physician-diagnosed diseases and stress. Height and weight were measured in subjects wearing lightweight clothing and without shoes and BMI was calculated (kg m^{-2}). Waist circumference was measured on bare skin during midrespiration at the bending point and at the narrowest indentation. Subjects who were on antihypertensive medication were considered to have hypertension. Blood pressure was measured twice per patient with 5 min intervals in the sitting position after 30 min of rest and the mean recorded. Blood specimens were obtained after 8-14 h overnight fast. Serum fasting glucose, total cholesterol, triglycerides and High Density Lipoprotein (HDL) cholesterol were measured by enzymatic methods using an ATAC 8,000 Random Access Chemistry autoanalyzer (élan diagnostics, A4-001-1198) and its reagent kits. Low Density Lipoprotein (LDL) cholesterol was calculated using Friedewald (1972) formula. Glycosylated haemoglobin (HbA_{1c}) levels were measured by an inhibition of latex agglutination test simultaneously with total haemoglobin by haemoglobin thiocyanate method, using DCA 2000+ analyzer (Bayer model 5031, USA) and its reagent kits. Insulin, ICA, GAD ab and IAA were determined by enzyme-linked immunosorbent assay (ELISA) technique using DRG International Inc., USA EIA kit reagents, an ELISA reader (Tipo model N Matricola Cotruito manufactured, Italy) and ELISA washer (Murex, Dynatech Med Prod. Ltd., Guensey Channel Islands, Great Britain). The standards were calibrated against international WHO approved reference material NIBSC 66/304.

Statistics: Statistical analysis were performed using the statistical package for social sciences (SPSS) for windows programme version 11.0.

RESULTS

This prospective study covered the period from July 2006 to June 2007. It involved a total of 120 recently diagnosed (<1 year) Ghanaian diabetes mellitus patients [17 (14.2%) were insulin requiring and 103 (85.8%) were non-insulin requiring] and 60 reference individuals. The mean age of the patients was 48.2 ± 13.4 years. The mean age of onset of diabetes was 47.9 ± 13.4 years. The mean value of basal metabolic index was $25.7 \pm 5.4 \text{ kg m}^{-2}$. It was observed that two (3.3%) of the reference individuals had autoantibodies (i.e., 1 ICA and 1 GAD ab). No reference individual had autoantibodies to IAA.

Out of the 17 insulin requiring patients, six (35.3%) were positive for ICA or GAD ab or both autoantibodies. ICA was detected in five (30%) and GAD ab in three (18%) of this diabetic population. The remaining 11 (64.7%) insulin-requiring patients were negative for autoantibodies. Insulin autoantibodies (IAA) were not measured in this group since they were on insulin therapy.

Out of the 103 recently diagnosed non insulin-requiring diabetic patients, 17 (16.5%) were positive for ICA and/or GAD ab and/or IAA. This consisted of 10 (9.7%) who were positive for ICA, nine (8.7%) who were positive for GAD ab and three (2.9%) who were positive for IAA. The three (2.9%) patients who were positive for IAA were also positive for ICA and/or GAD ab. Thus 86 (71.7%) of the non-insulin requiring patients, were autoantibody-negative. 14 (13.5%) of the recently diagnosed non-insulin requiring diabetic patients were positive for one or more of ICA, GAD ab or IAA, with age of onset of diabetes >35 years and insulin therapy not indicated in the first 6 months after diagnosis. Eleven of the above group of patients had single autoantibody positivity and three multiple (two or more) autoantibody positivity. However, three (2.9%) of the recently diagnosed non-insulin requiring diabetic patients, who were positive for one or more of ICA, GAD ab or IAA, had age of onset of diabetes = 35 years and insulin therapy not indicated in the first six months after diagnosis. The total number of the recently diagnosed diabetic population with age of onset < 35 years who were autoantibody positive was therefore 9 (7.5%).

Six of the nine autoimmune type 1 diabetic patients had single autoantibody positivity, whilst three had multiple autoantibody positivity. In all, 23 (19.2%) autoantibody-positive patients (including those with

onset > 35 years) were demonstrated in the total recently diagnosed diabetic patients, including 15 (12.5%) who were positive for ICA and 12 (10.0%) who were positive for GAD ab. It was found that 17 of the total autoimmune diabetic patients had single autoantibody positivity and six had multiple autoantibody positivity.

The mean values of the clinical and metabolic parameters (Table 1) of the 23 (19.2%) autoimmune diabetic patients and the 86 (71.7%) autoantibody-negative diabetic patients did not differ ($p = ns$); Compared with autoantibody-negative type 2 diabetic patients, autoimmune diabetic patients in Ghana were less likely to be hypertensive (17.0% vs. 26.0%, $p = 0.001$) and less likely to have central obesity (34.0 vs. 43.0%, $p = 0.01$) but more likely to have high HbA_{1c} (8.2 vs. 7.7%) values.

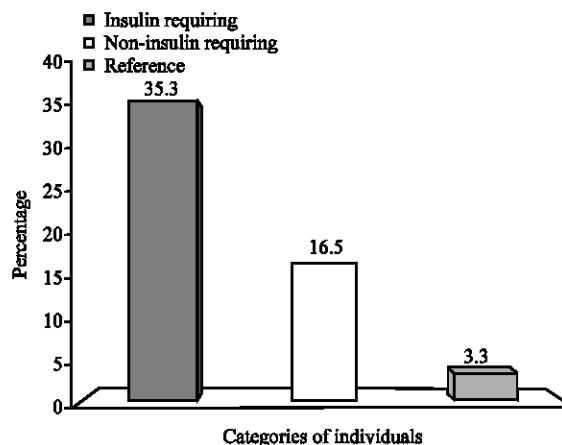


Fig. 1: Prevalence of autoantibodies in insulin-requiring and non-insulin requiring recently diagnosed diabetic patients and reference individuals in Kumasi, Ghana

Table 1: Clinical and metabolic characteristics of diabetes with and without autoantibodies among recently diagnosed diabetes mellitus patients in Kumasi, Ghana

Clinical and metabolic parameters	Autoimmune diabetes (N = 23)	Type 2 diabetes (N = 86)	p-values
Mean age (years)	45.4±15.5 (13.0-70.0)	50.4±11.7 (25.0-80.0)	0.090
Mean age of onset (years)	44.8±15.6 (12.5-69.6)	50.2±11.6 (24.4-79.8)	0.060
Preprandial glucose (mmol L ⁻¹)	9.0±5.9 (3.5-27.3)	7.5±4.0 (3.3 -22.2)	0.160
HbA _{1c} (%)	8.2±3.0 (4.4-14.0)	7.7±2.2 (4.6-14.0)	0.010
BMI (kg m ⁻²)	25.2±4.5 (18.7-33.2)	26.2±5.3 (15.1-45.0)	0.260
Hypertension No. (%)	4 (17.0%)	23 (26.0%)	0.001
Waist circumference (cm)	87.9±11.5 (65-104)	89.9±10.4 (66-131)	0.430
Central obesity No. (%)	8 (34.0%)	33 (43.0%)	0.010
Mean total cholesterol (mmol L ⁻¹)	4.3±1.1 (2.2-6.5)	4.7±1.3 (1.5-7.4)	0.280
Triglycerides (mmol L ⁻¹)	1.3±0.5 (0.60-2.65)	1.3±0.6 (0.20-3.98)	0.850
HDL cholesterol (mmol L ⁻¹)	1.3±0.5 (0.39-2.16)	1.4±0.5 (0.43-2.80)	0.400
LDL cholesterol (mmol L ⁻¹)	2.4±0.9 (0.40-4.75)	2.7±1.1 (0.07-5.03)	0.350
Insulin (μU mL ⁻¹)	7.8±3.1 (1.5-16.5)	8.5±4.8 (1.5-19.5)	0.490

DISCUSSION

For a clinician, the distinction between types 1 and 2 diabetes is very important but it is not always straightforward. The presence or absence of islet cell autoantibodies is one of the more direct ways to distinguish between types 1 and 2 diabetic patients. It is now believed that among the non-insulin requiring diabetic subjects at diagnosis, a significant minority are islet cell antibody-positive (Schiel and Mueller, 2000). These patients who clinically are difficult to distinguish from type 2 diabetic subjects test positive for those markers that characterize patients with type 1 diabetes. The term latent autoimmune diabetes in adults (LADA) was introduced to define adult diabetic patients initially non-insulin requiring but with immune markers of type 1 diabetes that, in a number of cases, progress to insulin dependency (Tuomi *et al.*, 1993). Autoantibodies against islet antigens allow one to clearly distinguish autoimmune diabetes in adults from autoantibody-negative type 2 diabetes and provide the strongest evidence that autoimmune diabetes are autoimmune disorders. The prevalence of ICA (0.017) and GAD ab (0.017) observed in healthy individuals in this study were low and comparable to earlier reported values (Takeda *et al.*, 2002). Thus, when these autoantibodies are found in diabetic patients, they are specific for the diabetic condition.

Earlier reports had found ICA and GAD ab to be rare among Nigerians, Filipinos or patients of African origin (Oli *et al.*, 1980). In contrast, slightly more than one-third of the insulin-requiring (apparent type 1 diabetes) recently (< 1 year) diagnosed diabetic patients in this study were positive for ICA and/or GAD ab and therefore, have autoimmune diabetes. The prevalence rate of ICA and GAD ab among Caucasian (70-80%) new-onset type 1 diabetic patients (Manjula *et al.*, 2002) is far higher than that (35%) in recently diagnosed Ghanaian insulin-requiring diabetic patients. The Ghanaian prevalence rate of ICA (29%) is also markedly lower than that (70%) observed among Caucasians (Irvine *et al.*, 1977). Similarly, the GAD ab prevalence (18%) among recently diagnosed non-insulin requiring Ghanaian diabetics is lower than that (32%) observed among South African Black and Indian subjects with type 1 diabetes (Motala *et al.*, 1999) and markedly lower than that (70-90%) in Caucasian type 1 diabetic patients (Vandewalle *et al.*, 1995). These results confirm the presence of ICA and GAD ab or autoimmune diabetes in recently diagnosed insulin-requiring or apparent type 1 diabetic patients in Ghana, though roughly at a lower prevalence than that reported for Caucasian type 1 diabetic patients.

The present study identified 16.5% non insulin-requiring recently diagnosed (< 1 year) Ghanaian diabetic patients with ICA and/or GAD ab and/or IAA, a value that lies within the percentage range of 10-20% reported for the world population (Niskanen *et al.*, 1995). The results indicate that IAA is rare in both Ghanaian healthy individuals and non-insulin requiring recently diagnosed Ghanaian diabetics. The three (2%) of the patients who were positive for IAA, were also positive for ICA and/or GAD ab. Therefore measuring IAA does not provide any additional information and hence it is not beneficial. It had been documented that- the presence of ICA and/or GAD ab is the best predictor of the early insulin requirement (Turner *et al.*, 1997). The autoantibody-negative non-insulin requiring recently diagnosed Ghanaian diabetic patients and therefore, type 2 diabetes, was found to be 71.7%. The 11 insulin-requiring patients who tested negative for autoantibodies could be referred to as unclassified patients and they form 9.1% of the total diabetic population in this study. The autoantibody to GAD ab positivity of 8.7% obtained in this study for recently diagnosed Ghanaian non-insulin requiring diabetes lies within the range of 6-10% obtained for Caucasian non-insulin dependent diabetic patients (4.17). Additionally, autoantibodies to GAD ab had been found to be a useful predictive marker for the development of insulin dependency in apparent type 2 diabetes (Turner *et al.*, 1997, 1999).

The prevalence of LADA (i.e., the non-insulin requiring patients, age of onset of diabetes >35 who were autoantibodies positive) in the total recently diagnosed diabetes population (11.7%) is consistent with values of 2-12% reported by Turner *et al.* (1997). The prevalence of single autoantibody positivity in the LADA group of recently diagnosed diabetic patients in Ghana was 79% and multiple (two or more) autoantibody positivity was 21%. The prevalence of autoimmune type 1 diabetes in the recently diagnosed diabetes population in Ghana (7.5%) provides evidence that autoimmune type 1 diabetes occurs in the Ghanaian diabetic population.

The prevalence of GAD ab in the Ghanaian recently diagnosed diabetic population (10%) was found to be roughly higher than that reported for newly diagnosed diabetic patients (8%) in a Swedish study (Wroblewski *et al.*, 1998) and a population-based Cremona study (2.8%) in Italy (Bosi *et al.*, 1999) in contrast, a roughly higher prevalence of GAD ab positivity (>20%) was observed in Northern Italy (Bruno *et al.*, 1999). The differences in the values could be attributed to the differences in the inclusion criteria (age, duration and category of diabetes) and assay methods.

In this study, single autoantibody positivity was slightly higher in LADA (three-quarters) than autoimmune type 1 diabetes (two-thirds). Conversely, multiple by autoantibody positivity was higher in autoimmune type 1 diabetes (one-third) than LADA (one-quarter). However, Hosszufalusi *et al.* (2003) reported that single autoantibody positivity was significantly higher ($p = 0.0001$) in LADA (59%) than in autoimmune type 1 diabetes (23%). They concluded that the presence of single autoantibody positivity rather than low titre indicates the less aggressive destruction of islet cells. Single autoantibody positivity (ICA or GAD ab) was mainly responsible for both LADA and autoimmune type 1 diabetes rather than multiple autoantibody positivity among the diabetic patients used in this study. This implies that both ICA and GAD ab tests should be used to identify autoimmune diabetes among diabetic patients in Ghana. In addition, it had been reported that, at diagnosis, both ICA and GAD ab were shown to be predictors of insulin dependency, but GAD ab persisted for a longer time and had higher sensitivity as predictors than ICA (Tuomi *et al.*, 1999; Kobayashi *et al.*, 1996), whilst ICA had a higher prevalence. Therefore, combined measurement of ICA and GAD autoantibodies provide more useful information. Autoimmune diabetes among recently diagnosed diabetic patients in Ghana was mainly due to the presence of ICA and GAD autoantibodies which indicate slow disease progression or less aggressive destruction of islet cells (Seissler *et al.*, 1998); hence autoimmune diabetes among Ghanaian diabetics would be expected to progress slowly rather than have an acute onset.

In this study, clinical and metabolic parameters that were comparable ($p = ns$) in autoimmune diabetes and autoantibody-negative type 2 diabetic patients in Ghana included (Table 1) age, age of onset, pre-prandial glucose, BMI and waist circumference; others were total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and insulin; the exceptions were hypertension and central obesity which were more likely in autoantibody-negative type 2 diabetes than autoimmune diabetes ($p = 0.001$, $p = 0.01$, respectively) and HbA_{1c} which was higher ($p = 0.01$) in autoimmune diabetes than type 2 diabetes. Therefore, generally, clinical and metabolic markers cannot be used to distinguish Ghanaian autoimmune diabetes from type 2 diabetes. It had been reported by Lohmann *et al.* (2001) that only diabetic patients with multiple autoantibody positivity had lower BMI and lower frequency of hypertension compared with autoantibody-negative type 2 diabetic patients by Lohmann *et al.* (2001). The autoimmune diabetic patients in this study were mostly single

autoantibody-positive and this may explain why there was no difference in the clinical and metabolic markers.

In summary, the findings in this study demonstrate the presence of autoimmune diabetes in insulin-requiring recently diagnosed diabetic patients in Ghana, though at a prevalence lower than Caucasian type 1 diabetic patients. The prevalence of autoimmune diabetes in the non-insulin requiring recently diagnosed diabetic patients in Ghana was comparable to that reported for the world population. Similarly, the prevalence of LADA in the non-insulin requiring and in the overall recently diagnosed diabetes population were consistent with values reported for Caucasians. Further, autoimmune type 1 diabetes and the overall autoimmune diabetes occur in recently diagnosed diabetic patients in Ghana at prevalence that is comparable to that in developed countries. Such prevalence is of value in developing early intervention strategies, correct classification of diabetes and for public health purposes. Single autoantibody positivity (ICA or GAD autoantibodies) was mainly responsible for autoimmune diabetes, including LADA and autoimmune type 1 diabetes, rather than multiple autoantibody positivity, among recently diagnosed diabetic patients in Kumasi, Ghana. In this study, clinical and metabolic parameters were comparable in autoimmune diabetes and autoantibody-negative type 2 diabetic groups of the recently diagnosed diabetic patients in Ghana. The exceptions were hypertension and central obesity, which had higher prevalence in autoantibody-negative type 2 diabetes than autoimmune diabetes and HbA_{1c} which was higher in the latter than the former.

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