AUTOIMMUNE PROFILE IN INSULIN DEPENDENT DIABETES MELLITUS

AT KENYATTA NATIONAL HOSPITAL

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A dissertation presented as part of fulfillment for the degree of Master of Medicine (Medicine) of the University of Nairobi.
DECLARATION

This dissertation is my original work and has not been presented for a degree in any other University.

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131 patients with IDDM attending K.N.H. were studied. The mean age of onset of IDDM was 20.1 years. There was a male predominance with M:F ratio of 1.4:1. There was a low prevalence of IDDM among first degree relatives of diabetic probands. Definitive history of viral infection preceding the onset of IDDM was found in one patient. None of the patients had thyroglobulin antibodies; thyroid microsomal antibodies were found in 0.7% of the patients. Parietal cell antibodies were found in 4.6% of the patients. Islet cell antibodies were found in 3.7% of patients with IDDM. The prevalence of autoantibodies in Kenyan Africans with IDDM is much lower when compared with Caucasians and Black Americans with IDDM. In view of the low prevalence of autoantibodies in Kenyan Africans with IDDM, primary autoimmune diabetes mellitus is considered rare in Kenyan Africans with IDDM.
INTRODUCTION

Diabetes mellitus is a common disease affecting approximately 2 - 3% of the population in developed countries (1). Diabetes mellitus is defined as a state of chronic hyperglycaemia. The hyperglycaemia may be due to lack of insulin or to an excess of factors and hormones such as growth hormone, glucocorticoids, glucagon and somatostatin that oppose the action of insulin. Several studies have shown that a wide variety of environmental and genetic factors may cause the diabetic state. There are also different clinical manifestations of diabetes mellitus. Diabetes mellitus is not a single disease, rather, it may be viewed as an heterogenous group of diseases, the common denominator being chronic hyperglycaemia. Recognition of the heterogeneity of diabetes mellitus has prompted the Diabetes Data Group of the National Institutes of Health (NDDG) U.S.A. to propose a new classification based on clinical and aetiological grounds (2). The NDDG classification of diabetes mellitus was accepted by the World Health Organization in 1980 (3).

Type 1 or insulin dependent diabetes mellitus (IDDM) is characterised by hyperglycaemia, dependence on insulin to sustain life, florid symptoms, younger age of onset and tendency to develop ketoacidosis without insulin therapy. The mere use of insulin in the treatment of a diabetic is not adequate in itself to fulfil the classification requirement. A history of clinical onset with ketoacidosis is the strongest supporting evidence. However, this may not always be available. Type II diabetes or non-insulin dependent diabetes mellitus (NIDDM) defines a group of patients who do not require insulin. It usually occurs in older, obese persons and is characterised
by the absence of ketoacidosis though they may become ketotic during acute medical or surgical stress. A distinct subgroup known as maturity onset diabetes mellitus of youth (MODY) may be diagnosed in patients in their teens (4). Though these patients are usually treated with insulin, they are able to survive without it (5).

Genetic predisposition, autoimmunity and environmental factors acting independently or together have been implicated in the pathogenesis of IDDM (6). It has long been recognised that insulin dependent diabetes mellitus shows a strong familial tendency. Tattersall and Pyke in their study of monozygotic twins found a 93% concordance rate for adult onset disease (greater than age 40 at diagnosis) and a 50% concordance rate for juvenile onset disease (7).

This suggests that genetic factor tends to predominate in NIDDM but not in IDDM. Another approach to the study of inheritance of IDDM apart from twins studies is human leukocyte antigens (HLA) typing. These cell surface antigens are associated with rejection of tissue transplants. The genes coding for HLA antigens -A, -B, -C, and -DR (D-related) occupy four loci along the short arm of chromosome 6 (8, 9).

Initial population studies in IDDM found a higher proportion of HLA antigens B8 and B15 (10,11). Subsequent studies have demonstrated that in Caucasian populations an increased relative
risk for developing IDDM is associated with HLA DR3 and DR4 (12,13). Current evidence suggests that the strongest association of IDDM lies with HLA DR3 and HLA DR4 (14). The previously reported findings with respect to HLA B antigens are secondary to linkage disequilibrium within the HLA system (14). HLA B8 is in linkage disequilibrium with DR3 while HLA B15 is in linkage disequilibrium with DR4 (14). Though most of the studies on HLA antigens in IDDM have been in Caucasian populations in Europe and North America, there is one study which reports an association between HLA DR3 and HLA DR4 in American Blacks with IDDM (15).

HLA studies in siblings of patients with IDDM have shown a statistically significant association between the sharing of HLA identical haplotypes and development of IDDM (16, 17). Rubinstein (16) studied HLA types in 31 families having one or more children with type I diabetes mellitus. In 23 families, 50% of the HLA identical siblings were diabetic whereas only 6% of the non-identical siblings were diabetic. Gorsuch (17) in his study of 288 siblings of 160 patients with IDDM taking part in Barts Windsor Family Study found that statistically significant number of HLA identical siblings developed IDDM. Ginsberg - Fellner et al (18) in their study of IDDM found that there was an increased risk of developing IDDM in siblings of patients with IDDM if they were HLA identical. These studies support the concept that the genetic susceptibility to IDDM is HLA linked.

Autoimmunity was implicated as an aetiological factor in pathogenesis of type I diabetes mellitus when it was noted that there was a higher prevalence of insulin dependent
diabetes mellitus in patients with Addison's disease as compared with normal population (19). Subsequent studies showed that there was a significantly higher prevalence of primary hypothyroidism in patients with IDDM as compared to NIDDM (20). Other investigators also noted that there was an increased incidence of thyroid autoantibodies and gastric parietal cell antibodies in patients with IDDM as compared with normal controls (21).

Antibodies against the cytoplasm of pancreatic islet cells, as detected by indirect immunofluorescence using sections of blood group O human pancreas were first isolated in 1974 in diabetic patients with organ specific autoimmune disease (22, 23). Subsequent studies showed a high prevalence of islet cell antibodies in newly diagnosed cases of type I diabetes mellitus (24, 25). The prevalence of islet cell antibodies falls rapidly; 60% of newly diagnosed cases of type I diabetes mellitus have islet cell antibodies as compared with 15 - 20% of patients with type I diabetes mellitus of more than two years duration (24). About 10 - 15% of patients with type I diabetes mellitus have persistent islet cell antibodies for many years (26). Islet cell antibodies have also been detected before clinical onset of disease by as many as five years (27). Islet cell antibodies have also been detected in unaffected first degree relatives of patients with IDDM more frequently than in normal controls (17). Islet cell antibodies have also been helpful in detecting apparently normal persons who are at risk of developing IDDM (28, 29, 30). A separate species of islet cell antibodies which fixes complement has been identified (29).
These complement fixing islet cell antibodies are distinct from the conventional islet cell antibodies and are more closely related to the onset of clinical disease than conventional islet cell antibodies (29).

Viruses have been implicated in the pathogenesis of IDDM. The most direct evidence of the role of viral infection has been the Coxsackie B4 virus which was isolated from the pancreatic tissue of a child who died with meningoencephalities and new onset diabetes mellitus (31). The isolated virus was then injected into a susceptible species of mice. This resulted in damage to the beta cells of the islet of Langerhans of the pancreas with subsequent diabetes mellitus (31). Gamble (32) reported that within the first three months of the onset of IDDM, higher titres of neutralising antibodies to Coxsackie B4 virus were found as compared to those in normal subjects or in patients with diabetes mellitus of a duration of longer than three months. Encephalomyocarditis (EMC) virus has been shown experimentally to produce diabetic like state in mice (33). Venezuelan equine virus has also been shown to produce a diabetic like state in mice (34). However, only a few strains of mice develop a diabetic like disease after viral infections whereas other strains seem to be resistant to the development of diabetes - like state. This suggests that there is a genetically determined susceptibility to develop diabetes - like state (33). Gamble described a seasonal incidence of insulin dependent diabetes mellitus in patients up to the age of 19 most marked in October with a smaller peak in July (35). These peaks showed a significant positive correlation with
annual prevalence data for Coxsackie B4 virus but not for other types of viral infections (35). However, other workers have not been able to confirm these findings (36). There have also been several reported cases of IDDM following mumps infection (38, 39).

Chemicals may be responsible for diabetes. Streptozocin when injected into some strains of mice leads to pancreatic islet cell damage and hyperglycaemia (40). N-nitroso derivatives which are food additives have been shown to induce diabetes in offspring of mice which were fed large amounts of N-nitroso compounds (41). However, there is no direct evidence that N-nitroso compounds are important in pathogenesis of type I diabetes mellitus in human beings (41).

There have been several reports that autoimmunity may be an important aetiological factor in the pathogenesis of IDDM in Caucasians (42, 43). However, studies from Africa are scanty. One study from West Africa in patients with new onset diabetes mellitus showed a low prevalence of islet cell antibodies and organ specific antibodies (44). Studies from Black Americans with IDDM indicate that they have a lower prevalence of thyroid auto antibodies and islet cell antibodies when compared with Caucasian populations with IDDM (45). These differences among the various population groups prompted this study with the following objectives.
OBJECTIVES

1. Study the prevalence of islet cell antibodies in Africans with insulin dependent diabetes mellitus (IDDM) attending the Kenyatta National Hospital.

2. Study the prevalence of thyroid microsomal, thyroglobulin and parietal cell antibodies in Africans with IDDM attending the Kenyatta National Hospital.

3. Study the prevalence of islet cell antibodies in non diabetic hospital controls.
PATIENT SELECTION: This study was carried out in African patients with IDDM according to NDDG criteria (2) who were attending the Kenyatta National Hospital between September 1982 and July 1984. Patients were included in the study if they had IDDM of less than five years duration and were not on corticosteroids for any other disease. Due to financial and technical constraints, the sample size was limited to 131 patients.

The age of the patient and the duration of the disease from the onset of symptoms was noted. Detailed history of diet, alcohol consumption and family history of IDDM was taken. The clinical and laboratory protocol employed for the patients is given in Appendix A.

CONTROL GROUP: The control group consisted of 51 non diabetic, age and sex matched hospital patients. All the controls had a random blood glucose of less than 8.0 mmol/L.

BLOOD COLLECTION: 10 ml. of blood was collected from patients by venepuncture and the serum was stored at -70°C.

LABORATORY STUDIES:

Thyroid microsomal antibodies were detected using haemagglutination with tanned turkey erythrocytes using a commercial kit (Thymune M - Welcome Reagents) as described (46). The test was considered positive if there was haemagglutination in a dilution of 1:40 and onwards.
Thyroglobulin antibodies were detected using haemagglutination with tanned turkey erythrocytes using a commercial kit (Thymune T - Welcome Reagents) as described (46). The test was considered positive if there was haemagglutination in a dilution of 1:40 and onwards.

Islet cell antibodies were detected by indirect immunofluorescence using unfixed, cryostat frozen sections of blood group O human pancreas as described by Bottazzo (22). The result (scale 0 to ++++) was considered positive if the test sample showed one plus (+) reaction to endocrine cells.

**STATISTICAL ANALYSIS**

Statistical analysis was done using mean and statistical significance was calculated using Student t test. Statistical significance was assumed if the P value was equal to or less than 0.05.
RESULTS

131 patients were studied. There were 77 males and 54 females, a male to female ratio of 1.4 to 1. The age distribution of patients studied is shown in Figure 1. The peak age was 10 - 30 years, the mean age was 21.8 years. The age distribution at the onset of IDDM is shown in Figure 2. The peak age of onset of IDDM was between 10 - 30 years. The mean age at the onset of IDDM was 20.1 years. The duration of the disease from the onset of symptoms is indicated in Table 1. 49% of the patients studied had disease for less than one year.

Family history from 131 diabetic probands revealed that they had 60 siblings. None of the 30 siblings available at the time of the study had IDDM from history. 30 siblings were not available at the time of the study due to separation from their parents or due to death; the cause of death could not be ascertained in the dead siblings. Of the 151 parents of diabetic probands available at the time of the study only one parent had IDDM. 111 parents were not available at the time of the study.

Detailed history about alcohol revealed that none of the patients studied had significant (more than three bottles of beer per day) history of alcohol consumption.
Autoantibody profile: The autoantibody profile of patients with IDDM and non-diabetic control is shown in Table 2. None of the non-diabetic hospital controls had thyroid microsomal or thyroglobulin antibodies. 0.7% of the patients with IDDM had thyroid microsomal antibodies and none had thyroglobulin antibodies. The differences in these two groups were not significant ($P > 0.05$). 4.6% of patients with IDDM had parietal cell antibodies as compared with 1.9% in the control group. This difference was not considered statistically significant ($P > 0.05$).

Due to technical constraints in obtaining fresh human pancreas, islet cell antibody could only be done in 80 patients with IDDM and 51 controls. None of the controls had islet cell antibodies. 3.7% of the patients with IDDM had islet cell antibodies. The results in these two groups did not reach a level of statistical significance ($P > 0.05$). The prevalence of islet cell antibodies in relation to the duration of the disease is indicated in Table 3. 5.8% of the patients with IDDM of less than one year's duration had islet cell antibodies as compared with 7.1% in patients with disease duration of between one and two years which is not statistically significant. None of the patients with IDDM of more than two year's duration had islet cell antibodies. Age, sex and autoantibody profiles of patients who had islet cell antibodies is shown in Table 4.
FIGURE I

AGE DISTRIBUTION OF 131 PATIENTS WITH IDDM
FIGURE 2

AGE DISTRIBUTION AT THE ONSET
OF IDDM IN 131 PATIENTS

NUMBER OF PATIENTS

AGE IN YEARS
TABLE 1

Duration of the disease from the onset of symptoms.

<table>
<thead>
<tr>
<th>Sex of patients</th>
<th>Duration of IDDM (in months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 12</td>
</tr>
<tr>
<td>Males</td>
<td>30</td>
</tr>
<tr>
<td>Females</td>
<td>34</td>
</tr>
<tr>
<td>Total Number</td>
<td>64 (49%)</td>
</tr>
</tbody>
</table>
TABLE 2

AUTOANTIBODIES IN PATIENTS WITH IDDM AND NON DIABETIC HOSPITAL CONTROLS.

<table>
<thead>
<tr>
<th>ANTIBODY</th>
<th>TMA</th>
<th>TGA</th>
<th>PCA</th>
<th>ICA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDDM</td>
<td>M</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1/131</td>
<td>0/131</td>
<td>6/130</td>
<td>3/80</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CONTROL</td>
<td>M</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0/51</td>
<td>0/51</td>
<td>1/51</td>
<td>0/51</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

KEY:

TMA : Thyroid microsomal antibodies
TGA : Thyroglobulin antibodies
PCA : Parietal cell antibodies
ICA : Islet cell antibodies
NS : Not significant
TABLE 3

Prevalence of islet cell antibodies in 80 cases of IDDM in relation to duration of the disease.

<table>
<thead>
<tr>
<th>DURATION OF IDDM IN MONTHS</th>
<th>Islet cell antibodies positive</th>
<th>Islet cell antibodies negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>2 (5.8%)</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>12-24</td>
<td>1 (7.1%)</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>24-36</td>
<td>0 (0%)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>36-48</td>
<td>0 (0%)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>48-60</td>
<td>0 (0%)</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>
### Table 4

**AGE, SEX AND ANTIBODY PROFILES OF IDDM PATIENTS WITH ISLET CELL ANTIBODIES.**

<table>
<thead>
<tr>
<th>AGE</th>
<th>SEX</th>
<th>TMA</th>
<th>TGA</th>
<th>PCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 years</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22 years</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>36 years</td>
<td>F</td>
<td>+ve</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**KEY:**

- **TMA**: Thyroid microsomal antibodies
- **TGA**: Thyroglobulin antibodies
- **PCA**: Parietal cell antibodies
<table>
<thead>
<tr>
<th>STUDY</th>
<th>POPULATION</th>
<th>TMA</th>
<th>TGA</th>
<th>PCA</th>
<th>ICA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lendrum (24)</td>
<td>Caucasians</td>
<td>12%</td>
<td>-</td>
<td>-</td>
<td>38%</td>
</tr>
<tr>
<td>Irvine (25)</td>
<td>Caucasians</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22%</td>
</tr>
<tr>
<td>Neufield (45)</td>
<td>Caucasians</td>
<td>20%</td>
<td>-</td>
<td>8.5%</td>
<td>36.1%</td>
</tr>
<tr>
<td>Neufield (45)</td>
<td>U.S. Blacks</td>
<td>4%</td>
<td>-</td>
<td>10%</td>
<td>22%</td>
</tr>
<tr>
<td>Riley (52)</td>
<td>Caucasians</td>
<td>20.1%</td>
<td>-</td>
<td>7.8%</td>
<td>-</td>
</tr>
<tr>
<td>Riley (52)</td>
<td>U.S. Blacks</td>
<td>5.5%</td>
<td>-</td>
<td>9.4%</td>
<td>-</td>
</tr>
<tr>
<td>Del Prete (59)</td>
<td>Caucasians</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24.2%</td>
</tr>
<tr>
<td>PRESENT STUDY</td>
<td>Kenyan Africans</td>
<td>0.7%</td>
<td>0%</td>
<td>4.6%</td>
<td>3.7%</td>
</tr>
</tbody>
</table>

KEY:

TMA: Thyroid microsomal antibodies
TGA: Thyroglobulin antibodies
PCA: Parietal cell antibodies
ICA*: Islet cell antibodies irrespective of the duration of the disease.
DISCUSSION

All the patients studied had abrupt onset of symptoms, random blood glucose of more than 14.0 mmol/L on more than three occasions and at least one documented episode of diabetic ketoacidosis. Their selection criteria would satisfy the NDDG criteria of type I diabetes mellitus or insulin dependent diabetes mellitus (2). The same selection criteria would effectively exclude maturity onset diabetes of the young described by Tattersall and Fajan (5).

AGE AND SEX: The peak age of onset was between 10 - 30 years and the mean age of onset of IDDM was 20.1 years. This is a much higher age of onset than reported by other investigators (18, 47). Ginsberg - Fellner et al (18) in their study found that the mean age of onset of IDDM was 13.7 years. The Pittsburg study found that the mean age of onset of IDDM was 7.8 years (47). Neufeld (45) in his study found that the mean age of onset of IDDM in U.S. Caucasians was 8.1 years and 10.2 years in U.S. Blacks. The reason for the high age of onset of IDDM in this study is not clearly known but may be due to the fact that most of the patients included in the study were attending the Diabetic Clinic which is manned by physicians while some paediatricians follow few diabetic children in the paediatric clinics. A male predominance was noted in this study. A male predominance has also been noted by some investigators (17), however, male predominance is not a universal finding in studies of IDDM. The Pittsburg study found an equal male to female ratio (47). However, it must be noted that the male predominance in our study may be due to a bias in the number of
females attending the Diabetic Clinic which is situated in Nairobi. It is possible that many women may be attending hospitals near their homes in rural areas and thus be missed in this study.

ALCOHOL AND DIET: This study reveals no association between alcohol consumption and IDDM in Kenyan Africans. Mngola (49) has recently described a ketosis prone diabetic state in patients over the age of twenty associated with high consumption of a local alcoholic brew. It is very unlikely that any of the patients included in this study had the pancreatic diabetes described by Mngola. Detailed dietary history revealed that Cassava consumption was uncommon in patients with IDDM. Cassava consumption has been associated with Pancreatic Fibrosis Calcification Syndrome (PFC Syndrome) (49). Pancreatic Fibrosis Calcification Syndrome has been described in several countries and is characterised by recurrent abdominal pain, onset of diabetes at a young age, male predominance, large insulin requirements for control and resistance to develop ketoacidosis on insulin withdrawal. In view of the lack of cassava consumption and the tendency of the patients to develop ketoacidosis in this study, it is most unlikely that any of the patients included in this study had PFC Syndrome.
RELATION TO FAMILY HISTORY: Family studies in Caucasians with IDDM showed that there is an increased prevalence of IDDM among first degree relatives of patients with IDDM. Gorsuch (50) in his study of first degree relatives of patients with IDDM found that 3.5% of the parents and 5% of the siblings of IDDM probands developed IDDM. The risk of developing IDDM in Gorsuch's study (50) was greatest among HLA identical siblings, suggesting that the genetic susceptibility to IDDM is HLA linked. Warram et al (51) have also reported higher prevalence of IDDM among offspring of diabetics, 1.3% of the offspring of women with IDDM and 6.1% of the offspring of men with IDDM were diabetic by the age of 20. In our study only one parent of a diabetic proband had IDDM. None of the offspring of the diabetic patients had IDDM. This low prevalence of IDDM among first degree relatives of patients with IDDM in this study may be due to the fact that in many cases definitive history of IDDM among first degree relatives could not be ascertained due to unavailability of first degree relatives either because of separation or death. The other factor may be that this was a short term study and may have missed many first degree relatives who were at risk of developing IDDM but at the time of study did not have IDDM. The low prevalence of IDDM among first degree relatives of patients with IDDM needs to be confirmed by further long term studies.
THYROID AUTOANTIBODIES: None of the patients with IDDM in this study had thyroglobulin antibodies and only 0.7% of the patients with IDDM had thyroid microsomal antibodies. The prevalence of thyroid autoantibodies in this study is much lower than reported by other investigators (24, 25) (Table 5). Lendrum (24) in his study of 750 patients with IDDM found that 12% of the patients with IDDM had thyroid microsomal antibodies. He also found that the prevalence of thyroid microsomal antibodies increased with age and duration of the disease. Irvine (25) in his study of 1032 diabetics found that there was an increased prevalence of thyroid autoantibodies when compared with normal controls and patients with NIDDM. Neufield (45) in his study of IDDM found that Caucasians with IDDM had significantly higher prevalence of thyroid microsomal antibodies as compared with Black Americans with IDDM and normal controls. 20% of Caucasians with IDDM in Neufield's study had thyroid microsomal antibodies as compared with 4% of Black Americans with IDDM. Riley (52) in his study has reported that Black Americans with IDDM have a lower prevalence of thyroid microsomal antibodies (5.5%) as compared with 20.1% in Caucasians with IDDM. This difference was considered statistically significant. Several studies have shown that there is also an increased prevalence of thyroid autoantibodies among relatives of patients with IDDM (18, 53). Ginsberg - Fellner et al (18) in their study showed that 9.7% of first degree relatives of patients with IDDM had evidence of autoimmune thyroid dysfunction and 48% of patients with IDDM had evidence of autoimmune thyroid dysfunction as manifest by thyroid
microsomal antibodies or thyroglobulin antibodies. Fialkow et al (53) found a 23% prevalence of thyroid autoimmunity in first degree relatives of patients with IDDM. A low prevalence of thyroid autoantibodies in patients with IDDM was found in this study despite using a more sensitive assay than used in other studies (46). Though it is often suggested that Black populations have a lower propensity to form organs specific autoantibodies, Bowry (54) in her study of thyrotoxicosis in Kenyan Africans found that the commonest cause of thyrotoxicosis was Graves' disease and thyroid microsomal antibodies were found in 54.2% of patients with Graves' disease indicating that Kenyan Africans have the same propensity to develop thyroid autoantibodies in thyrotoxicosis. Thus the low prevalence of thyroid autoantibodies in this study cannot be solely due to reduced propensity to form organ specific autoantibodies as suggested (45, 52). The low prevalence of thyroid autoantibodies in this study does confirm the suggestion of Gorsuch (55) who found that the presence of thyroid antibodies in healthy siblings was independent of HLA haplotypes possessed by the IDDM proband and suggested that the genes controlling the production of thyroid autoantibodies are different from those controlling the susceptibility to IDDM.

PARIETAL CELL ANTIBODIES: The prevalence of parietal cell antibodies among Kenyan Africans with IDDM in this study was 4.4%. Riley (52) in his study found that 9.4% of Black Americans with IDDM had parietal cell antibodies while 7.8% of Caucasians with IDDM had parietal cell antibodies. Neufield (45) in his study found that 10% of Black Americans with IDDM
had parietal cell antibodies as compared with 8.5% in Caucasians with IDDM. Previous studies have demonstrated that gastric mucosa in patients with parietal cell antibodies is invariably abnormal with mild to severe atrophic changes (56, 57). Presence of parietal cell antibodies has also been associated with achlorhydria. Riley (52) has observed that 54% of patients with IDDM and parietal cell antibodies had achlorhydria and 9% of patients with IDDM and parietal cell antibodies had overt pernicious anaemia. Though we did not study gastric mucosa and gastric acidity, it is suggested that IDDM patients with parietal cell antibodies should be followed up regularly and be screened for atrophic gastritis and achlorhydria.

ISLET CELL ANTIBODIES: The prevalence of islet cell antibodies in this study within the first one year of onset of the disease was 5.8% and the overall prevalence of islet cell antibodies was 3.7%. The prevalence of islet cell antibodies in this study is much lower than reported elsewhere (24, 25) (Table 5). Lendrum (24) in his study of IDDM found a strong association between IDDM and islet cell antibodies. The prevalence of islet cell antibodies within the first three months of onset of IDDM was 60 - 80%, and at the end of two years, the prevalence of islet cell antibodies was 10 - 20%. Irvine (25) in his study of IDDM found that 56% of patients with IDDM but without evidence of other autoimmune disease had islet cell antibodies within the first year of onset of IDDM. Irvine (25) also demonstrated that 22% of patients with
IDDM but without evidence of other autoimmune disease have islet cell antibodies irrespective of the duration of IDDM. The prevalence of islet cell antibodies in patients with IDDM and with another autoimmune disease was 38% Del Prete (58) in his study found that the overall prevalence of islet cell antibodies in patients with IDDM was 24.2%. The prevalence of islet cell antibodies was highest within the first year of the onset of the disease and prevalence of islet cell antibodies decreased as duration of IDDM increased.
A lower prevalence of islet cell antibodies has been noted in U.S. Blacks with IDDM as compared with U.S. Caucasians with IDDM (45). Neufield (45) found that 22% of Black Americans with IDDM has islet cell antibodies as compared with 36% in Caucasians with IDDM. Furthermore, islet cell antibodies were present in 74% of Caucasians with IDDM within the first three months of the onset of the disease as compared with 33% in Black Americans with IDDM within the first three months of onset of IDDM. Our study confirms that the prevalence of islet cell antibodies in Kenyan Africans with IDDM is much lower when compared with Caucasian populations and Black Americans with IDDM even though a similar assay was used in detecting islet cell antibodies in this study. Islet cell antibodies as detected by indirect immunofluorescent assay described by Bottazzo (22) are found in less than 0.5% of normal population and non diabetic hospital controls (29). Diabetics who do not have islet cell antibodies do not develop islet cell antibodies at a later date (28, 59). However, patients with NIDDM or normal subjects with islet cell antibodies will progress given time to IDDM (28, 60). Thus islet cell antibodies have a very high prevalence in newly diagnosed clinically overt disease and a very low but significant prevalence in a large control population (28). Islet cell antibodies have also been detected using Bouin's fixed human pancreas (61). However, it is not yet established whether these two methods of estimating islet cell antibodies are comparable (62). Islet cell surface antibodies detected using indirect immunofluorescence test with dispersed islet cells from rats are different from cytoplasmic islet cell antibodies with
different specificities and different prevalence among the patients studied (63, 64).

Though islet cell antibodies are found in high prevalence in newly diagnosed Caucasians with IDDM, their role in the pathogenesis of IDDM is undefined. Islet cell antibodies react with all the endocrine cells of islets of Langerhans. However, it is only the beta cells which are selectively destroyed in IDDM. Cross sectional studies suggest that there is no obvious relationship between islet cell antibodies and pancreatic cell function (65, 66). As has been suggested by Irvine (19), the primary insult to the beta cells must be in relation to the cell membrane of B cells with release of cytoplasmic antigens, this would make islet cell antibodies a secondary phenomenon to the processes which initially initiated injury to the beta cells. Del Prete (58) has also suggested that islet cell antibodies have no direct pathogenic role in diabetes and represent a marker of other immune or autoimmune mechanism which induce beta cell damage.

A low prevalence of islet cell antibodies in this study may be due to a delay in seeking medical attention as is common here which would lead to the disappearance of islet cell antibodies as islet cell antibodies in contrast to other antibodies are transient and tend to disappear with the duration of the disease (24). However, in 10 - 15% of patients with IDDM, islet cell antibodies tend to persist for several years after the onset of IDDM. These patients with persistent islet cell antibodies show a female predominance, increased prevalence of other organ specific
autoantibodies without biochemical evidence of other endocrine disease and a later age of onset (17). These patients are thought to have "primary" autoimmune diabetes mellitus (67). Several studies have shown that patients with persistent islet cell antibodies have increased incidence of HLA B8 (25, 17). As it has been mentioned before, HLA B8 occurs in increased frequency in Caucasian diabetics due to linkage disequilibrium with HLA DR3. Studies from various African populations with IDDM have shown that HLA B8 occurs in reduced frequency when compared with Caucasian populations with IDDM (68, 69). A reduced frequency of HLA B8 has also been noted in U.S. Blacks with IDDM (15). Though HLA antigens were not studied, it may be that HLA B8 occurs at a reduced frequency in Kenyan Africans with IDDM as noted in other African populations with IDDM.

Definitive clinical evidence of viral infection preceding the onset of diabetes could only be found in a three year old boy who developed diabetic ketoacidosis one week after mumps. The other siblings in the family who also had mumps at the same time did not develop diabetes, however islet cell antibodies were not found present in this case. Islet cell antibodies have been found in virus-induced diabetes due to mumps. Epstein Barr virus and Coxsackie B4 virus (39, 70). Though definitive evidence of viral infection could only be found in one case in this study, it is possible that many of the patients had viral infections preceding the onset of IDDM, which may have been treated as malaria as it is common practice here to treat any febrile illness as malaria. A study is in progress to look into the role of Coxsackie B4 virus in the pathogenesis of IDDM in Kenyan Africans.
The low prevalence of islet cell antibodies and other organ specific autoantibodies in this study suggest that "primary" autoimmunity is not a significant factor in aetiology of IDDM in Kenyan Africans. The other important aetiological factors suggested in pathogenesis of IDDM include viruses and toxins which lead to B cell destruction in genetically susceptible individuals (71). The precise mechanism by which viruses and toxins lead to beta cell damage are not well defined. However, Reeves (72) has suggested a model whereby viruses and toxins in genetically susceptible individuals initiate B cell destruction through cytotoxic antibodies and/or T cells.

However, proof of viral aetiology in our study is lacking as viral neutralizing antibodies and HLA types were not studied. Further studies in Kenyan Africans with IDDM are needed to study the role of HLA antigens and the susceptibility to develop insulin dependent diabetes mellitus.
CONCLUSIONS

The data presented in this study has suggested the following:

1. Prevalence of islet cell antibodies in Kenyan Africans with IDDM is much lower than reported elsewhere.

2. Prevalence of thyroid microsomal, thyroglobulin and parietal cell antibodies is much lower than reported elsewhere.

3. Unlike the Caucasian cases of IDDM where concurrence of thyroid autoantibodies, which are indicators of autoimmune thyroiditis, occur in 20% of IDDM cases, it is observed in only 0.7% of Kenyan Africans with IDDM confirming that the genetic susceptibility to these diseases differs in two races.

4. In view of the low prevalence of autoantibodies, primary autoimmune diabetes is considered rare in Kenyan Africans with IDDM.

5. Islet cell antibodies are rare in non diabetic hospital controls.
From the study, several recommendations can be made:

1. **Long term prospective studies are needed to evaluate the prevalence of IDDM in first degree relatives of IDDM probands.**

2. **Further family and epidemiological studies are necessary to evaluate the role of islet cell antibodies as an early marker of IDDM in Kenya.**

3. **HLA studies are needed to determine the genetic susceptibility to IDDM in Kenya.**

4. **Viral studies are needed in newly diagnosed cases of IDDM to determine the role of viruses in initiating beta cell damage.**
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APPENDIX A

AUTOIMMUNE PROFILES IN IDDM

NAME __________________________  UNIT __________________________
AGE __________________________  DATE __________________________
SEX __________________________

Date of onset of IDDM __________________________
Insulin Requirements __________________________
Clinical presentation at onset of disease __________________________
Episode of ketoacidosis __________________________
Diet __________________________
Alcohol Type __________________________
Amount __________________________
Duration __________________________

Family history Parents __________________________
Children __________________________

LABORATORY DATA
BLOOD SUGAR __________________________

IMMUNOLOGICAL DATA
TMA  TGA  PCA  ANA  AMA  ISAB