Postgraduate Institute of Medicine Oration 2008

SPECTRUM OF DIABETES AMONG YOUNG ADULTS IN SRI LANKA – AETIOLOGY AND IMPLICATIONS

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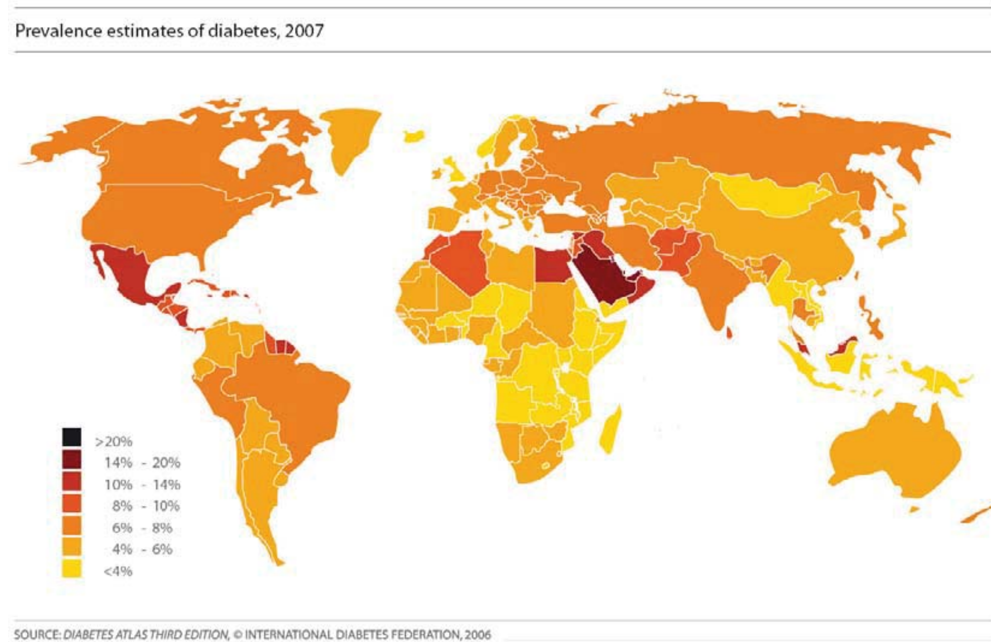
Introduction - Global epidemic

The disease that led people to pass excessive amounts of sweet urine and hence termed diabetes mellitus has become a worldwide pandemic. According to the International Diabetes federation 246 million people worldwide has diabetes mellitus [1](Figure 1 – World Diabetes Atlas).

Figure 1. World Diabetes Atlas



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Pathogenesis of diabetes

Diabetes occurs due a deficiency of insulin hormone secreted from the beta cells of the islets of the pancreas or due to the tissue resistance to insulin or both [2](Figure 2). The deficiency of insulin secretion can occur due to destruction of beta cells of the pancreas as commonly seen in type 1 diabetes, due to defects in glucose sensing or due to post receptor mechanisms (Figure 3).

Figure 2. Major pathways of pathogenesis of hyperglycaemia. The sites of defects shown by (a) - reduced beta cell sensitivity to glucose, b – reduced insulin secretion, c – peripheral insulin sensitivity

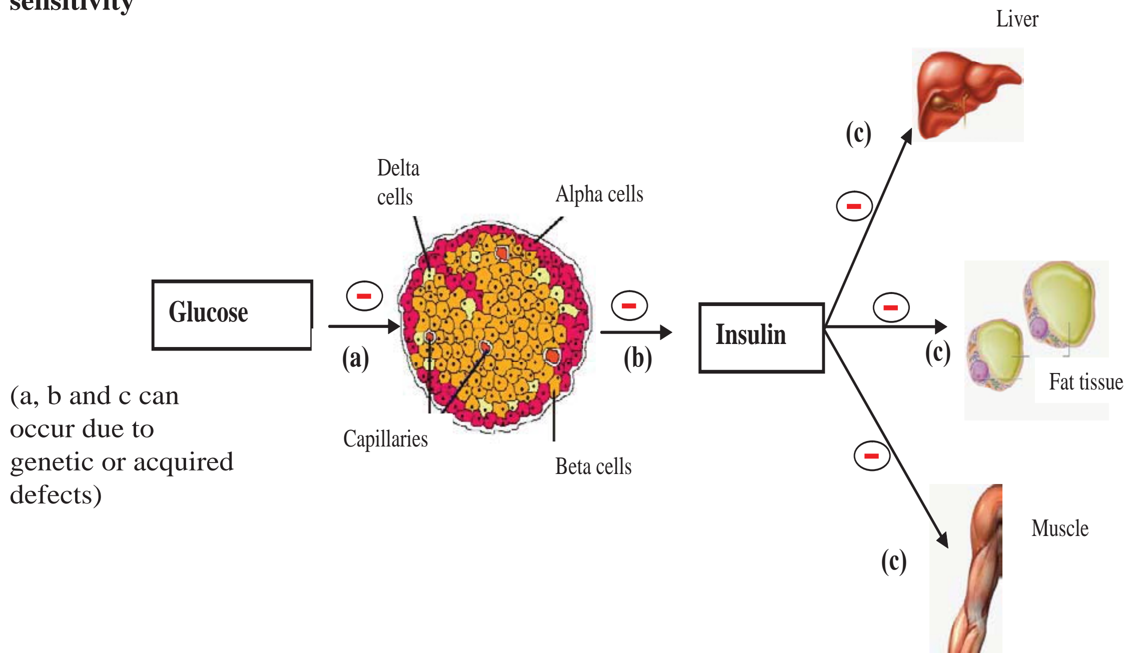
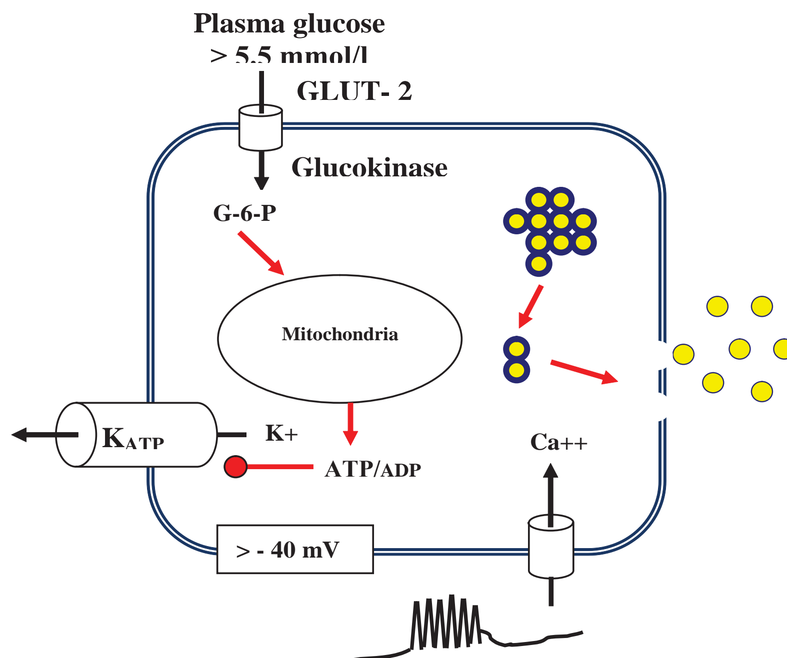


Figure 3 Molecular mechanisms of insulin secretion in the pancreatic beta cell

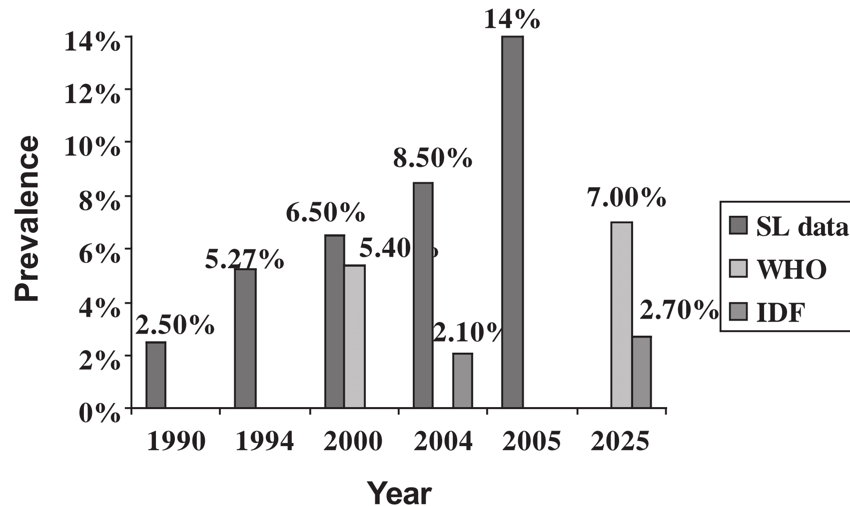


[GLUT – 2: Glucose Transporter II, G-6-P: Glucose – 6 – Phosphate, ATP – Adenosine triphosphate, ADP – Adenosine diphosphate, K_{ATP}: Potassium ATP channel]

Diabetes in Sri Lanka

Sri Lanka is facing an epidemic of diabetes mellitus (Figure 4). The epidemiology and aetiology of diabetes in young adults in Sri Lanka is under studied. However, when the data from South India is considered, it is highly likely that diabetes is an important emerging health problem among young adults in Sri Lanka.

Figure 4 The prevalence data from the epidemiological studies in Sri Lanka and the IDF and WHO estimates for 2000 and 2025*



[Based on data from studies carried out from 1990 to 2005 ([3-10] and from IDF and WHO estimates [8, 10]. * According to data available by the year 2005]

Aetiological Subtypes of diabetes

The recent international classifications have recognised the importance of the pathophysiological basis of different subtypes of diabetes [11, 12]. The subtypes of diabetes according to the World Health Organisation and American Diabetes Association Classification is shown in Table 1.

Table 1 Aetiological
Classification of Disorders of Glycaemia

I. Type 1 diabetes (cell destruction, usually leading to absolute insulin deficiency)
   A. Immune mediated
   B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types
   A. Genetic defects of beta cell function
      1. Chromosome 12, HNF-1 (MODY3)
      2. Chromosome 7, glucokinase (MODY2)
      3. Chromosome 20, HNF-4 (MODY1)
      4. Mitochondrial DNA
      5. Others
   B. Genetic defects in insulin action
   C. Diseases of the exocrine pancreas
   D. Endocrinopathies
   E. Drug-or chemical-induced
   F. Infections
   G. Uncommon forms of immune-mediated diabetes
   H. Other genetic syndromes sometimes associated with diabetes

IV. GESTATIONAL DIABETES MELLITUS (GDM)

Type 1 diabetes

Type 1 diabetes due to autoimmune destruction of the pancreatic beta cells leads to early insulin dependency and is characterised by the presence of autoantibodies to islet cell antigens (Glutamic Acid Decarboxylase [GAD_{65}], islet cell cytoplasm [ICA] and islet antigen 2 [IA-2A]) [11].

Type 2 diabetes

Type 2 diabetes results from a combination of insulin secretory deficiency, resistance and is not typically associated with islet autoantibodies and those affected can be initially managed by a combination of lifestyle measures and oral therapy. Type 2 diabetes can be prevented by lifestyle modification in high risk patients.

Latent autoimmune diabetes in adults (LADA)

Antibodies to GAD_{65} (GADA) and other islet autoantibodies are present in a subset of adult subjects presenting clinically with type 2 diabetes [13]. Due to the slowly progressive nature of this subtype of diabetes to insulin dependency, it was called, Latent Autoimmune Diabetes in Adults (LADA) [14, 15]. Islet antibody positive and slowly progressive diabetes has also been described in patients younger than 30 years of age and is called Latent Autoimmune Diabetes in Young (LADY) [16].

Monogenic diabetes - Maturity onset diabetes in the young (MODY) and mitochondrial diabetes

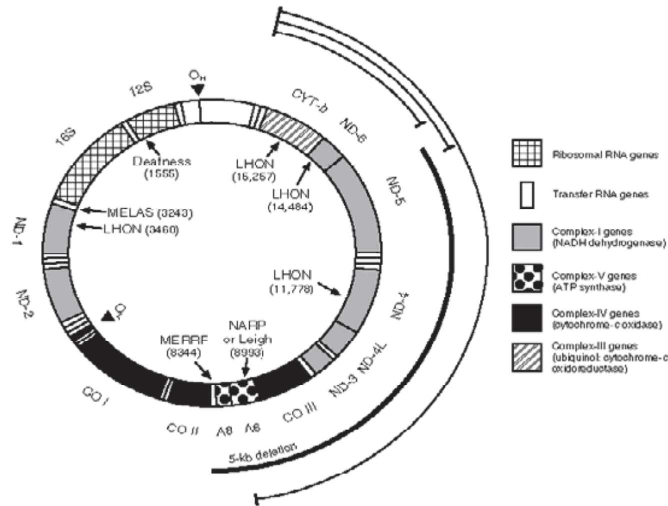
Advances in molecular research have led to the identification of several forms of diabetes due to single gene defects (monogenic diabetes)[17]. These include Maturity-onset diabetes of the young (MODY)[18] and mitochondrial diabetes[19]. The commonest mutation in the mitochondrial DNA that causes diabetes is the mt.3243A>G mutation in the mitochondrial DNA-encoded tRNA (Leu, UUR) gene [20]. Since the mutation is maternally inherited and sensori-neural deafness is a recognised feature, it is termed maternally inherited diabetes and deafness (MIDD)[19].

Out of the six subtypes of MODY characterised so far, the glucokinase MODY or GCK – MODY (also called MODY 2) is due to a gene mutation that encodes Glucokinase (GCK) which is a vital enzyme in glucose homeostasis [21]. The other MODY subtypes are due to mutations in genes that encode transcription factors: Hepatocyte nuclear factor 4 α (HNF-4α) associated with MODY 1, HNF-1 α (MODY 3), Insulin Promoter Factor 1 (IPF-1) [MODY 4], HNF-1β (MODY 5) and Neurogenic Differentiation Factor 1 (NeurodD1) also known as beta-cell E-Box transactivator 2 (BETA2) [MODY 6] [22, 23].

The MODY patients are generally characterised by non- ketotic diabetes mellitus, young age-of-onset (less than 25 years) and an autosomal dominant pattern of inheritance of diabetes in three or more successive generations [24]. In addition they are less obese, more insulin sensitive, have lower prevalence of dyslipidaemia and hypertension compared to the patients with type 2 diabetes [17]. Those with MODY do not carry autoantibodies against islet antigens [18]. Specific diagnosis of MODY can only be performed by identification of the specific genetic mutations. The HNF - MODY patients are very sensitive to sulphonylurea treatment and remain so for a long period compared to those with type 2 diabetes [25]. The GCK - MODY causes a mild disease with good prognosis and rarely needs pharmacological treatment [26].

The maternally inherited mt.3243A>G mutation is associated with a variable clinical phenotype including diabetes, deafness (MIDD) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)[19]. A genetic diagnosis can assist with clinical management and is important for genetic counselling. The structure of the mitochondrial DNA is shown in figure 5.

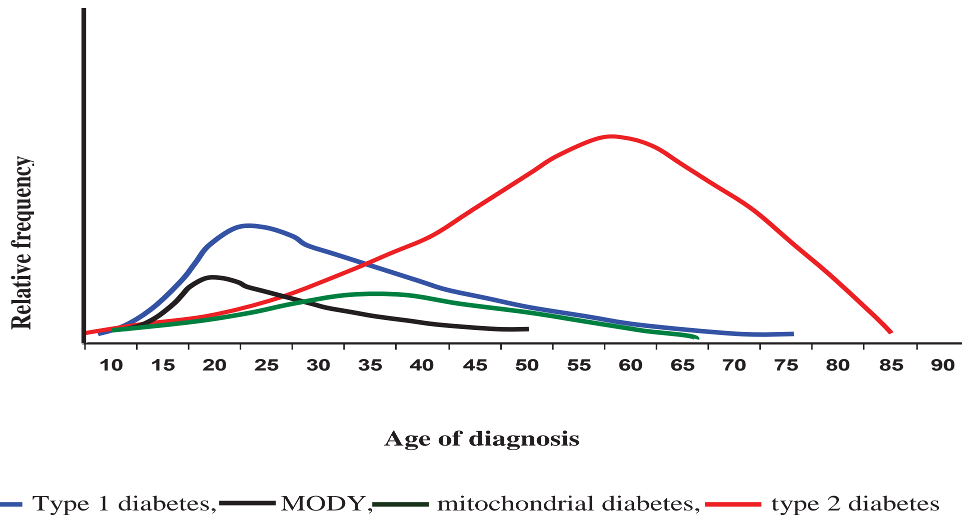
Figure 5
Structure of the mitochondrial DNA
[Adapted from New England Journal of Medicine [27]]



Diabetes in young adults

During the recent past diabetes mellitus is increasingly diagnosed among young adults worldwide [28]. Diabetes in children is still largely due to type 1 diabetes (although type 2 diabetes is increasingly reported in children in high risk ethnic groups) [29]. Similarly in older adults the predominant type of diabetes is type 2 diabetes [1]. As shown in the Figure 6 diabetes among young adults (Age 20 – 40 years) is aetiologically and phenotypically heterogeneous compared to the small children and older adults [30, 31]. This poses many diagnostic and therapeutic challenges to clinicians [32].

Figure 5 Schematic illustration of the common aetiological subtypes of diabetes according to the age of diagnosis



[Constructed based on data from [17, 23, 30, 32-34]]

Patients who develop diabetes at a young age are theoretically at a higher risk of development of diabetes related complications during their lifetime due to the higher duration of hyperglycaemia. Diabetes occurring in young adults has been reported to be more aggressive than that of older age groups [35].

Implications of a diagnosis of subtype of diabetes

Studying the aetiology of diabetes in young adults is important due to several reasons.

First, it will help understand the aetiology of the disease in young adults with potential for better clinical care and prevention by practicing individualized patient care. The specific characteristics of common subtypes of diabetes and the specific therapeutic interventions are illustrated in Table 2. Second, it will clarify the future burden of diabetes in the older population describing both the specific aetiological types and diabetes related complications.

Rationale of this study

In the present context, a clinician needs to consider several differential diagnoses in a young onset (<40 years) diabetic patient [30, 36]. Accurate aetiological diagnosis on clinical grounds may not be straightforward and would need expensive biochemical, immunological or genetic tests [28]. Nevertheless accurate aetiological diagnosis can help therapeutic decision making and prognostication. It can guide screening of family members and allow accurate genetic counseling. The knowledge of the relative prevalence of the diabetes subtypes in a population can be helpful in strategies for the prevention of diabetes.

The clinical spectrum of diabetes among young adults had not been adequately characterised in Sri Lanka or in other South Asian populations.

Objectives

Overall objective : To describe the spectrum of diabetes among young adults in Sri Lanka based on clinical phenotypes, autoimmune markers and genotyping.

Table 2.
The characteristics of subtypes of diabetes that necessitate specific interventions

Subtype of diabetes	Characteristic	Specific interventions
Type 1 diabetes	Absolute insulin deficiency	Insulin therapy for survival
Type 2 diabetes	High insulin resistence/ higher prevalence of metabolic syndrome and CVD risk/ late onset beta cell failure. Preventable.	Lifestyle intervention for glycaemic control and for CVD risk reduction. Detection of deterioration of beta cell failure. Specific measures for CVD risk reduction.
LADA	Early beta cell failure in a majority	Close follow up to detect insulin requirement
MODY 1	Beta cell secretory defect that can be corrected by sulphnylureas	Low dose sulphonylurea treatment
MODY 2	Mild diabetes. Microvascular complications occur rarely.	Usually no need of pharmacological treatment
Mitochondrial diabetes	High prevalence of insulin requirement	Close follow up to detect those needing insulin

Specific objectives

1. To identify different phenotypes of young-adult-onset diabetes in Sri Lanka
2. To establish aetiological breakdown of the young-adult-onset diabetes in Sri Lanka based on the clinical, immunological biochemical and genetic tests
3. To predisposition to disease progression and diabetic related complications among different subtypes of diabetes

Terms and classifications

Those in whom insulin treatment was started within 6 months from diagnosis were classified as having type 1 diabetes and these subjects were further sub-classified as GADA-positive type 1 diabetes and GADA-negative type 1 diabetes. Those who were GADA-positive but insulin independent during the first six months from diagnosis and were ≥ 30 years at diagnosis were considered to have Latent Autoimmune Diabetes in Adults (LADA) [37]. Those with GADA antibody positivity, insulin dependence within six months of diagnosis and less than 30 years were termed latent Autoimmune Diabetes in Young (LADY). Subjects who were GADA negative and insulin independent within six months from diagnosis were considered to have type 2 diabetes. Metabolic syndrome was diagnosed according to the International Diabetes Federation criteria [38]. Those who had mt.3243A>G mutation were termed to have Maternally Inherited Diabetes and deafness (MIDD).

Methods

Detailed study protocol is illustrated in Figure 6.

Study design

Sri Lanka Young Diabetes Study was a multi centre descriptive cross sectional study.

Participants

Inclusion criteria

- Definitive diagnosis of diabetes according to the ADA or WHO criteria (irrespective of type of treatment)
- Males and females with the age-of-diagnosis of diabetes between 16 to 40 years
- Current age <45 years

Exclusion criteria

- Pregnancy
- Acute illness at the time of recruitment
- Refusal or inability to provide informed written consent

Study setting

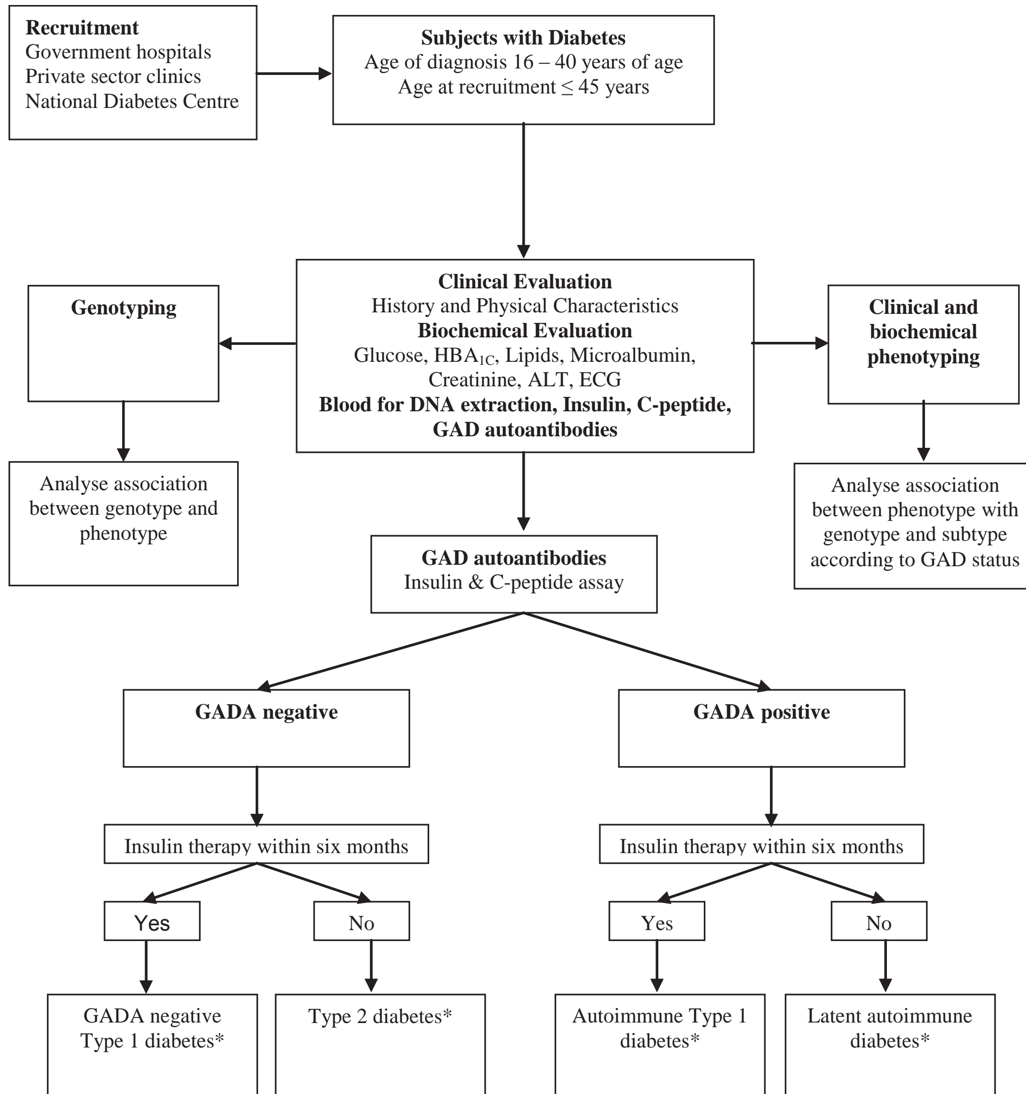
The SLYDS was undertaken as a multi-centre study in order to recruit a representative sample of young adult onset diabetic subjects in Sri Lanka. Recruitment was carried out in the largest three hospitals in Sri Lanka (National Hospital of Sri Lanka, Colombo South Teaching Hospital and Colombo North Teaching Hospital), diabetes and medical out-patient clinics of selected government hospitals, the National Diabetes Centre and through referrals from physicians or general practitioners between June 2005 and February 2006. In these centres the total number of diabetic patient visits of all ages exceeded 20000 per month. During the recruitment period 1214 eligible subjects were invited and 1007 of them attended for data collection (response rate was 83%).

Data collection

In SLYDS, the data collection was carried out by a team of trained medical graduates. The recruited participants attended the Diabetes Research Unit or National Diabetes Centre for data collection after an overnight fast. Data collected through interviews, physical examinations and biological samples according to standard techniques (Figure 7).

Figure 6

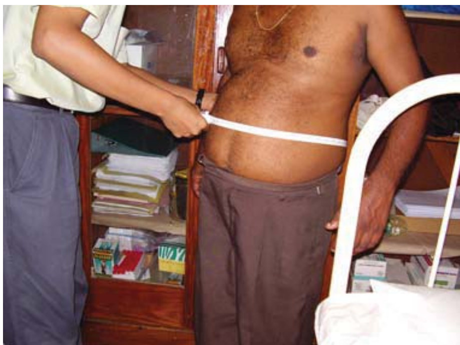
Plan of Investigation for aetiological diagnosis among young adult diabetic subjects



(*Provisional diagnoses – may change in future with genetic analysis)

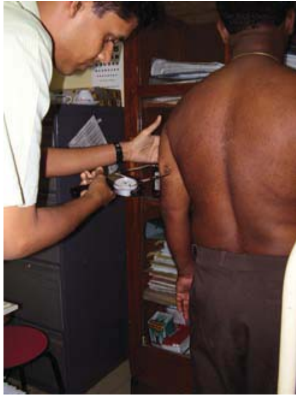
Figure 7.
Pictures illustrating clinical features and data collection

Measuring central obesity in a male

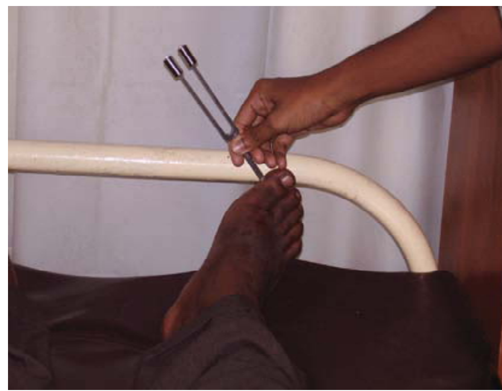


Acanthosis nigricans in the neck

Measuring skinfold thickness

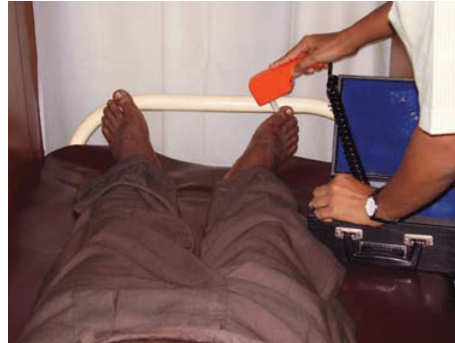


Examining vibration sensation



Retinopathy screening

Assessment of vibration perception



Biochemical assays

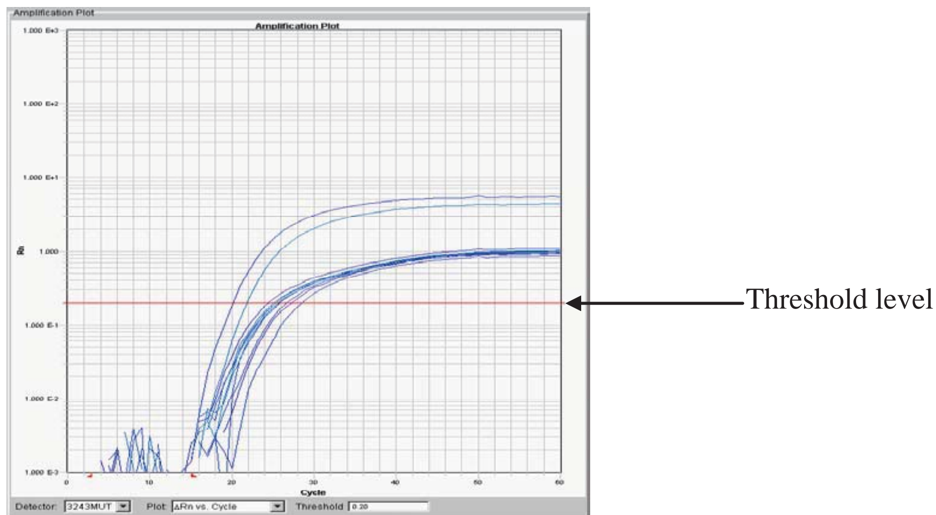
The biochemical assays were carried out according to standard protocols under internal and external quality control in the laboratories in Sri Lanka and UK. Fasting plasma glucose was measured by a glucose oxidase method (Roche Diagnostics, Mannheim, Germany) [20]. The total cholesterol, HDL-cholesterol (by precipitation) and triacylglycerol were measured by an enzymatic colorimetric method (Roche Diagnostics, Mannheim, Germany) in a Hitachi 704 chemical autoanalyser. LDL-cholesterol was estimated using the Friedwald equation [21]. HbA_{1C} was measured by a HPLC technique standardised to DCCT [22] (Biorad, Hercules, CA, USA).Insulin, C-peptide and GADA measurements were undertaken in UK.

Genetic studies

Genetic studies were undertaken in the Oxford Centre for Diabetes and Endocrinology in Oxford. Genomic DNA was isolated from whole blood using the QIAamp® 96 DNA blood kit (Qiagen, UK).

The entire cohort (n = 1007) were screened for the mt3243A>G mutation using a previously described quantitative real time PCR (QRT-PCR) technique on an ABI 7900HT system using sequence specific TaqMan MGB probes [39]. Samples with levels of heteroplasmy >5% were considered mutation positive.

Figure 8 RT-PCR amplification plot illustrating C_T values for mutant and wild type amplification reactions in a mutant positive sample



(C_T - threshold cycle. Red arrow and blue arrows indicate C_T values for amplification profiles of the mutant and wild type DNA respectively in a mutant positive sample)

Mutation positive individuals were invited to attend a local research centre for a repeat blood test for DNA extraction and to undergo a Pure Tone Audiogram

Mutation screening for MODY 2 (glucokinase MODY)

Mutation screening for GCK was undertaken using a High resolution DNA melting technique and DNA sequencing. When there is no sequence variation in DNA, complementary strands form completely matched duplexes and are termed homoduplexes. In the presence of sequence variation, pairs of non matched hybrids are formed and these are called heteroduplexes. The melting temperatures of homoduplexes and heteroduplexes usually shows minor variations [40]. When used with double stranded DNA (dsDNA) binding fluorescent dyes these changes of melting profiles can be detected as changes of fluorescence over time (Figure 9). This techniques is called Hi-Res melting™ and used in mutation scanning in the LightScanner (Figure 10) system which I used in for screening of GCK mutations combined with DNA sequencing.

Figure 9. Melting profiles of a mixture of mutant and wild type DNA detected using LCGreen plus dsDNA binding fluorescent dye

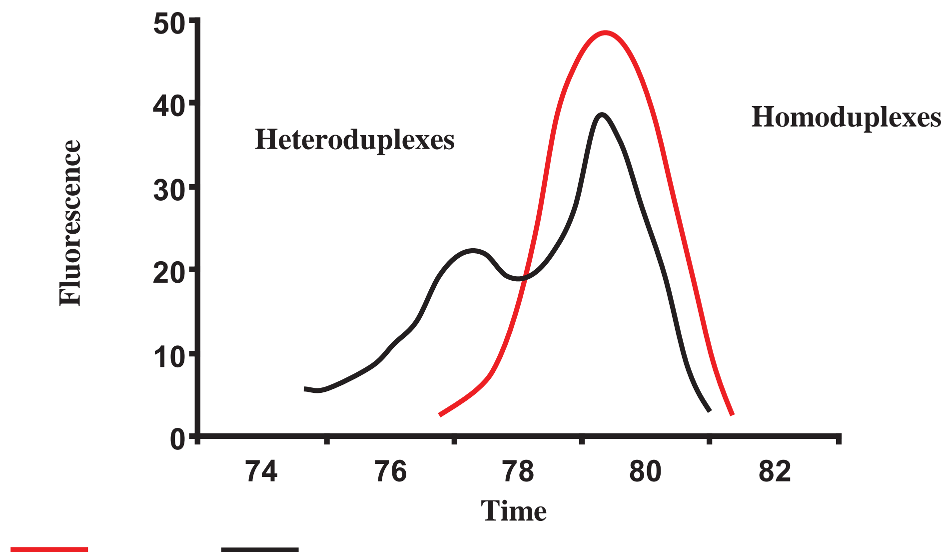


Figure 10. Light scanner system

DNA sequencing

The process in which the order of the nucleotide bases[1*] in a DNA fragment is determined is called DNA sequencing. The DNA sequencing for this research work was performed in ABI Prism 3700 capillary sequencer (Applied Biosystems, Warrington UK).

Results

Among the 1007 participants 42.1% were males. The medians (IQR) of age, age of diagnosis and duration of diabetes in years were 38.0 (33.1 – 41.1), 33.0 (29.0 – 36.1) and 4.0 (1.1 – 7.1) respectively. The treatment patterns were; lifestyle alone 13.6%, oral therapy alone 70.5% and insulin 15.9% (Table 3). The mean HBA_{1C} was 8.1% (6.5 – 9.3), BMI 24.7 kg/m^2 (22.1 – 26.8) and waist circumference 87.3cm (81.5 – 93.0). Characteristics of the all study participants, males and females are shown in Table 4.

In our cohort eight individuals (0.8%) had a history of pancreatic disease. Females had higher BMI, fasting insulin, diabetes duration and metabolic syndrome compared to males. Males had higher waist circumference, waist-hip-ratio, total cholesterol and triglycerides.

Table 3.
Treatment patterns in young adult diabetic subjects at diagnosis and at recruitment

	Initial treatment	Treatment at recruitment
Diet and exercise	**17.8%**	**13.6%**
Tablets	**66.2%**	**70.5%**
Insulin	**16%**	**15.9%**

Table 4
Characteristics of the study sample

Characteristic	All	Male	Female	p
Male (%)	42.1%	–	–	
GADA positivity (%)	5.4% (4.0 – 6.9)	7.4% (4.9 – 9.9)	4.0% (2.4 – 5.6)	0.028
Insulin therapy (%)	15.9% (13.7 – 18.3)	14.6% (11.2 – 18.0)	16.9% (14.0 – 20.2)	0.001
Hypertension (%)	31.7% (28.8 – 34.6)	30.1% (25.7 – 34.6)	32.8% (28.9 – 36.6)	0.37
Metabolic syndrome (%)	60.1% (57.0 – 63.1)	38.5% (33.8 – 43.2)	75.9% (72.2 – 79.3)	<0.001
Age at recruitment (years)	38.4 (33.1 – 41.0)	37.0 (32.1 – 40.0)	39.0 (35.0 – 41.1)	<0.001
Age at diagnosis (years)	33.0 (29.0 – 36.1)	33.0 (28.0 – 36.0)	33.0 (29.0 – 37.0)	0.214
Diabetes duration (years)	4.0 (1.1 – 7.1)	3.1 (1.0 – 6.1)	4.1 (2.0 – 7.1)	<0.001
BMI (kg/m2)	24.3 (22.1 – 26.8)	24.1 (21.6 – 26.3)	24.6 (22.3 – 27.1)	0.004
Waist circumference (cm)	87.0 (81.5 – 93.0)	88.0 (82.0 – 94.0)	86.0 (81.0 – 92.0)	0.009
WHR	0.91 (0.88 – 0.95)	0.92 (0.88 – 0.95)	0.91 (0.88 – 0.95)	0.048
Total cholesterol (mmol/l)	5.0 (4.4 – 5.7)	5.1 (4.4 – 5.9)	4.3 (4.3 – 6.0)	0.007
HDL-C (mmol/l)	1.0 (0.9 – 1.2)	1.0 (0.9 – 1.2)	1.0 (0.9 – 1.2)	0.444
LDL-C (mmol/l)	3.2 (2.6 – 3.8)	3.3 (2.7 – 3.9)	3.2 (2.6 – 3.7)	0.100
Triglycerides (mmol/l)	1.4 (1.1 – 2.0)	1.5 (1.1 – 2.2)	1.4 (1.0 – 1.9)	<0.001
HBA1C %	7.8 (6.5 – 9.3)	8.0 (6.5 – 9.4)	7.7 (6.5 – 9.2)	0.161
Fasting insulin (pmol/l)	82.3 (54.0– 122.6)	73.1 (50.5 – 113.3)	57.9 (57.9– 125.1)	0.001
Fasting C-peptide (nmol/l)	0.6 (0.4 – 0.8)	0.62 (0.43 – 0.84)	0.59 (0.38 – 0.79)	0.471
GADA level (WHO units)	4.3 (3.3 – 5.6)	4.6 (3.6 – 5.8)	4.1 (3.2 – 5.4)	0.078

Data are % (95% CI) or median (IQR). P for differences between males and females – x^2 and Mann Whitney tests for comparison of percentages and medians respectively.

Prevalence of GADA positivity and subtypes of diabetes

The prevalence of GADA-positive diabetes in this cohort was 5.4% (Table 5). Prevalence of GADA positivity was much higher in those who were young and had lower BMI compared to those older and more obese (Figure 12). GADA positivity among males and females were 7.4% and 4.0% respectively, p=0.028. In this cohort, type 1 diabetes, type 2 diabetes and LADA formed 7.0% , 88.8% and 2.6% respectively (Figure. 11 and Table 6). Another 7 patients (0.7%) who had been diagnosed

at a younger age than 30 years were GADA positive and insulin independent during first six months from diagnosis (LADY) and 0.9% had mitochondrial diabetes due to mt.3243A>G mutation. Type 1 diabetes was present in 8.8% of males and 5.6% in females (p=06). GADA were positive in 29.6% of type 1 diabetic subjects which comprised 2.1% of the total sample. Males had a higher prevalence of GADA-positive type 1 diabetes (M=3.8%, F=0.9%, p=0.003).

Table 5.
GAD antibody positivity according to age and gender

Prevalence of GADA positivity	5.4%
GADA positivity in males	7.4%
GADA positivity in females	4.0%
GADA positivity in < 30 years of age	10.2%
GADA positivity in ≥ 30 years of age	3.6%

Figure 11. Prevalence of different subtypes of diabetes among young adults in Sri Lanka

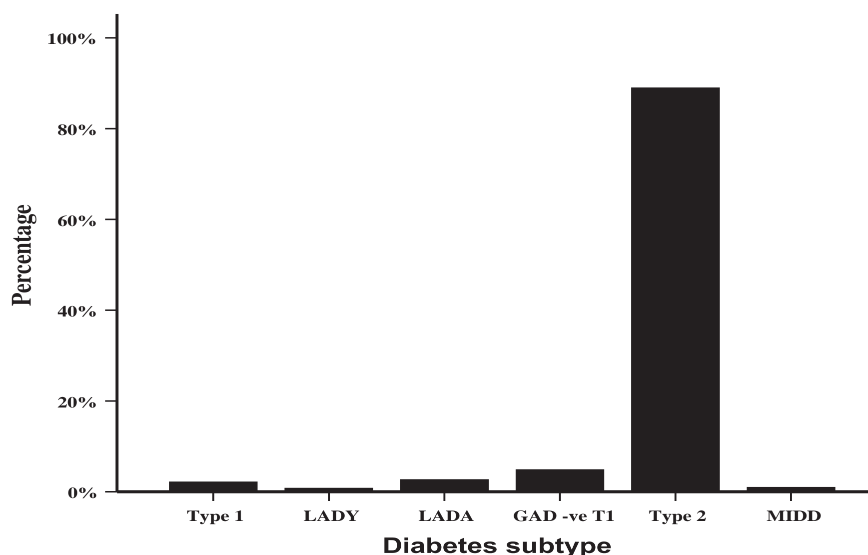
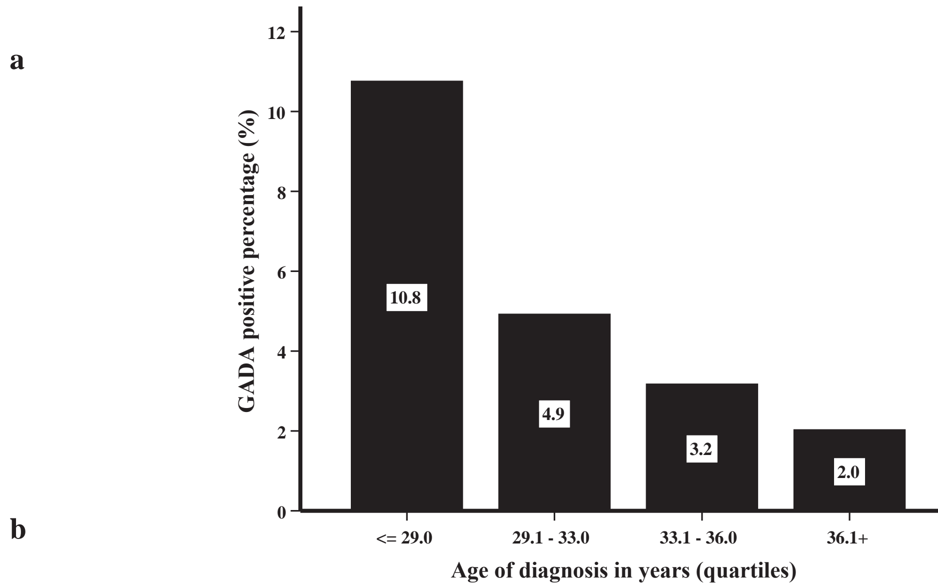


Table 6. Prevalence of subtypes of diabetes according to GADA positivity among young adults with diabetes in Sri Lanka

Subtype of diabetes		All - n (%)	Male - (%)	Female - (%)	p - value
Type 1 diabetes	GADA +ve	21 (2.1%)	3.8%	0.9%	0.003
	GADA -ve	48 (4.9%)	5.0%	4.7%	0.464
LADY		7 (0.7%)	0.9%	0.5%	0.332
LADA		26 (2.6%)	2.6%	2.6%	0.569
Type 2 diabetes		881 (88.8%)	86.7%	90.5%	0.036
MIDD		9 (0.9%)	0.9%	0.9%	0.575

Figure 12 GADA positivity according to quartiles of (a) age-of-diagnosis and (b) BMI among young adult onset diabetic subjects in Sri Lanka

a



b

Clinical features associated with GAD autoantibody positivity and insulin therapy

In all patients GADA levels negatively correlated with the age of diagnosis ($r = -0.224$, $p<0.0001$), fasting C-peptide levels ($r= -0.241$, $p<0.0001$), BMI ($r = -0.210$, $p<0.0001$) and time to insulin treatment ($r= -0.220$, $p=0.006$) (Figure 13).

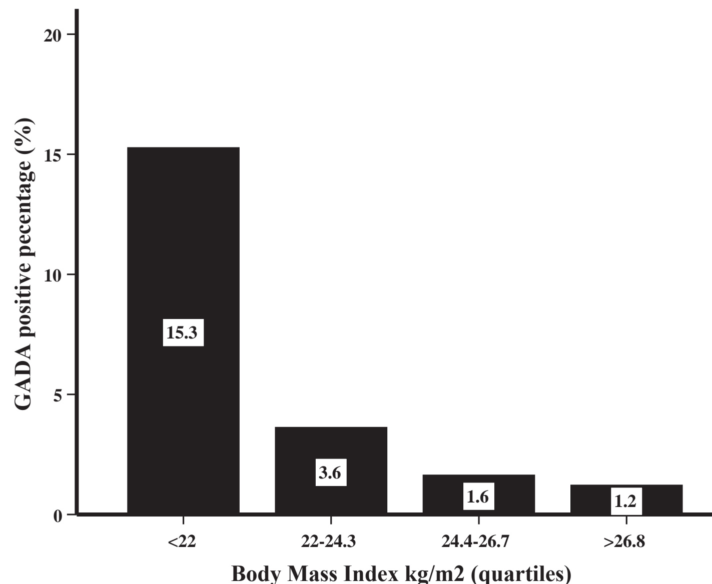
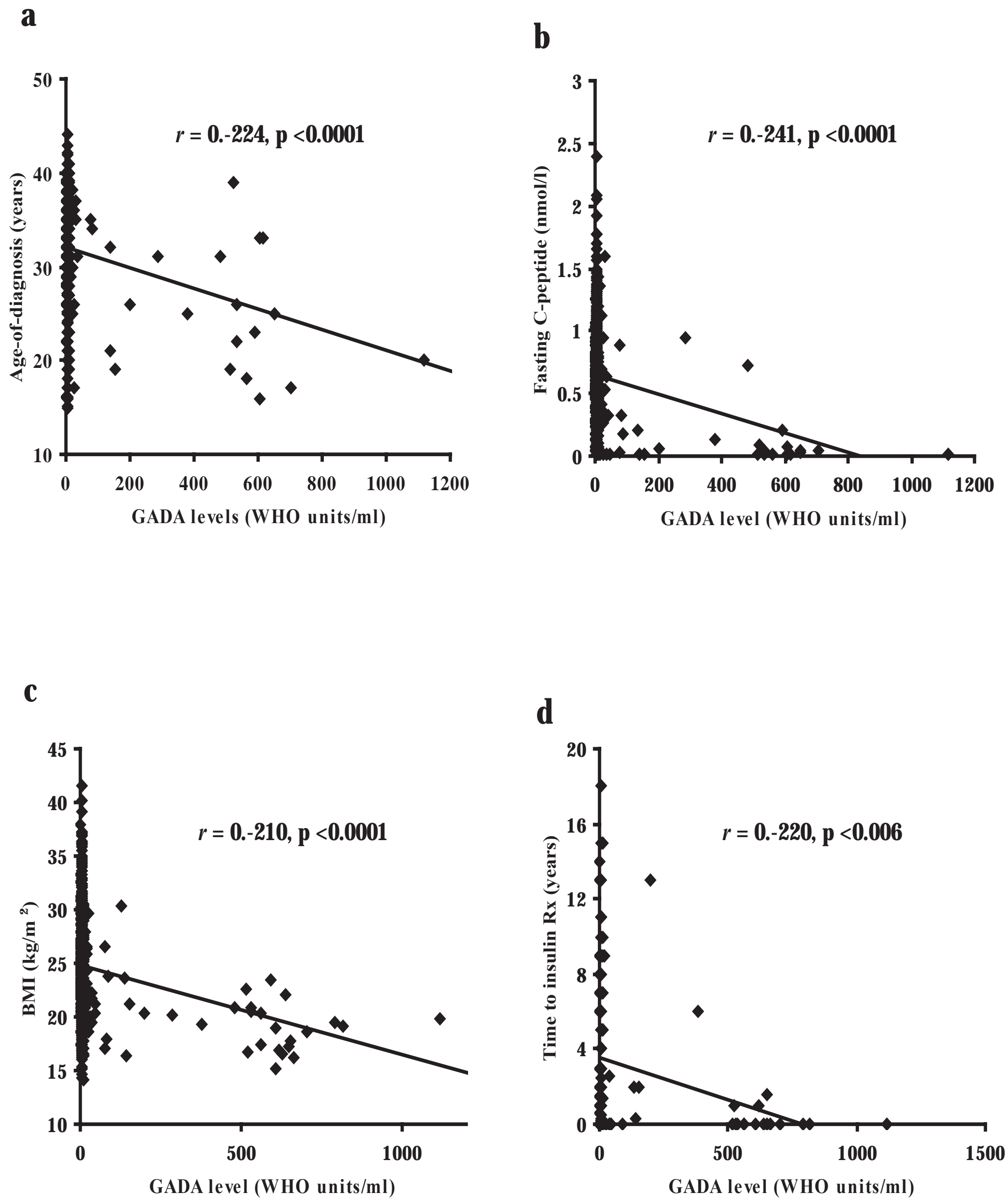
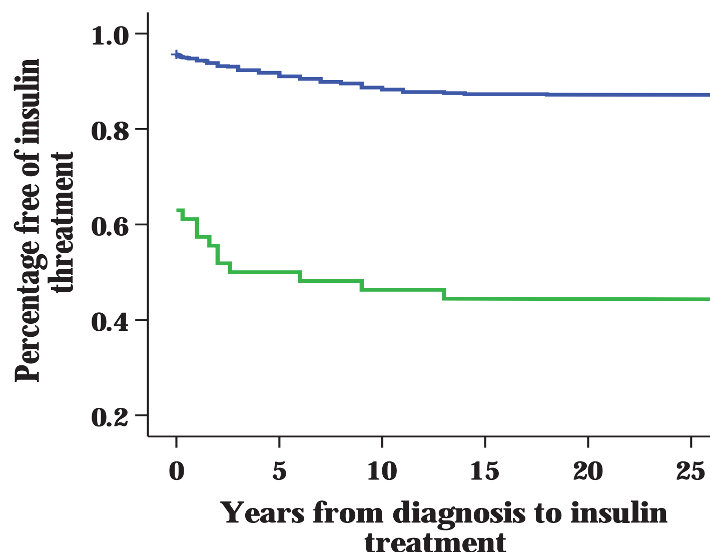


Figure 13
Scatterplots illustrating the associations of GADA levels with (a) age-of-diagnosis, (b) fasting C-peptide, (c) BMI and (d) time from diagnosis to insulin treatment among young adult onset diabetic subjects in Sri Lanka (Rx – treatment)



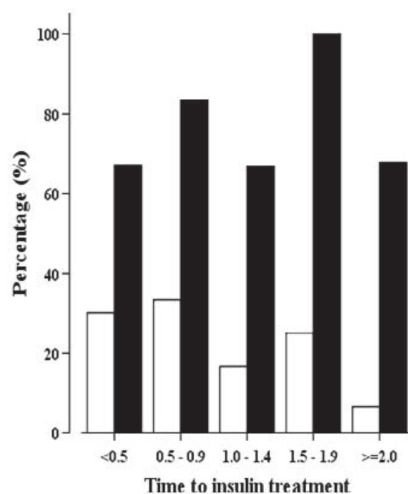
Overall, 57.4% of GADA positive subjects were on insulin treatment compared to 13.6% of GADA negative subjects ($p<0.0001$). GADA positivity (corrected for the age-of- diagnosis) correlated with insulin treatment ($r=0.213$, $p<0.0001$). The Figure 14 illustrates the Kaplan- Meier survival plot for insulin treatment among the GADA positive and negative subjects (based on retrospective data).

Figure 14 Kaplan- Meier survival plot for remaining free from insulin treatment among the GADA positive and negative subjects



When the frequency of low fasting C-peptide (being in the lowest quartile of fasting C-peptide) and GADA positivity was compared in patients categorised according to the time taken for initiation of insulin treatment, the frequency of low C-peptide was higher compared to the GADA positivity in all categories (Figure 15). Overall, among those treated with insulin 69.2% had low fasting C-peptide compared to 19.5% being GADA positive ($p<0.0001$).

Figure 15 Frequency of GADA positivity and low fasting C-peptide according to the duration for initiation of insulin treatment



Phenotypic characteristics among subtypes of diabetes

The phenotypic characteristics among GADA positive and negative subjects are compared in Table 7. The comparison of phenotypic characteristics among individual subtypes is shown in Table 5. Significant differences were seen between GADA-negative and GADA-positive categories in all parameters examined but for LDL cholesterol. Those with GADA-positive compared to GADA-negative had been diagnosed young, were lean, less hypertensive, presented more frequently with symptoms and commonly had a history suggestive of ketoacidosis. GADA-positive patients had lower prevalence of metabolic syndrome and its components, lower markers of beta cell function (fasting C-peptide and HOMA %B), less family history of diabetes and lower prevalence of acanthosis nigricans compared

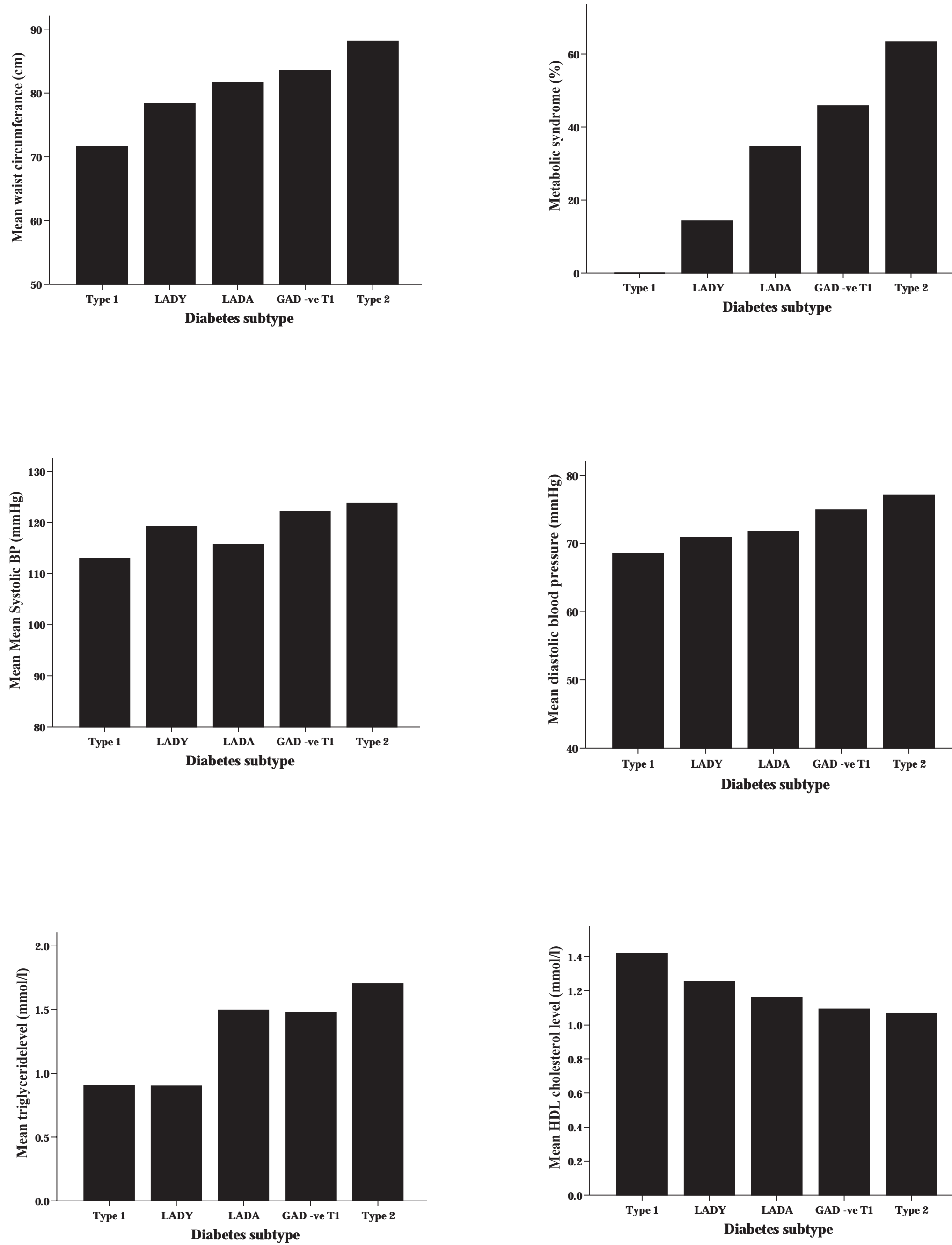
to those GADA negative. Metabolic syndrome, its components and the other phenotypic characteristics progressively changed when the diabetes subtypes were ordered as GADA-positive type 1 diabetes, LADY, LADA, GADA-negative type 1 diabetes and type 2 diabetes (Table 8 and figure 16).

Table 7. Clinical and metabolic characteristics of GADA positive and GADA negative subjects with young adult onset diabetes in Sri Lanka

Classification	GADA-positive diabetes	GADA-negative diabetes	p
Number	54	938	
Age at recruitment (years)	31.6 (24.1 – 39.1)	38.1 (34.0 – 41.1)	<0.0001[a]
Age at diagnosis (years)	26.5 (21.1-33.0)	33.0 (29.0-36.1)	<0.0001[a]
Duration of diabetes (years)	4.0 (1.1 – 6.1)	4.0 (1.1 – 7.1)	0.708 [a]
BMI (kg/m^2)	20.5 (18.9-22.6)	24.5 (22.3-26.9)	<0.0001[a]
Waist circumference (cm)	74.8 (71.0-81.5)	87.0 (82.0-93.0)	<0.0001[a]
WHR	0.86 (0.81-0.91)	0.92 (0.88-0.95)	<0.0001[a]
Body fat %	21.2 (17.5-29.3)	29.9 (21.5 -35.4)	<0.0001[a]
HDL-C (mmol/l)	1.1 (0.9-1.5)	1.0 (0.9-1.2)	0.001[a]
LDL-C (mmol/l)	3.2 (2.7-3.8)	3.2 (2.6-3.8)	0.865[a]
Triglycerides (mmol/l)	0.9 (0.8-1.5)	1.5 (1.1- 2.1)	<0.0001 [a]
HBA_{1C} %	9.2 (7.1-11.4)	7.8 (6.4-9.2)	<0.0001[a]
Fasting C-peptide (nmol/l)	0.15 (0.02-0.51)	0.61 (0.43-0.82)	<0.0001[a]
HOMA-B%	17.6 (2.1-42.8)	44.4 (26.1-72.4)	<0.0001[a]
Current insulin treatment (%)	57.4 (44.2 – 70.6)	13.6 (11.4 – 15.8)	<0.0001[b]
Definite or probable DKA (%)[c]	25.9 (14.2 – 37.6)	7.2 (5.5 – 8.9)	<0.0001[b]
Hypertension (%)	7.4 (0.4 – 14.4)	33.0 (30.0 – 36.0)	<0.0001[b]
Family history of diabetes (%)	51.9 (38.6 – 65.2)	74.6 (71.8 – 77.4)	<0.0001[b]
Acanthosis nigricans (%)	7.4 (0.4 – 14.4)	21.6 (19.0 – 24.2)	0.020[b]
Symptomatic presentation (%)	94.4 (88.3 – 100.0)	72.1 (69.2 – 75.0)	0.001[b]
Metabolic syndrome (%)	18.5 (8.1 – 28.9)	62.5 (59.4 – 65.6)	<0.0001[b]

Data are median ± IQR or Percentage (%) and 95% CI. P values are according to [a]Mann-Whitney *U* test and [b]Chi squared test, [c] – Episodes suggestive of and including diabetes ketoacidosis (DKA)

Figure 16. Metabolic syndrome and its components among subtypes of diabetes in Sri Lankan young adults with diabetes



(Data are % for metabolic syndrome and means for its components)

Table 8 Clinical and metabolic characteristics in different subtypes of diabetes among young adult diabetic subjects in Sri Lanka

Classification	GADA +ve T1DM	LADY	LADA	GADA –ve T1DM	T2DM	p
Number	21	7	26	48	890	
Age at recruitment (years) [c]	25.1 (23.0 – 28.0)	24.1 (23.1 – 27.1)	39.1 (34.1 – 41.1)	30.6 (25.6 – 36.5)	38.1 (34.1 – 41.4)	<0.0001[a]
Age at diagnosis (years) [c]	21.1 (18.0-27.0)	23.0 (19.0-25.0)	32.5 (30.0-35.0)	25.6 (20.5-31.5)	33.0 (29.1-36.1)	<0.0001[a]
Duration of diabetes (years)	3.0 (1.1 – 6.0)	1.9 (0.1 – 5.1)	4.5 (2.9 – 9.1)	3.5 (0.5 – 9.5)	4.0 (1.9 – 7.0)	0.25 [a]
BMI (kg/m^2)	19.5 (18.6-20.9)	21.2 (17.1-23.4)	21.3 (19.6-24.3)	23.4 (21.8-26.5)	24.5 (22.4-27.0)	<0.0001[a]
Waist circumference (cm)	72.0 (68.0-74.0)	78.0 (71.0-82.0)	80.5 (73.5-91.5)	84.0 (79.0-89.0)	87.3 (82.0-93.5)	<0.0001[a]
WHR	0.82 (0.79-0.84)	0.87 (0.77-0.93)	0.89 (0.86-0.96)	0.88 (0.87-0.93)	0.92 (0.88-0.95)	<0.0001[a]
Body fat %	19.6 (17.3-23.7)	17.2 (14.1-24.3)	24.6 (20.8-35.0)	25.8 (20.1 -32.4)	30.2 (21.6-35.5)	<0.0001[a]
HDL-C (mol/l)	1.2 (1.0-1.8)	1.1 (0.9-1.8)	1.1 (0.9-1.4)	1.1 (0.9-1.3)	1.0 (0.9-1.2)	0.007[a]
LDL-C (mmol/l)	3.3 (2.5-3.9)	3.0 (2.7-3.5)	3.2 (2.7-3.8)	3.4 (2.8-4.0)	3.2 (2.6-3.8)	0.86[a]
Triglycerides (mmol/l)	0.8 (0.5-0.9)	0.9 (0.6-1.1)	1.4 (0.9-1.8)	1.3 (1.0-1.8)	1.5 (1.1-2.1)	<0.000 [a]
HBA_{1C} %	10.1 (8.0-11.3)	7.8 (5.2-10.8)	9.0 (16.5-11.7)	8.4 (7.3-10.0)	7.7 (6.4-9.1)	0.0002[a]
Fasting C-peptide (nmol/l)	0.02 (0.02-0.05)	0.16 (0.02-0.40)	0.47 (0.14-0.72)	0.36 (0.15-0.63)	0.63 (0.44-0.83)	<0.0001[a]
HOMA-B%	2.1 (1.0-21.7)	18.1 (8.8-43.4)	26.1 (6.7-51.7)	34.6 (21.6-68.0)	45.0 (27.1-72.9)	<0.0001[a]
Insulin Rx (%) at recruitment	100 (0.0)	28.6 (33.5)	30.8 (17.7)	100 (0.0)	9.1 (1.9)	<0.0001[b]
Definite or probable DKA	42.9 (21.2)	28.6 (33.5)	11.5 (12.3)	20.8 (11.5)	6.5 (1.6)	<0.0001[b]
Hypertension (%)	0.0 (0.0)	0.0 (0.0)	15.4 (13.9)	31.3 (13.1)	33.1 (3.1)	0.002[b]
Family history of diabetes (%)	33.3 (20.2)	28.6 (33.5)	73.1 (17.0)	64.6 (13.5)	75.2 (2.8)	<0.0001[b]
Acanthosis nigricans (%)	0.0 (0.0)	0.0 0.0)	15.6 (13.9)	20.8 (11.5)	21.6 (2.7)	0.085[b]
Symptomatic presentation (%)	100 (0.0)	100 0.0)	88.5 (12.3)	77.1 (11.9)	71.8 (2.9)	0.006[b]
Metabolic syndrome (%)	0.0 (0.0)	14.3 (25.9)	46.2 (19.2)	52.1 (14.1)	67.2 (3.1)	<0.0001[b]

Data are median ± IQR or Percentage (%) and 95% CI. P values are for differences between all subtypes according to [a]Kruskall – Wallis test and [b]Chi squared test, [c] – Study had restrictions of age: Age of recruitment was ≤ 45 years and the age of diagnosis 16 – 40 years. DKA - Diabetes ketoacidosis.

Treatment patterns and glycaemic outcome in patients with latent autoimmune diabetes

In the subgroup with latent autoimmune diabetes (both LADY and LADA), 70% (23/33) were on lifestyle modification or oral hypoglycaemic agents (OHA) and 35% of these (8/23) had HBA_{1C} ≤ 7% (good glycaemic control) compared to only 20% (2/10) in patients requiring insulin Table 9. Those

with $HBA_{1C} \leq 7\%$ while on lifestyle modification or OHA had higher beta cell function (median HOMA %B 57.9; IQR 46.4 – 61.1)) despite having a longer duration of diabetes (median duration 5.6 years; IQR 3.6 – 9.1).

Table 9 Characteristics of subjects with Latent Autoimmune Diabetes (of all ages) according to the treatment and glycaemic control

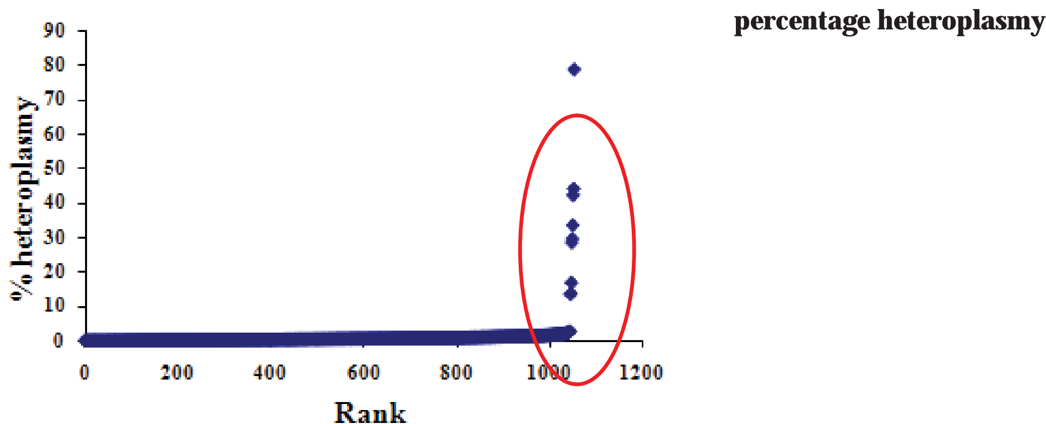
Treatment	Insulin		OHA and/or lifestyle mdification		P
HBA_{1c} category	$HBA_{1c} \leq 7$	$HBA_{1c} > 7$	$HBA_{1c} \leq 7$	$HBA_{1c} > 7$	
N (%)	2 (6.1%)	8 (24.2%)	8 (24.2%)	15 (45.5%)	
Age of diagnosis (years)	32.5 (26.0-39.0)	25.0 (21.0-29.0)	31.5 (24.5-31.5)	31.1 (29.0-37.0)	0.073
GADA (WHO units/ml)	527 (521-533)	177 (87-498)	26 (20-80)	30 (20-481)	0.159
C-peptide (nmol/l)	0.06 (0.02-0.09)	0.05 (0.02-0.17)	0.48 (0.38-0.62)	0.62 (0.35-0.95)	0.072
HOMA %B	5.55 (2.8-8.3)	5.7 (1.9-13.4)	57.9 (46.4-61.1)	25.3 (11.2-38.0)	0.002
Duration (years)	3.6(0.9-7.1)	7.5(4.1-11.1)	5.6(3.6-9.1)	2.9 (1.0-4.0)	0.073

(Data are median and Inter Quartile Range, Latent Autoimmune Diabetes comprises of all LADY and LADA subjects)

Derivation of a cut-off level for mutant positivity in mitochondrial diabetes due to mt.3243A>G mutation

At present there is no widely accepted cut-off level for the percentage heteroplasmy of mt3243A>G mutations to classify mutant positivity. When all the samples were according to the percentage heteroplasmy (Figure 17) and heteroplasmy data were plotted against the rank, a percentage heteroplasmy of 5% could be identified as the cut-off point. Therefore, samples with heteroplasmy >5% were considered mutant positive.

Figure 17 Graphical representation of the ranked data for all subjects (n= 994) for percentage heteroplasmy



(Samples determined as mutation positive are highlighted by the red ring. A dotted line indicates the threshold of >5% for mutation positive samples.)

Associations of mt3243A>G heteroplasmy and clinical presentation

The percentage heteroplasmy showed a negative correlation with the current age of the subjects (r=-0.71) and the age of diagnosis (r =-0.53) which was significant for the current age (p=0.033) but not for the age of diagnosis (p=ns).

Clinical characteristics of patients with MIDD in Sri Lanka

The clinical and metabolic characteristics among mt3243A>G mutation carriers and non mutant carriers (NMCs) are compared in Table 10. All mutation positive subjects were negative for GAD autoantibodies. A total of 44.4% of carriers required insulin within the first six months following diagnosis compared to 6.9% of NMCs, p=0.002. The mean fasting plasma glucose and HbA_{1C} were not significantly different between the carriers and NMCs. The carriers had lower BMI (18.7 Kg/m^2 ± 2.7 vs. 24.7 Kg/m^2 ± 4.1%, p<0.001) and waist circumference (72.9 cm ± 7.8 vs 87.4cm ± 9.7, p<0.001) compared to NMCs. Only one (11.1%) of the carriers had metabolic syndrome compared to 641 (64.2%) of NMCs, p=0.002. The mutation positive subjects had lower beta cell function as indicated by low fasting insulin (median 60.3 pmol/l vs. 82.3 pmol/l, [normal range 18.0-77.0] p=0.028), c-peptide (median 0.3 nmol/l vs. 0.6 nmol/l, [normal range 0.27-1.28] p=0.002) and HOMA % B (median 33.6 vs. 54.3, p=0.053) compared to NMCs although HOMA % B did not reach statistical significance.

Table 10. Clinical and metabolic characteristics of subjects with the mt3243A>G mutation (n=9) compared to the remainder (n=998) of the cohort.

	mt3243A>G positive subjects	Non- carriers	P – value
Number	9	998	
Mean (SD) age of diagnosis (years)	25.9 (4.8)	31.9 (5.6)	0.002*
Family history of diabetes (%)	89	73	0.266†
Maternal family history of diabetes (%)	66.7	43.1	0.354†
Paternal family history of diabetes (%)	31.9	33.3	0.726†
Personal Hearing impairment (%)	44.4	7.5	0.003†
Family history of hearing impairment (%)	33.3	3.2	0.003†
Maternal history of hearing impairment (%)	22.2	0.9	<0.001†
Mean BMI Kgm^{-2} (SD)	18.7 (2.7)	24.7 (4.1)	<0.001*
Mean waist circumference (cm)	72.9 (7.8)	87.4 (9.7)	<0.001*
Mean HbA_{1C} (SD)	7.9 (1.8)	8.0 (2.0)	0.866
% Metabolic syndrome	11.1	64.2	0.002†
Acanthosis nigricans (%)	0.0	21.0	0.130†
Insulin therapy within six months (%)	44.4	6.9	0.002†
Peripheral neuropathy (%)	22.2	3.3	0.036†
Retinopathy (%)	22.2	18.3	0.673†
GFR <60ml/min (%)	44.4	5.0	0.001†
Median microalbuminuria mg/l (IQR)	7.5mg/l (4.3-91.3)	9.1mg/l (4.1-28.9)	0.688‡
Median fasting insulin (IQR) (pmol/l)	60.3 (21.1-79.5)	82.3 (54.4 – 122.9)	0.028‡
Median fasting C-peptide (SD) (nmol/l)	0.3 (0.2 – 0.4)	0.6 (0.4- 0.8)	0.002‡
Median HOMA % B (IQR)	33.6 (15.7-55.8)	54.3 (31.8 – 95.7)	0.053‡
Median HOMA % S (IQR)	53.3 (43.6 – 170.4)	47.4 (32.2 – 71.0)	0.071‡
Median HOMA IR (IQR)	1.9 (0.6 – 2.3)	2.1 (1.4-3.1)	0.072‡

Microvascular complications

Diabetic peripheral neuropathy was higher among mutant positive subjects compared to NMCs (22.2% vs. 3.3%, p=0.036). More mt3243A>G positive carriers had a GFR < 60ml/min compared to NMCs (44.4% vs. 5.0%, p=0.001). There was no significant difference in microalbuminura among carriers and NMCs (33.1% vs. 33.3%, p=ns). When diabetic peripheral neuropathy and low GFR were tested as dependant variables in the multiple regression models, with mt3243A>G mutation, duration of diabetes and HbA_{1C} as independent co-variates, mt3243A>G positivity remained significantly associated with retinopathy (β coefficient 2.4; p=0.009) and low GFR. (β coefficient 3.3; p<0.001).

(Continuous variables are shown as mean with standard deviation (SD) or median with inter-quartile range (IQR) and categorical variables as percentages. †- χ^2, *- *t*-test, ‡ - Mann-Whitney *U* Test.)

Family history of diabetes and hearing impairment

A maternal family history of diabetes was present among 66.7% of carriers compared to 43.1% of NMCs (p=ns). A personal self-reported history of hearing impairment was seen among 44.4% of carriers compared to 7.5% of NMCs (p=0.003). The carriers showed a higher frequency of positive family history (33.3% vs. 3.2%, p=0.003) and maternal family history of hearing impairment (22.2% vs. 0.9%, p<0.001) compared to NMCs. A combined screening criteria of any two of maternal history of diabetes, personal and/or family history of hearing impairment identified only 5 (55%) of the carries with a positive predictive value of 7.4% (5/68). Of the 9 subjects with mt3243A>G mutation, 8 underwent a pure tone audiogram after their genetic diagnosis. Figure 18 illustrates a normal audiogram and an audiogram of a subject with MIDD with sensori-neural deafness. In addition to confirming hearing loss in all those who reported hearing impairment, the audiograms detected sub-clinical hearing impairment in 3 out of the five subjects (60%) who had no self reported hearing impairment.

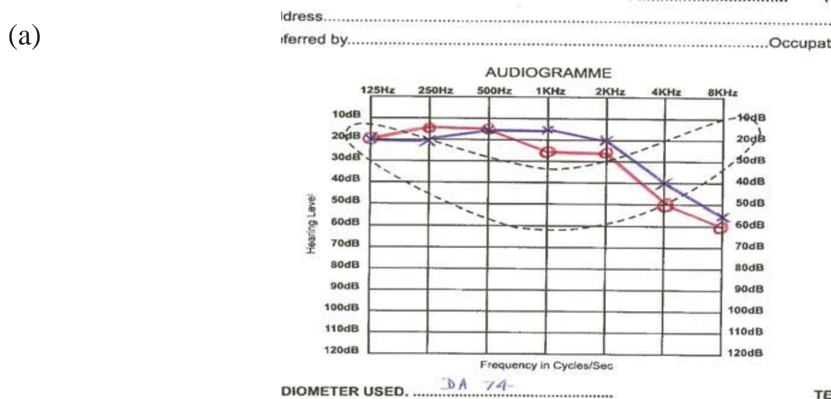
Prevalence of mt3243A>G mutation in family members

In eight of the nine families of mutant positive subjects, 9 family members presented for mutation screening. Among them 7 subjects were found to have mt3243A> positivity (heteroplasmy level >5%). Results of the mutation screening and phenotypic data of the family members are shown in Table 6. Among the mutation positive family members 2 had diabetes, 3 had IFG and 4 had normal glucose tolerance. Three of the 7 mutant positive family members had abnormal audiograms and one had hearing impairment. One of the family members who did not carry the mutation also had abnormal audiographic findings despite having normal glucose tolerance. In the 5 family members of a mutant negative proband who underwent screening, none were positive for the mutation nor had hearing or audiographic abnormalities.

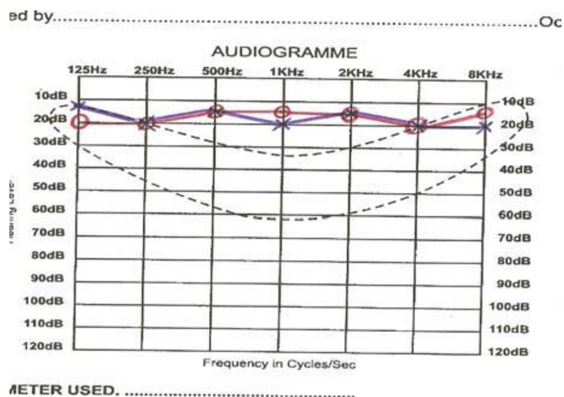
Pedigrees of eight of the mutant positive subjects are illustrated in Figure 19. Five of the eight pedigrees (pedigrees b, c, d, e, h) of the mutant positive subjects had characteristic pattern of maternally inherited diseases with the phenotype being present in mothers and siblings. In one family (pedigree g), that did not have maternal history of diabetes, the maternal grandmother had diabetes. Hearing impairment among the family members was reported only in three families.

Figure 18 Pure tone audiograms showing a normal audiogram (a) and a audiogram with sensori-neural deafness (b)

(a)



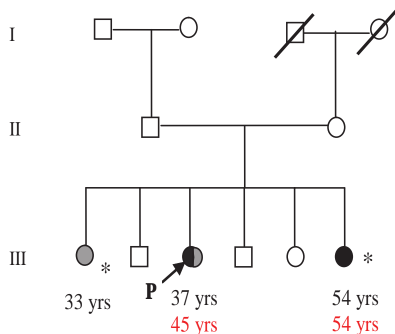
(b)



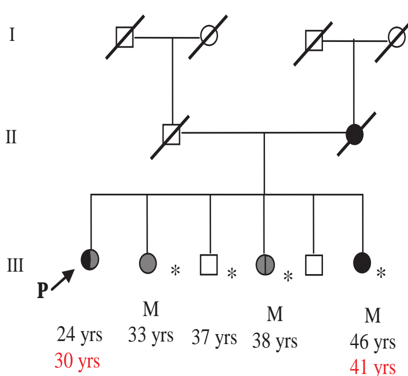
(Sensori-neural deafness at high frequency sounds are shown in the audiogram b. Right ear (red) – o, left ear (blue) - x)

Figure 19. Pedigree charts of mt3243A<G positive subjects – family history of diabetes and hearing impairment in three generations are shown

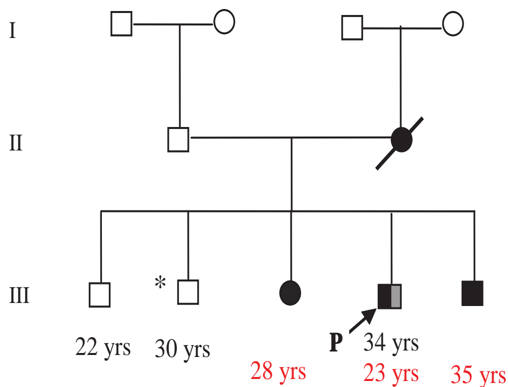
(a)Pedigree of subject Y0120



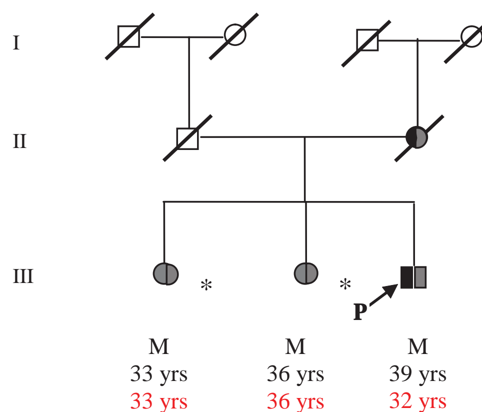
(b) Pedigree of subject Y0303



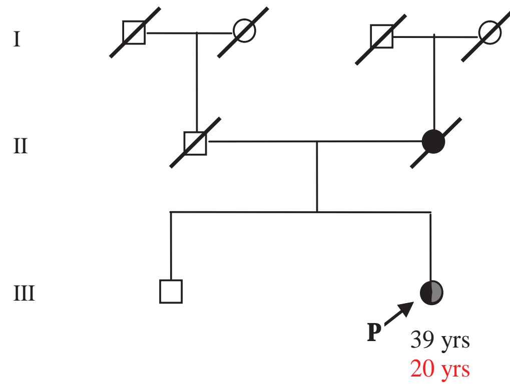
(d) Pedigree of subject Y0533



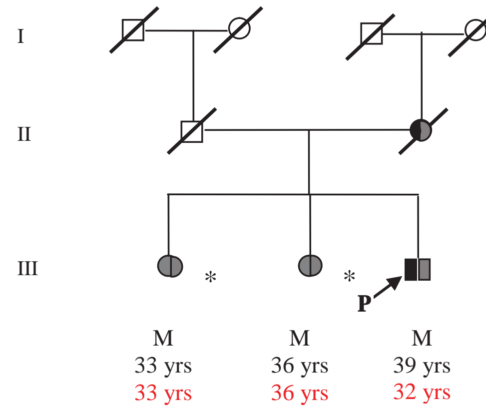
(c)Pedigree of subject Y0522



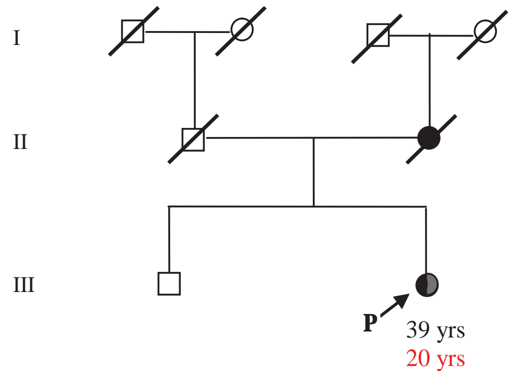
(e) Pedigree of subject Y0803



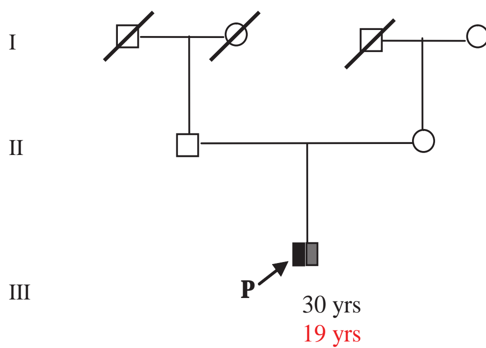
(f) Pedigree of subject Y0635



(g)



(h)



GCK – MODY

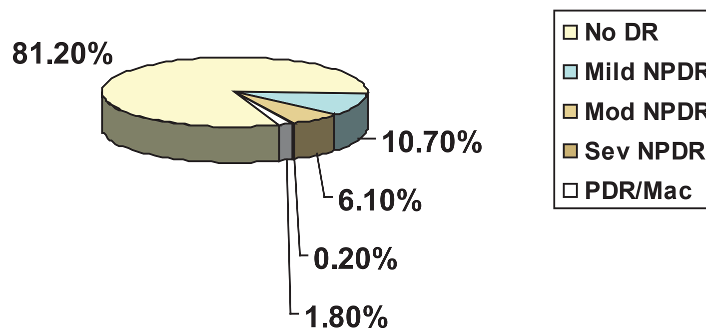
No cases of GCK MODY was detected in 91 subjects who were subjected to screening. Five of the 91 subjects had non pathogenic DNA polymorphisms.

Complications among young ault diabetic subjects in Sri Lanka

Retinopathy

In our series 18.2% of patients had diabetic retinopathy (Figure 20)

Figure 20 Retinopathy among young adults with diabetes



(DR- Diabetic retinopathy, NPDR – non-proliferative diabetic retinopathy, PDR – Proliferative diabetic retinopathy, Mac – macular oedema)

Diabetic neuropathy

In our sample 30% had neuropathic symptoms and 3.2% had both symptoms and abnormal physical examination findings (Table 11).

Table 11. Prevalence of neuropathy

Abnormal neurological symptoms	30%
Abnormal neurological signs	5%
Abnormal signs and symptoms	3.2%

Prevalence of sexual dysfunction

In this series 27% of sexually active males and females had some form of sexual dysfunction (pain, desire, arousal or orgasm). In males who are sexually active, 20% had erectile dysfunction.

Table 12.
Prevalence of erectile dysfunction and any form of sexual dysfunction

Any form of sexual dysfunction (males and females)	27%
Erectile dysfunction (males)	20%

Prevalence of nephropathy

Among the young adult diabetic subjects 30% had microalbuminuria and 5.4% had a glomerular filtration rate less than 60ml/mt (Table 13).

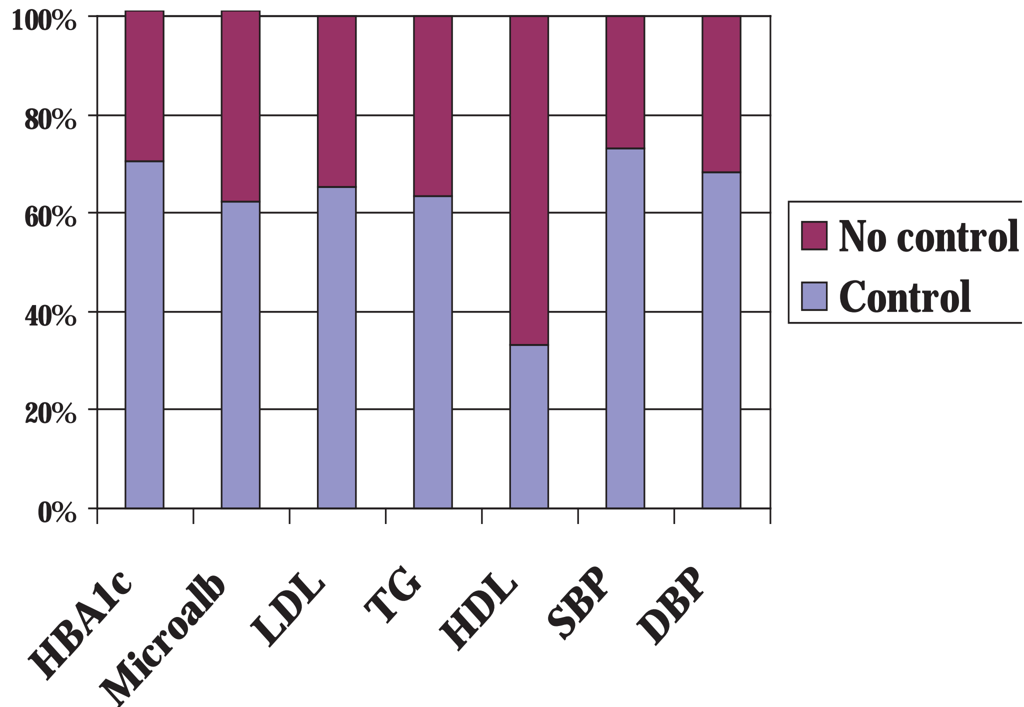
Table 13.
Prevalence of nephropathy

Microalbuminuria	30%
Creatinine clearance <60ml/min	5.4%

Metabolic control among young adults with diabetes in Sri Lanka

We evaluated the metabolic control of the study subjects according to the American Diabetes Association recommendations (HBA1C <7%, absence of microalbuminuria, LDL cholesterol <100mg/dl, triglycerides <150mg/dl, HDL cholesterol >40mg/dl, systolic blood pressure <130mmHg, diastolic blood pressure <80mmHg). Nearly 30% of the patients did not have optimal control in all parameters except HDL cholesterol which showed about 70% below desired levels (Figure 21).

Figure 21.
Metabolic control among young adult diabetic patients in Sri Lanka



Conclusions

* Nearly 90% of young adult subjects in Sri Lanka have type 2 diabetes
* Remaining 10% have specific aetiological subtypes
* 7% of the young adults with diabetes have type 1 diabetes and among them 2.1% have autoimmune type 1 diabetes
* Latent autoimmune diabetes comprises 3.3% of young adults with diabetes. Among them 2.6% were diagnosed after 30 years (LADA) and 0.7% younger than 30 years (LADY)
* Mitochondrial diabetes account for 1% of diabetes in young adults

There are marked phenotypic and clinical differences between subtypes of diabetes.

* Those with type 2 diabetes have preserved beta cell functions and features of insulin resistance and metabolic syndrome compared to other subtypes
* Those with autoimmune type 1 diabetes has severe beta cell failure
* GAD antibody negative type 1 diabetic subjects form a heterogeneous group with some having similarities for type 2 diabetes while some have typical features of autoimmune type 1 diabetes
* More patients with LADA progress to insulin treatment compared to others. However some of them have preserved beta cell function and insulin independence as long as 5 years from diagnosis
* Many patients with mitochondrial diabetes develop insulin requirement compared to the type 2 diabetic subjects and have an atypical nephropathy

A considerable proportion of patients with young adult onset diabetes already have diabetes related complications at a relatively young age

* Retinopathy – 18.8%
* Neuropathy 3.2% to 30%
* Nephropathy – 30%
* Sexual dysfunction – 27%

Metabolic control of the young onset diabetic subjects is not satisfactory.

* About 30% has not acheievd recommended levels of glycaemic, lipid and blood pressure control.
* Metabolic control was worse in those with type 1 diabetes

With increasing number of younger adults developing diabetes this can lead to a serious public health problem with the potential to overwhelm the healthcare system

Clinical implications and recommendations

* Since type 2 diabetes account for 90% of diabetes burden in young adults aggressive lifestyle modification programmes need to be introduced to prevent the epidemic of diabetes in Sri Lanka
* Necessary measures need to be taken to optimize the metabolic control and control of cardiovascular risk factors in young adult diabetic subjects who will otherwise develop microvascular and macrovascular complications.
* Insulin treatment in patients with type 1 diabetes needs attention since they have the worse glycaemic control.
* Physicians need to be aware of the presence of subtypes of diabetes especially among young adults with phenotypic characteristics different to typical type 2 diabetic subjects. These patients need to be identified early for optimization of their overall diabetes care.

Future

* Facilities need to be established in Sri Lanka for the diagnosis of different sub types of diabetes – GAD antibody assay, genetic testing
* Simple clinical protocols, based on clinical criteria need to be developed to identify patients for further diagnostic evaluation including genetic testing to minimize costs.

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