CHARACTERIZATION OF LATENT AUTOIMMUNE DIABETES IN ADULTS IN A REGION OF INDIA

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CHARACTERIZATION OF LATENT AUTOIMMUNE DIABETES IN ADULTS IN A REGION OF INDIA

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Abbreviations:

AAs	Auto antibodies
AC	Abdominal circumference
ADA	American Diabetes Association
APC	Antigen presenting cell
BMI	Body mass index
CAD	Coronary artery disease
CVD	Cerebro vascular disease
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DASP	Diabetes antibody standardization programme
DM	Diabetes Mellitus
DM1	Type 1 Diabetes Mellitus
DM2	Type 2 Diabetes Mellitus
DM1-A	Type 1 Diabetes Mellitus (Autoimmune)
DM-1B	Type 1 Diabetes Mellitus (Idiopathic)
DRAs	Diabetes related antibodies
FABP5	Fatty acid-binding protein 5
GDM	Gestational Diabetes Mellitus
GAD	Glutamic acid decarboxylase
GADA	Glutamic acid decarboxylase (GAD-65) autoantibodies
GWAS	Genome-wide Association Studies
GCT	Trinucleotide repeat polymorphism
HbA1c	Glycated Haemoglobin A1C
HDL	High density lipoproteins
HLA	Human leukocyte antigen
HSPA1A	Heat shock protein A1A
IA	Insulin Antibody
IAA	Insulin Autoantibody
IDS	Immunology of Diabetes Society
IA-2A	Protein tyrosine phosphatase-like protein
IBW	Ideal body weight
ICA	Islet cell auto antibodies
ICAM	Intercellular adhesion molecule (ICAM)
IDDM	Insulin dependent Diabetes Mellitus
IGT	Impaired glucose tolerance
IgG	Immunoglobulin G
IDF	International Diabetes Federation
INS	Insulin
IL	Interleukin
IL2RA	Interleukin 2 receptor alpha

IR	Insulin resistance
LADA	Latent autoimmune diabetes in adults
LADC	Latent autoimmune diabetes in children
LADY	Latent autoimmune diabetes in young
MAPK	Mitogen activated protein kinase
MHC	Major Histocompatibility complex
MICA	MHC class 1 related A (MICA)
MS	Metabolic syndrome
NDDG	National diabetes data group
NIDDM	Non-insulin dependent Diabetes Mellitus
NODM	Non-obese diabetic mice
NK	Natural Killers
NSGP	National Haemoglobin Standardization Program
OHA	Oral hypoglycaemic agent
OxPTM	Oxidative post-translational modification
PBMC	Peripheral blood mononuclear cell
PDE4B	Putative gene phosphodiesterase 4B
PTPN 22	Protein tyrosine phosphatase non receptor type 22
SEA	South East Asia
SU	Sulfonylureas
TCF7L2	Transcription factor 7-like 2
TCR	T cell receptor
TNF	Tumor necrosis factor
TNFAIP3	Tumor necrosis factor alpha-induced protein 3
TPO	Thyroid peroxidase
UAE	United Arab Emirates
UKPDS	United Kingdom Prospective Diabetes Study
VCAM	Vascular cell adhesion molecule
VNTR	Variable number of tandem repeat polymorphisms
WHO	World Health Organisation
WHR	Waist Hip Ratio
ZnT8	Zinc T8 transporter antibody

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ABSTRACT

Introducción: Una proporción de pacientes con diabetes de la edad adulta, que inicialmente no requieren insulina, presentan autoanticuerpos contra la decarboxilasa del ácido glutámico (GADA) en el suero y diferencias fenotípicas con la diabetes clásica tipo 2 (DM2). Se ha propuesto la designación de diabetes autoinmune latente del adulto (LADA) para esta categoría.

Objetivos de la investigación: La diabetes autoinmune de la edad adulta es heterogénea. Este hecho ha sido demostrado en Europa y ciertos países no europeos. Los estudios epidemiológicos han encontrado variaciones en la prevalencia de LADA. En el sur de Asia, los datos sobre LADA son escasos. Se ha observado una discrepancia muy marcada en la frecuencia de LADA en la población de la India, entre 2.6% y 58%, que no puede atribuirse a diferencias étnicas o ambientales; más bien a metodologías conflictivas/distintas. El diagnóstico precoz de LADA es importante por sus implicaciones terapéuticas. Con el fin de caracterizar adecuadamente LADA y evaluar su prevalencia real se requiere un criterio de diagnóstico fiable con procedimientos validados.

Métodos: Se realizó una investigación transversal en la región norte de la India en 139 sujetos. Los criterios de reclutamiento incluyeron: a) diagnóstico de diabetes; b) edad al diagnóstico, 30-70 años; c) duración conocida de la enfermedad entre 6 meses y 5 años. Se determinaron en muestras de suero/plasma, tras un ayuno de más de 10 horas, las concentraciones de glucosa, hemoglobina glicosilada, perfil lipídico, creatinina, péptido C y GADA. Los pacientes con positividad a GADA insulinizados desde el diagnóstico, o antes de un mes desde el diagnóstico, se definieron como DM1. Los individuos con resultado negativo en la determinación de

GADA fueron diagnosticados como DM2. El grupo de sujetos con diabetes y positividad a GADA que no requirieron insulina durante al menos 6 meses tras diagnóstico fueron definidos como LADA.

Resultados y Conclusiones:

1- LADA representó el 6.5% de los casos entre las personas adultas con diagnóstico de diabetes, frecuencia considerablemente superior a la informada en estudios previos para esta población. En sujetos diabéticos diagnosticados a los 31-40 años de edad, la frecuencia de LADA fue del 13,9%. El estudio sugirió una tendencia decreciente de LADA con el aumento de edad.

2- LADA fue el subtipo prevalente de diabetes autoinmune de inicio en la edad adulta, dato que convendría contrastar con observaciones previas publicadas de una menor prevalencia de DM-1A entre niños y adolescentes en el norte de la India. En esta población investigada, la prevalencia de LADA fue considerablemente inferior a la informada en el sur de la India.

3- El grupo de sujetos con diagnóstico de LADA es más joven y presenta niveles inferiores de circunferencia abdominal, péptido C sérico y triglicéridos en ayunas, que el grupo de sujetos con DM2 de la misma zona del norte de la India.

4- Los pacientes con LADA con títulos más elevados de GADA en el momento del diagnóstico eran preferentemente varones, más delgados, y necesitaban tratamiento insulínico, presentando menor riesgo de hipertensión sistólica y síndrome metabólico.

5- Los pacientes con LADA con títulos bajos de GADA eran preferentemente mujeres, y no mostraron diferencias fenotípicas con las pacientes con DM-2, en

concordancia con datos publicados en la población asiática china, y en contradicción con los publicados para la población europea.

6- En el presente estudio, los niveles séricos de péptido C en ayunas al diagnóstico fueron inferiores en los pacientes LADA que en los pacientes con DM2. Esta diferencia se mantuvo durante 36 meses, contrariamente a los datos del Estudio LADA en España.

ANTECEDENTS

1. DEFINITION OF DIABETES AND GENERAL BACKGROUND

Diabetes Mellitus (DM) is a chronic disorder caused by relative or absolute insulin deficiency and characterized by chronic hyperglycaemia. It is also associated with insulin resistance in skeletal muscles, adipose tissues and liver. Diabetes can lead to microvascular complications like nephropathy, retinopathy, neuropathy and macrovascular complications like cerebrovascular disease (CVD) and coronary artery disease (CAD). Clinical manifestation of diabetes occurs when anti islet autoimmunity in Type 1 Diabetes (DM1) or non-autoimmune β-cell dysfunction in Type 2 Diabetes (DM2) decrease insulin secretory capacity below a threshold determined by insulin resistance.(1)

An estimated 415 million people worldwide have diabetes and by 2040, this number is expected to rise to 642 million.(2) Data from India and China demonstrated the rise in prevalence of diabetes from 3% to 9.4 % and 1% to 7.8 % respectively from 1970s to 21st century.(3) In Asians, decline in traditional dietary practices, increase in dietary fat, sugar, high glycemic index foods and lack of physical activity have contributed to the rise in diabetes. India is situated in South East Asia (SEA), the most populous region in the world. Prevalence of Diabetes in SEA according to recent World health organization (WHO) global report is shown below (*Table 1)*. About 85% to 95% of total diabetic population have type 2 diabetes which is characterized by insulin resistance with relatively reduced insulin secretion.(4–6) Asian Indians are at higher risk of diabetes at much lower body mass index (BMI) compared to Europeans.(7,8)

Indian diabetic patients are more at risk of developing CAD characterized as dyslipidemia and low levels of high density lipoproteins (HDL) cholesterol due to genetic predisposition at an early age as compared to European population.(9)

Country	Prevalence (%)
Afghanistan	8.4
India	7.8
Sri Lanka	7.9
Bangladesh	8.0
Bhutan	9.2
Nepal	9.1
Maldives	8.5
Pakistan	9.8

Table 1: Prevalence of Diabetes in South East Asian (SEA) region in 2016 (3)

A significant proportion of diabetic patients with adult-onset, initially non-requiring insulin treatment, depict diabetes-associated autoantibodies in their sera. A new subclass of diabetes with the nomination of latent autoimmune diabetes of adult-onset (LADA) has been proposed for this category of subjects.(10,11) Preliminary studies have demonstrated that patients with autoimmune diabetes, characterized by the presence of glutamic decarboxylase autoantibodies (GADA) display a different clinical phenotype from classical type 2 diabetes without GADAs.(12–14)

Diagnosing LADA at an initial stage is important to facilitate improved glycemic control as well as the preservation of residual beta cell function. Ethnic variation and correct diagnosis of LADA may have relevant therapeutic implications.(15) Due to differences in dietary habits, environmental factors and phenotypic characteristics between European and Asian populations there may be heterogeneity in the prevalence and other characteristics of LADA in these two populations. LADA is discussed in greater detail below.

2. CLASSIFICATION OF DIABETES MELLITUS – PAST AND PRESENT

National diabetes data group (NDDG) in 1979 published the first categorization of DM.(16) Diabetes was recognized as a heterogeneous disease clinically and etiologically and classified as “insulin dependent diabetes mellitus” (IDDM) and “non-insulin dependent diabetes mellitus” (NIDDM). Later, in 1997 American Diabetes Association (ADA) recommended to eliminate IDDM and NIDDM terms and suggested DM1 and DM2. Impaired Glucose Tolerance (IGT) and Gestational Diabetes Mellitus (GDM) were additional terms included in this classification. DM1 was defined as a disease due to the destruction of pancreatic islet β-cells, prone to ketoacidosis and mostly occurring at young age but not restricted to any age of onset. Depending upon the aetiology, DM1 is subdivided into DM1-A (autoimmune) and DM-1B (idiopathic).(17–20). Various other specific types of diabetes due to genetic defects in β-cell, insulin action and other causes have been classified.(21) These specific subtypes will not be discussed further in the text.

3. MISSING POINTS IN PRESENT CLASSIFICATION OF DIABETES IN ADULTS

Diabetes is more heterogeneous than assumed. In adult-onset autoimmune diabetes, usually the presence of residual β-cell function makes the clinical presentation similar to DM2. Additionally, many patients with DM2 remain undiagnosed for years and may clinically debut with severe hyperglycemia requiring immediate insulin therapy. The present ADA classification does not include many patients with hybrid form of diabetes having genetic predisposition to both DM1 and DM2 with pancreatic autoantibodies. This form of diabetes is commonly called LADA or type 1.5. LADA patients do not require insulin therapy at least within first six

months after diagnosis but progress to insulin early as compared to antibody negative patients.(22) Such uncertainties mandate a revised and improved classification of diabetes to include new emerging types of diabetes.

4. DIAGNOSTIC CRITERIA OF DIABETES MELLITUS

WHO criterion for diagnosis of diabetes requires fasting plasma glucose ≥ 7.0 mmol/l or 2 hours plasma glucose ≥ 11.1 mmol/l after a standardized oral glucose tolerance test (OGTT).(7) Haemoglobin A1C (HbA1c) > 6.5% by standardised assays can be used as a diagnostic test for diabetes.(26)

5. TYPES OF DIABETES MELLITUS

Type 1 Diabetes

Though not in the same proportion as DM2, DM1 is also having an increasing trend with 3-5% increase per year. India is also witnessing a constant rise in the incidence of DM1 as evident in some European countries like Finland, Sweden and Germany. In the last 50 years in Finland the incidence of DM1 has increased from 10/100,000 to around 60/100,000 children. In Asia, the frequency of DM1 is low.(23) Worldwide the lowest incidence has been reported from China (0.1/100,000 per year).(23) The epidemiological data on type 1 diabetes in India is sparse. Studies from southern Indian states have shown the prevalence of type 1 diabetes to be 3.2 cases / 100,000 children in Chennai (24) A study from northern state of India reported the incidence of DM1 to be 10.2 cases / 100,000 per year.(25) Other studies from southern India have suggested the frequency of DM1 to be around 2-5% of

diabetes.(26,27) Although, DM1 could be idiopathic, classified as type 1B diabetes,(17) mostly, DM1 is caused by autoimmune destruction of insulin producing islet β-cells of pancreas, leading to absolute insulin deficiency known as classic Type 1 Diabetes (DM1-A). The autoimmune process is mediated by T-cells, as shown below in the recognized model of development of DM1. (Fig. 1) Auto reactive T cells, both CD4 and CD8 cells play active role in beta cell destruction. Various autoantigens like GAD, the protein tyrosine phosphatase-like protein IA-2 and most recently zinc transporter SI 30A8 (ZnT8) have been identified in the insulin secretory granule of beta cells.(28) Autoimmune process is triggered by interaction between susceptibility genes and environmental predisposing factors.

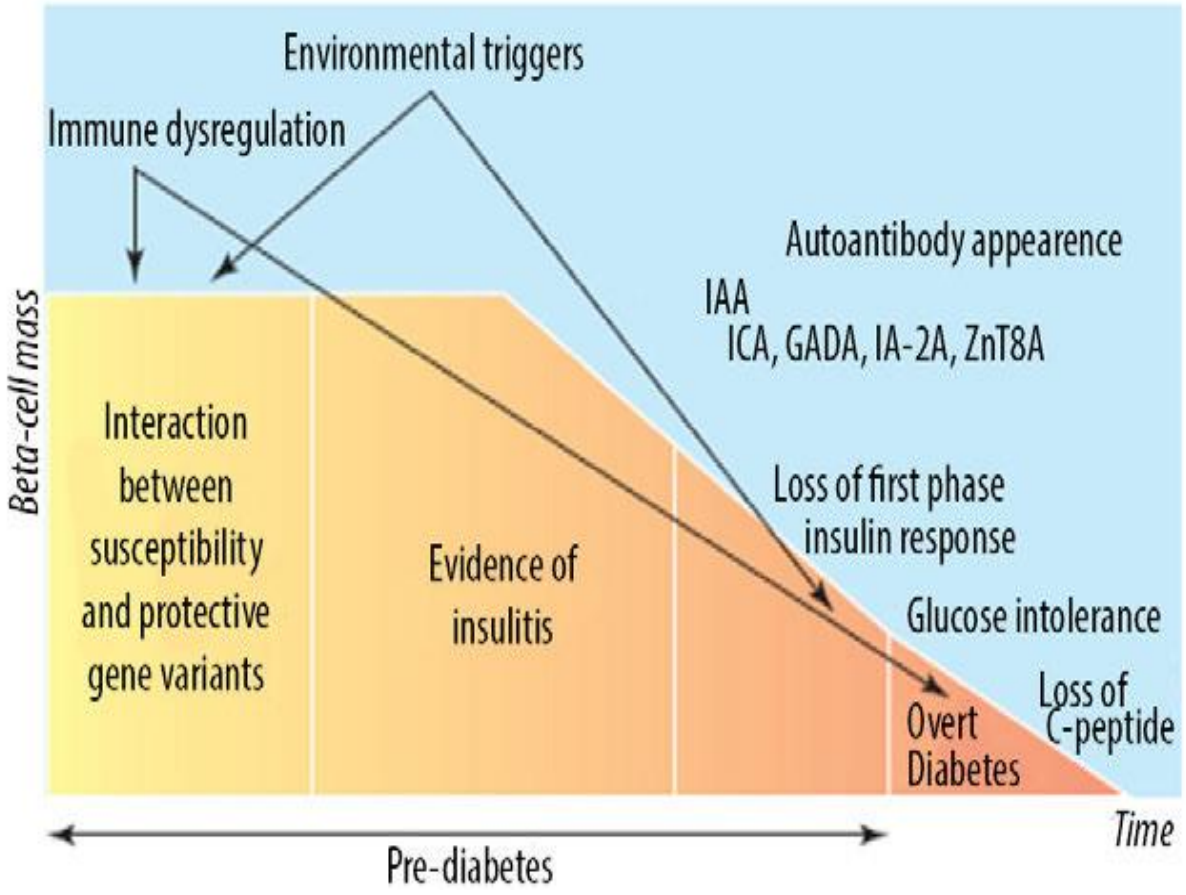

Figure 1: Model of the pathogenesis of DM1. Reproduced from [Type 1 diabetes: recent developments, Devendra et.al, Vol.328, Page.752, 2004(29)] with permission from BMJ Publishing Group Ltd

6. MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) / HLA REGION

Terms like Human leukocyte antigen (HLA) and major histocompatibility complex (MHC) are synonymous. MHC region on chromosome 6p21.31 is major susceptibility locus for DM1. MHC consists of 3 sub regions namely class 1, class II and class III with over 200 genes.(30) The class I region lies telemetric and are antigens expressed on the surface of almost all nucleated cells from the organism. (54) Class II region that contains HLA-DP, DQ and DR loci is most centrometric. (Fig. 2) These loci are found as pairs encoding α and β chains. These chain encode the heterodimeric class-II protein molecules expressed at cell surface of antigen presenting cells (APC) like macrophages and dendritic cells. Antigenic molecules presented by the class I HLA molecules are recognised by cytotoxic T lymphocytes (CD8+) while helper T lymphocytes (CD4+) recognize antigens presented by the class II HLA molecules.(54)

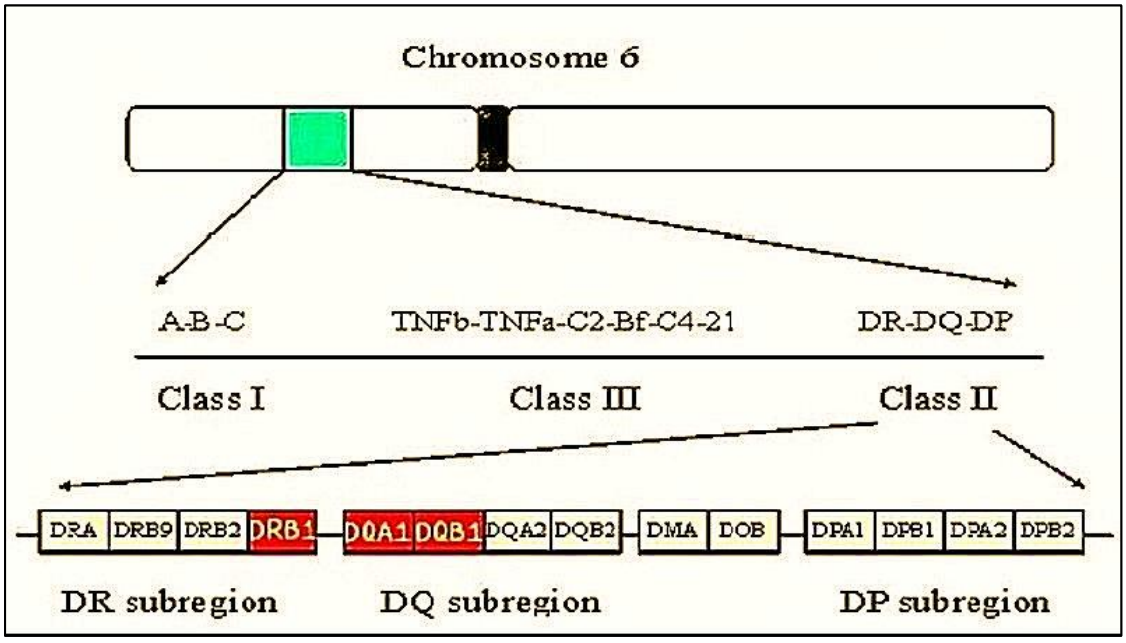

Figure 2: The HLA region on chromosome 6 showing DM1 associated haplotypes DRB1*03-DQB1*02 and DRB1*04-DQB1*0302(31) Reproduced with permission from Gillespie M.K Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy; The Genetics of Type 1 Diabetes, Chapter 24; Publisher: InTech, 2011(32)

7. AUTOIMMUNE PROCESS

7.1. Antigen presentation

Classical type-1A diabetes is caused by an autoimmune destruction of pancreatic beta cells that leads to progressive insulin loss.(29,33–35) DM1 is characterized by prodromal stage of islet autoimmunity. It is evident that children who develop islet auto antibodies against antigens Insulin, GAD-65, IA-2 or ZnT8 before 3-5 years of age have shorter prodrome prior to clinical onset of the disease as compared to older children or adults.(36) Multiple islet antibodies may be present for many years before clinical onset of DM1 *(Table 2)*.(37)

Autoantigen	Autoantibody
Insulin	IAA
Glutamic acid decarboxylase, 65kD	GAD65A
Insulinoma antigen-2	IA-2A
ZnT8 transporter	ZnT8A

Table 2. Islet autoantigens in type 1 diabetes.

LADA is characterized by presence of GAD65 not insulin autoantibodies.(29,33,35) In an individual until 80-90% of beta cells are lost, the clinical onset of the disease does not occur in spite of presence of islet autoantibodies for years.(38) The aggressive autoimmune process destroys pancreatic beta cells. DM1 is known as T-cell mediated disease as it is associated with T-lymphocyte autoimmunity.(39–41)

Autoimmune process in DM1 may get triggered in some cases when virus infected beta cells leads to lysis of these cells. After lysis, local dendritic cells, the most effective APCs engulf virus and beta cell debris. APCs processing the cell debris get

activated and move to the draining lymph nodes of pancreas through lymphatics. The β-cell autoantigen presentation takes place in the lymph nodes rather than in pancreatic islets. APC present autoantigen to the T cell receptor (TCR) of CD4+ T-helper cells. Activation of CD4+ T-helper cells with TCRs induce an immunological reaction with involvement of both CD8+ cytotoxic T cells and also antibody producing B cells. (65) (Fig. 3)

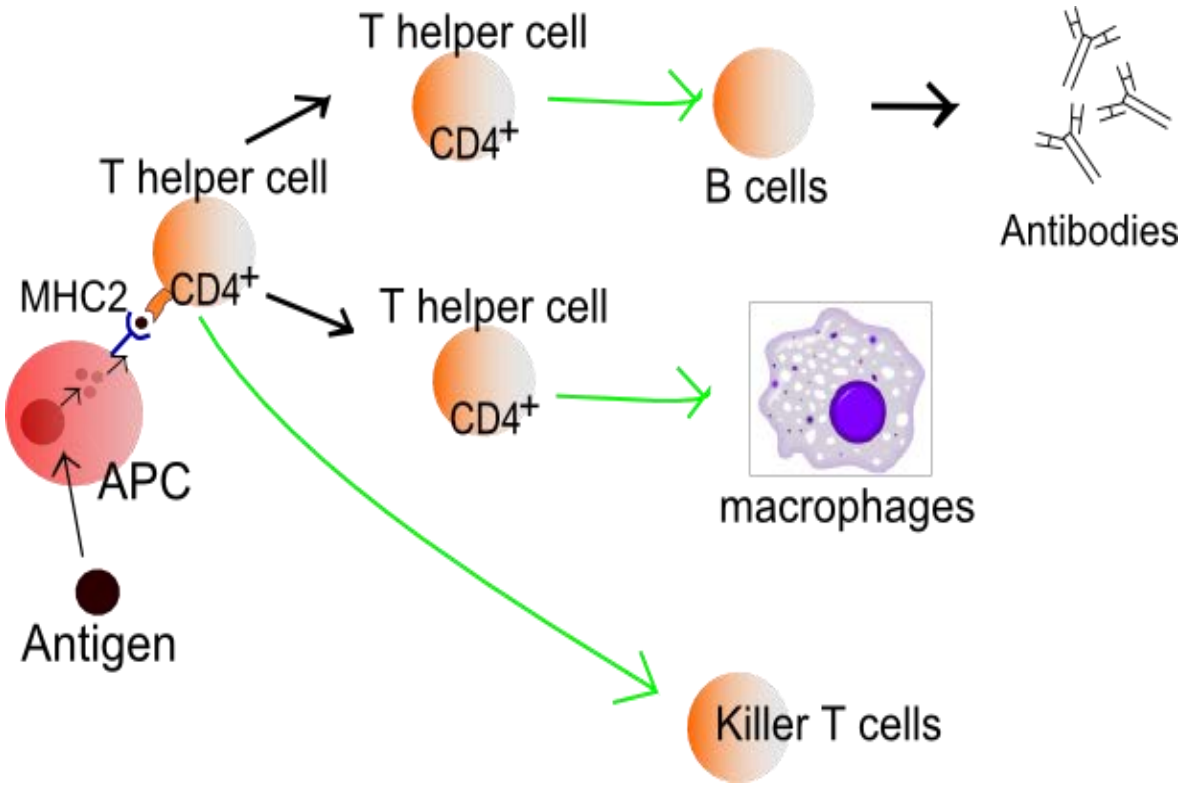

Figure 3: Cartoon showing process of Antigen Presentation (64)

Antigen presentation process consists of binding of single T cell receptor to a complex on the surface of APC consisting of MHC molecules and peptide fragment derived from the foreign antigen.

8. GENETIC SUSCEPTIBITY OF DM1-A

MHC region class II gene Human Leukocyte Antigen (HLA) complex, on short arm of chromosome 6p21.31, has been recognised as a major contributor followed by insulin Variable Number of Tandem Repeat polymorphisms (INS-VNTR) at 5' flanking region of the insulin gene on chromosome 11p15.5 (IDDM-2).(42)

About 18 regions of the genome has been linked to DM1. These regions have been labelled as IDDM1 – IDDM18. IDDM-1 that contains the HLA genes has been well studied. Association of other non HLA gene IDDM-2 and IDDM-3 that map close to cytotoxic T- Lymphocyte Associated Protein-4 (CTLA4), having regulatory role in immune response on chromosome 15q26 has been identified.(43)

Protein tyrosine phosphatase non receptor type22 (PTPN 22) and the region surrounding interleukin 2 receptor alpha (IL2RA/CD25) and interferon induced helicase 1 genes also show an association with DM1. Environmental factors have been associated with DM1 and include dietary factors in early infancy, vaccination, climate changes, toxins and stress.(44) Approximately 90% patients with DM1 carry either HLA-DR3, DQB1*0201 (also referred as DR3-DQ2) or HLA-DR4, DQB1*0302 (also referred as DR4-DQ8). Around 30% of these patients have both haplotypes (DR3/4 heterozygous) conferring highest susceptibility. HLA haplotypes conferring significant risk across various populations are shown below *(Table 3)*

By the presence of islet cell antibody (ICA) in sera of subjects with DM1-A in 1974, it became evident that β-cell destruction and dysfunction in DM1 is autoimmune in nature leading to insulin deficiency and generation of circulating antibodies to islet cell cytoplasm (ICA), and or to Glutamic decarboxylase (GAD-65) and or IA-2A. Usually, subjects with DM1-A depict multiple antibodies in their sera.(95)

Populations	HLA-Haplotypes
Swedish (45,46)	A1*03:01-B1*03:02; A1*05:01-B1*02:01
French (47)	DRB1*04:05-DQ A1*03-B1*02
Spanish (48)	DRB1*04:05-DQ A1*03-B1*02; DQA1*0101-DQB1*0501-TNFa2b1 DQA1*0201-DQB1*0202- BAT-2*2
Japanese(49–51)	DRB1*04:05-DQA1*03:01/02-DQB1*04:01; DRB1*08:02-DQA1*03:01DQB1*03:02 DRB1*04:05-DQA1*03:01-DQB1*03:02 DRB1*03:01DQA1*05:01-DQB1*02:01; DRB1*11:01-DQB1*03:01; DRB1*11:01-DQB1*03:02; DRB1*13:02-DQB1*06:04
Koreans (44,49)	DRB1*03:01-DQB1*02:01; DRB1*04:01-DQB1*03:02; DRB1*03:01DQB1*05:01; DRB1*04:01-DQB1*03:02; DRB1*04-DQA1*03:01- DQB1*03:02; DRB1*04:05-DQB1*03:02; DRB1*04:07-DQB1*03:02; DRB1*04:05-DQB1*04:01; DRB1*09:01 DQB1*03:03
Chinese (52,53)	DRB1*09:01-DQA1*03;01/02-B1*03:03; D Q A 1*03-DQB1*03:03; DQA1*03-DQB1*04:01; DQA1*05-DQB1*02:01
Indians (52,54–58)	DRB1*03:01-DQA1*05:01 B1*02:01; DRB1*04:01/02/04/05-DQA1*03:01/02-B1*02:01
Africans (Sub-Saharan) (59)	DRB1*03:01-DQA*05:01; DRB1*04-DQA*03; DRB1*04-DQB*03:02; DQA*05:01-DQB*02:01; DQA*03-DQB*03:02

Table 3 HLA haplotypes conferring significant risk across various populations (60)

9. TYPE 2 DIABETES

DM2 is prevalent in about 90% of all patients with diabetes. It is characterized by combination of insulin resistance and relative insulin deficiency. Not always but it is usually associated with obesity. Genetic and environmental factors like dietary habits and less physical activity play essential roles in etiopathogenesis of DM2.(61) Like in other populations, in India variants of transcription factor 7 like 2 (TCF7L2) gene are strongly associated with increased risk of DM2.(62,63)

10. GENETIC DIVERSITY RELATED TO TYPE 1 DIABETES AMONG ASIANS WITH SPECIAL EMPHASIS ON INDIAN POPULATION

In Asians commonly protective DR4 allele is associated with the susceptible DQ alleles while the neutral / protective DQ allele is associated with susceptible DR4 allele. Important factor responsible for low incidence of DM1 in Asians could be linked with counterbalancing influence between susceptible DRB1 and protective DQB1 and vice versa. In addition to DQB1*0302, DQB1*0401 on DR haplotype is positively associated with DM-1 in Asians.(50)

North Indian population differ from western population in frequencies of their HLA antigen.(64) North Indians are a subgroup of Indo-Europeans who invaded Europe, the Middle east, Iran and India around the second millennium B.C. North Indians belong to Indo Aryan race that is considered a subgroup of Caucasoid.(65) The variation in the frequency of HLA antigens in north Indians could be probably due to intermixing of genes of Indo-Aryans with original population of the region that was further invaded by various races like Mongols and Turks.(64,66) North Indians exhibit unique and strong association of HLA-BW21 with IDDM.(67)

Compared to western population HLA-DR3 showed a much greater and significant association with IDDM (RR 10.1) in North Indians.(68) No association of IDDM with HLA-DR4 (the second DR locus antigen) that confers susceptibility to IDDM was found in North Indian population.(54,68,69)

11. AUTOIMMUNE DIABETES IN ADULTHOOD

1. Introduction

Adult onset autoimmune diabetes consists of several subgroups. In the recent decades, it has been demonstrated that approximately 30% cases of classical DM1-A are diagnosed after 30 years of age (Late-Onset type 1 diabetes).(70,71) It has been recognized that around 10 % of adult subjects initially classified as DM2 depict autoantibodies to pancreatic autoantigens in their sera.(72,73) Eponym LADA (Latent Autoimmune Diabetes in Adults) was suggested to specify this new category of diabetic population.(10,11) These patients initially could be misdiagnosed as having DM2.(116) This subset of phenotypic DM2 subjects positive for islet autoantibodies tend to have sulfonylurea failure and need insulin treatment earlier in the disease process.(117) The distinction between both conditions is challenging. We have already reviewed etiopathogenesis and immunogenetic aspects of DM1-A. Here, we will review clinical, immunological and genetic complexities related to LADA. Recently, with the evidence of antibody-negative phenotypic DM2 patients' subgroup showing T-cell response, following classification of Adult-onset Diabetes has been suggested. (Table 4)

	Adult onset autoimmune diabetes			DM2
Diabetes Subtypes	**DM1**	**LADA**	**Autoimmune antibody Negative**	
Autoantibodies	Present	Present	Absent	Absent
Islet reactive T-cells	Present	Present	Present	Absent
Insulin requirement at diagnosis	Present	Absent	Absent	Variable

Table 4: Suggested classification of Adult onset Diabetes (74)

Mostly, DM1-A has been considered to be a childhood disease. However, increasingly autoimmune diabetes is being observed in adults. Parameters used in clinical practice to distinguish DM1 from DM2 are phenotypic characteristics like age, obesity and presence of other autoimmune disorders. However, this clinical distinction is not straight forward and not always correct.(75) Autoimmune diabetes in children and adults are differentiated by only relatively few age dependent genetic defects. Howson et al observed that the genetic load was inversely related to age at diagnosis. There was no convincing evidence of age at diagnosis effect except HLA.(76)

In adulthood, the distinction between classic DM1-A and LADA is a challenging issue. It is imperative to correctly classify these patients as misclassification may have therapeutic implications. Genetic immunological and functional complexities make it difficult to distinguish classic DM1-A, LADA and DM2 clinically.(77–79)

However, characteristically, patients with LADA progress slowly towards insulin requirement (within 5- 6 years) and older patients with LADA show even a slower progression.(11,80) Hawa et al defined classic DM1-A as subjects with diabetes and associated autoantibodies in whom insulin was started immediately at diagnosis or within 1 month of diagnosis.(81)

2. Epidemiology

Around 3-14 % of patients clinically diagnosed as DM2 depict islet autoantibodies in their sera. High frequency of 7-14 % GADA-positive DM2 has been reported in northern Europe.(80,82–84) Lower frequency of 4-6 % GADA positivity has been observed in studies from southern Europe, Northern America and Asia.(85–89) This varied frequency of GADA could be attributed to biases like selection criteria, age at onset of diabetes, disease duration and assays used.(74) As compared to DM1-A, LADA was far more frequent (OR 3.3) in a multi centred European Action LADA study.(81) In Europe, the prevalence of adult-onset autoimmune diabetes including LADA is more than childhood DM1. Similar observations have been documented in China where interestingly, childhood onset DM1 is rare.(87) Prevalence of LADA among different populations is shown below *(Table 5)*

In India, data assessing the prevalence of LADA are sparse. Some studies revealed controversial results and reported considerably high prevalence of LADA.(90–93) These studies included specific subgroup of subjects who were young, non-obese and had early onset of diabetes with higher probability of LADA. Such results may not represent the true prevalence of LADA in Indian population. Additionally, these studies do not specify the sensitivity and specificity as validated

Author/ Study Name	n	DM Duration	Prevalence of each antibody alone and in combination (%)				Diagnostic criteria of LADA		Prevalence LADA (%)
			ICA	**GAD**	**IA-2**	**GAD + ICA/IA2/ZnT8**	**Age at diagnosis (Years)**	**Insulin independence (months)**	
Zhou et al (87) LADA China	4880	< 1 y		5.9			> 30	> 6	5.9
Turner et al.(80) UKPDS	3672	< 1 y	5.8	9.8		GAD+ICA=4	25-65	≥ 3	12
Tuomi et al (83) Finland	1122	Any	0.5	9.3	0.4	GAD+IA=1.4 GAD+ICA=1.3	>35	≥ 6	10.1
Castleden et al (94) UK	2059						> 25	> 12	7
Fourlanos et al (95) Australia	130	< 2 m		7.7			30-70		7.7
Zinman et al (96) USA, Europe, Canada,	4134	< 3 y		4.2			30–75		4.2
Buzzetti et al (85) Italy	420	6 m - 5 y		4.4	0.9	GAD+IA2= 0.8	> 20		4.5
Radtke et al (82) Norway	1261			10				<12; >12 if c-peptide>150 mmol/l	10
Maioli et al (86) Sardinia	5568	< 5 y		4.9		GAD+IA2=0.9	35-70	8	4.9
Hawa et al (81) Europe ACTION LADA	6,156	< 5Y		8.8	0.5	GAD+IA2=0.7 GAD+Znt8=0.5	30-70	6	9.7
Kotulanda et al.(97) Srilanka	992			5.4			> 30	> 6	2.6
Takeda et al (88)Japan	4098	Any		3.8			> 20		3.8
Park et al (98) Korea	884	< 5Y		4.4		GAD+IA2=0.3 GAD+Znt8=0.3	35		4.4
Maddaloni et al (99) UAE	17,072	Any		1.7	0.8	GAD+IA2=0.1	30-70	**>** 6	2.6

Table 5: Prevalence of LADA among different populations

by Diabetes Antibody Standardization Program (DASP) of the methods used to measure GAD/IA-2 antibodies. Sensitivity and specificity of the methods used may not be ideal. It is assumed that by applying the diagnostic criteria suggested by Immunology of Diabetes Society (IDS) and European Action LADA group, the prevalence of LADA will be much lower Britten et al reported 2.6% of the south Asian population of North Indian Punjabi ancestry in Birmingham, UK with DM2 to be positive for pancreatic autoantibodies.(100) Another study from south India reported 17% GADA positivity in DM2 individuals.(101) Considerably low prevalence of GADA and/or IA-2 (3.2%) was reported in a considerably large study.(102) Interestingly, two studies that reported the use of DASP validated method with high specificity to measure GAD/IA-2 showed a much lower prevalence of LADA.(100,102) Prevalence of LADA among Indian population is shown below *(Table 6)*

Author	n	LADA (%)	Age at Diagnosis (Years)	Duration of DM (Years)	Other specific inclusion criteria
Sachan et al (102)	618	3.2	30-70	Any	
Kanungo et al (90)	214	42	>20	Any	
Britten et al (100)	500	2.6	Any	Any	
Shrivastava et al (91)	300	44.67	>20	Not specified	Age 25-40 yrs., BMI < 25 kg/m^2 SU failure
Chandni et al (92)	31	58	>30	<3	BMI<23 kg/m^2
Brahamkshatriya et al(103)	80	5			
Anil Kumar et al (101)	100	17	25- 65		
Unikrishnan et al (93)	83	25.3	30-70	< 2	BM1< 18.5 kg/m^2
Mohan et al (104)	118	5.9		Any	

Table 6: Prevalence of LADA in Indian population

3. DEFINITION AND DIAGNOSIS OF LADA

Initially, non-insulin requiring subgroup of patients with adult-onset diabetes and associated auto antibodies like GAD, IA-2, insulin, or ZnT8 are defined as having LADA. These subgroups of patients are at high risk of progression to insulin dependency. Clinically such patients are initially diagnosed with DM2.(22,105) The European Action LADA group and the IDS have proposed the following specific criteria for diagnosis of LADA:

1- At diagnosis patient should be at least 30 -70 years of age

2- Presence of at least one of the four islet cell autoantibodies i.e. ICA, autoantibodies to GAD65, IA-2 and insulin) in serum

3- At least, 6 months of non-insulin requiring diabetes (72,106)

Presence of circulating islet autoantibodies distinguishes LADA from DM2 and insulin independence at diagnosis distinguishes LADA from classic DM1-A.(106) However, the criteria of treatment with insulin within the first 6 months meant to distinguish LADA and classic DM1-A diagnosed after 30 years of age is subjective.(106) Initiation of insulin treatment is dependent on the judgment of the treating physician and should not be used to define patients with LADA.(107)

It has been argued that to define LADA, criteria of age > 30 years is arbitrary as there is group of obese children who are non-ketosis prone, initially non-insulin requiring but depict beta cell autoantibodies in their sera.(108,109) Increasingly, significant overlap between DM1 and DM2 has been noticed. Such observation has challenged the present classification broadly dividing adult diabetes into two major

types i.e. DM1 and DM2. Various eponyms have been suggested for autoimmune diabetes in adults *(Table 7)*

Eponym	References
Latent type 1 diabetes	(110)
Latent autoimmune diabetes in (LADA)	(10)
Slowly progressive IDDM (SPIDDM)	(111)
Slow-onset IDDM	(112)
Slowly progressive type 1 diabetes	(113)
Type 1 ½ diabetes	(114)
Latent autoimmune diabetes in youth (LADY)	(115)
Autoimmune diabetes not requiring insulin at diagnosis	(116)
LADA-type1 and type 2	(117)
Slowly progressive adult onset type 1 diabetes	(118)
Antibody-positive phenotypic type 2 diabetes with obesity	(119)
Latent autoimmune diabetes in children (LADC)	(120)

Table 7: Various eponyms suggested for autoimmune diabetes in adults.(121) “Copyright ©2005 American Diabetes Association from Diabetes, Vol. 54, 2005; S68-S72 Reprinted with permission from The American Diabetes Association

4.CLINICAL AND METABOLIC CHARACTERISTICS OF LADA

4.1 General features

Generally, LADA patients are older than 30 years of age, non-obese and non-insulin requiring at diagnosis. However, presence of obesity does not exclude LADA. These patients are initially non-insulin requiring but compared to DM2 subjects progress rapidly towards insulin dependency within a short period ranging from few months to years. As compared to patient with DM2, LADA patients have lower BMI, Waist/ Hip ratio,

lower total cholesterol, higher HDL and lower prevalence of hypertension.(122,123) In a Spanish study, LADA patients had intermediate phenotype between DM1 and DM2. (124)

4.2 β-Cell function in LADA

Studies assessing insulin secretion have reported an intermediate β-cell function in LADA i.e. between DM1 and DM2.(125,126) As in classic DM1, C-peptide response to glucagon injection in ICA positive DM2 patients was impaired at diagnosis and lower than in ICA negative DM2 patients. ($P<0.01$). Early impairment in β-Cell function is observed in LADA patients although not as severe as in classic DM1 patients. β-Cell function in adult onset diabetic patients with more than one antibody deteriorated faster within 5 years. However, in those with only GADAs, severe deterioration in β- Cell function occurred later in the disease process. (Figure 4)

Considering these findings Fourlanos et al suggested that LADA is not a latent form of autoimmune diabetes and *term autoimmune diabetes in adults* is more appropriate to define this group of patients.(106) In contrast to antibody negative patients with DM2, a faster decline in C-peptide levels is observed in LADA patients.(118,126,127)

In a Spanish LADA study, it was observed that fasting C-peptide concentration in LADA patients was higher than in DM1 subjects but was lower than in DM2 patients ($p < 0.01$). However, this difference in C-peptide was seen only during first 36 months of the disease. Thereafter, overlapping of C-peptide concentrations in LADA patients with that of DM1 and DM2 subjects was noticed. In comparison to patients with DM2, a faster progression to insulin treatment from the diagnosis of diabetes was observed in LADA patients. Hazard ratio for insulin treatment in LADA compared with DM2 was quite elevated (mean value of 8.34; $p<0.001$).(124)

Analysis of Stimulated C-peptide secretion using the mixed-meal tolerance test revealed that patients with LADA have a lower stimulated C-peptide response than the DM2 group and a higher response than the DM1 group.(128) An inverse relation between GADA titers and C-peptide has been reported in LADA patients with rapid progression to insulin therapy.(82,83,124).

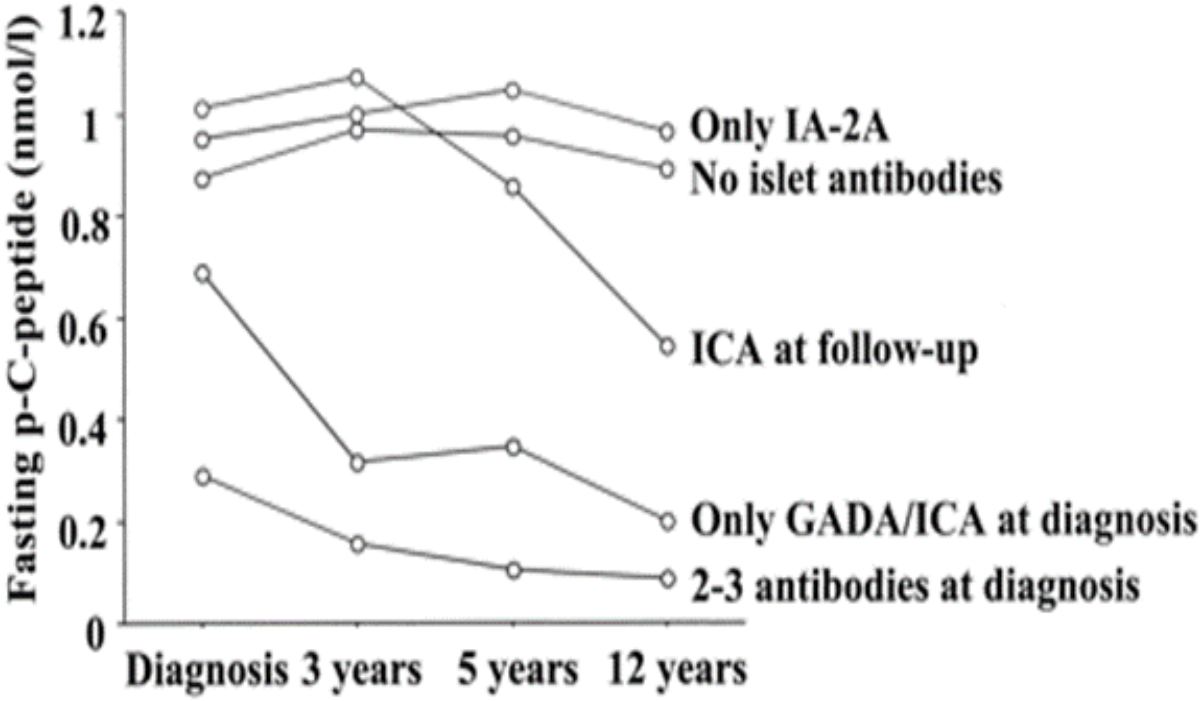

Figure 4: Showing association of β-cell function with duration of diabetes and antibody status.(121) Copyright ©2005 American Diabetes Association from Diabetes, Vol. 54, 2005; S68-S72 Reprinted with permission from The American Diabetes Association"

4.3 Insulin resistance and metabolic syndrome in LADA

It is known that insulin resistance (IR) does not have any significant role in the disease process of autoimmune DM1. However, there are controversies related to the contribution of IR in the pathophysiology of LADA. It has been hypothesized that

insulin resistance may play a unique role in LADA. In contrast to DM1 and DM2, in LADA, anti-islet autoimmunity, non-autoimmune β-cell dysfunction and increased IR are three mechanisms that are supposed to contribute in the disease process.(129) In an ongoing autoimmune destructive process of islet β-cells, diabetes occurs when insulin secretion is not able to meet the high demands rendered by IR that varies from low in DM1 to high in DM2.(129) Among Chinese population, Li et al showed a close association between LADA and MS.(130)

In Action LADA 3 study, significantly, higher prevalence of metabolic syndrome has been reported in patients with DM2 than in patients with LADA or adult DM1. Significant difference was noted in the frequency of MS in DM1 (31.9%) and LADA (41.9%) (P=0.015). Frequency in both groups was less than in DM2 patients (88.8%) (P<0.0001 for each). The same study concluded that metabolic syndrome does not characterize autoimmune diabetes.(131) In a study from Spain the prevalence of MS was higher among LADA patients than in patients with DM1, but lower than in patients with DM2.(124) Similarly, LADA China study exhibited the presence of MS in LADA, although the prevalence was less than in DM2 (62% vs 75.5%).(87) As a result of IR in LADA the risk of MS and cardiovascular complications may necessitate it to be considered as a therapeutic target.(129)

5. IMMUNOLOGY OF LADA

5.1 Humoral autoimmunity

Diabetes Related Autoantibodies (DRAs) are present in most subjects with autoimmune diabetes. There are four most described islet autoantibodies namely islet cell autoantibodies [ICAs]), to native insulin (IAAs), to GAD (GADA) and to

tyrosine phosphatases (insulinoma-associated antigens IA-2A and IA-2 β).(132–136) It was envisaged that these auto antibodies played a direct role in the destruction of islet β cells however, it is still not clear if these antibodies are only markers of an autoimmune process or have direct contribution in β-cell damage.(137)

Islet cell antibodies (ICA): Discovery of ICA in 1974 confirmed an autoimmune link in the etiopathogenesis of DM1.(138) Based on various studies, it has been shown that 80-90% of new onset DM1 depict ICA in their sera.(139,140) As the measurement of ICA is semi quantitative and time consuming, presently it is not routinely measured and used only if confirmation is required.

GADA: There are two isoforms of GAD i.e. GAD65 (65kDa; 585 amino acids) and GAD67 (67kDa; 593 amino acids). One of the major islet autoantigen in DM1 is GAD isoform 65 (GAD65). This is a biosynthesizing enzyme of an inhibitory neurotransmitter γ-amino butyric acid (GABA), that catalyzes the conversion of GABA from glutamate. In diverse cell types like neurons, epithelial cells of the Fallopian tube, and spermatozoa, this enzyme is expressed in β-cells of human islets. GADA are also found in other conditions like Stiff-man Syndrome due to autoimmune damage of GABAnergic neurons of brain(141), Autoimmune Polyendrocrine Syndrome(142) and Batten disease.(143) Therefore, the presence of GADA is not exclusive to DM1. However, unlike Stiff man syndrome in which GADA may be present against both isoforms, in DM1, GADA are targeted towards 65kDa isoform.(144–146) New–onset DM1 patients depict 60-70% GADA, 40% IA-2A and 20% IA-2β autoantibodies in their sera.(147,148) GADA/ IA-2A combination in adults and GADA/IAA in children are common.(149) GADA can persist in sera up to 12 years after diagnosis.(74) Non-isotopic immunoassays can be used for the

measurement of GAD65 and IA-2A autoantibodies to estimate the risk for predication and diagnosis of autoimmune diabetes.(150)

IA-2: IA2 antibody has two isoform IA-2A and IA-2β. IA-2A is a 979 amino acid protein weighing 106 kDa also known as its partial sequence ICA 512. It corresponds to 40k fragments immunoprecipitate and is considered as an atypical member of the transmembrane protein tyrosine phosphatases family due to lack of enzyme activity.(133,151) IA-2β is a precursor of 37K antigen. Compared to IA-2β, antibodies to IA-2A are more prevalent (55-75% patient with DM1 at diagnosis). In comparison with adult onset autoimmune diabetes, IA-2A is more frequently seen in childhood DM1.(152–156)

IAAs: IAAs are β-cell specific targets of autoimmunity in DM1. Presence of IAAs before initiation of insulin was reported in 1982.(157) IAAs are prevalent in 20-50% of newly diagnosed subjects with DM1. Similar to IA-2, IAA also shows strong negative association with age. Positivity of IAA in patients with DM1 above 12 years of age is 40%, much lower as compared to 90% in children before the age of 5 years.(47)

ZnT8 Ab: ZnT8 is a new antigenic target in DM1. It was discovered in 2007 by screening for highly expressed, islet beta-cell specific molecules. (158) ZnT8 is associated with membrane of secretory granules of islet β-cells and belongs to a large family of zinc transporters. ZnT8A are found in about 70% of patients with DM1. This is mostly detected in newly diagnosed childhood DM1 and has a tendency of decline rapidly after the onset of disease.(159)

GADA has high diagnostic sensitivity in older onset DM1.(160,161) IA-2A, and IAA, do not provide much information in adults (162,163). GAD 65 autoantibodies are the

most common autoantibody.(74) DM1 patients are usually positive for multiple autoantibodies.(72,74) Evidence shows that during the disease process new antibodies may develop and existing autoantibodies may get lost.(164) Exact mechanism of changing autoantibodies is not known completely; however; it has been suggested that potential time-varying anti-idiotypic antibodies may interfere in DAA assays.(164)

5.2 Cellular immune reaction

It would be imperative to add that autoimmunity in DM2 is not restricted to the presence of autoantibodies. Autoreactive T cells (Tregs) have been detected in autoantibody negative DM2 patients. T-cells represent a strong link between inflammatory and autoimmune alterations. It has been demonstrated that islet-reactive T-cells can be present in phenotypic DM2 patients and their presence is associated with more severe β-cell lesion and lower residual insulin secretion. (165,166) The eponym T- LADA has been used to describe this subgroup. (72) By establishing an assay to measure T-cell reactivity, Brook-Worrell el al discovered T-cells responsive to several islet antigens not only in LADA but also in phenotypic DM2 patients without antibodies establishing another important link between autoimmunity and DM2. (165)

T-cell responses in Ab (+) T (+) and Ab (-) T (+) type 2 diabetic patients was similar. T-cell responses to islet proteins demonstrated to fluctuate less than autoantibody responses. Interestingly, with respect to cellular reactivity to islet proteins i.e recognition of islet proteins by T-cells, a difference has been noted between antibodies positive adult phenotypic type 2 diabetic patients and classic DM1 patients. This finding hints towards a different pathogenic mechanism involved

in both categories of diabetes mellitus.(167) Recently, it was found that histone H3 acetylation in $CD4^+$ T lymphocytes of LADA patients were reduced significantly. Histone H3 acetylation may play a role in the pathogenesis of autoimmune diseases.(168)

5.3 Association of LADA with other autoimmune disorders

Action LADA 11 study demonstrated that compared to DM2 patients, LADA patients may also display non diabetes associated antibodies that include transglutaminase, thyroid peroxide autoantibodies (TPO-Abs) and parietal cell antibodies.(169) TPO-Abs are most frequent antibodies in patients with adult-onset autoimmune diabetes (32.5%) than in those with DM2 (13.58%). However, no significant difference in their frequency was observed between LADA (30%) and DM1-A (36.67%).(169) Compared to low GADA titer LADA and DM2 patients, higher frequency of these organ-specific antibodies was observed in high GADA titer LADA patients. This observation suggests increased severity of autoimmune process in high titer GADA patients.(170)

5.4 Diabetes-Related Autoantibodies in Gestational Diabetes Mellitus

ICAs: In comparison to control group, higher prevalence of ICA between (0.98 to 14.7%) has been reported in Caucasians GDM subjects(171–175)**,** higher than in the control group.(173,175,176)

IAAs: Low prevalence of IAAs (0 –5.9%) is reported in women with GDM.(175,177) Only one study has reported a higher prevalence than in the control population.(178) Among subjects with GDM treated with exogenous human insulin, 44% develop IAs, which can persist up to 24 months after delivery.(179)

GADAs and IA-2As: The prevalence of IA-2As in GDM ranges from 0 to 6.2%(178,180,181) IA-2As are not frequent in this age range (112) and are associated with rapid progression to severe insulinopenia.(182) Various studies have reported the prevalence of GADAs among women with GDM in the range between 0-10.8 %.(175,180) Combined presence of various autoantibodies (AAs) is infrequently seen in both LADA and GDM (175,181) justifying the concept of slow progressive autoimmune diabetes.(183)

6. GENETICS OF LADA

6.1 Association of HLA genes in LADA

Desai et al analysed the association of HLA-DRB1 AND HLA-DQB1 genotype with LADA in a European population and showed the difference in the distribution of HLA-DRB1 and HLA-DQB1 genotype between LADA and control subjects. HLA- DRB1 in the DRB1*0301/DRB1*0401 heterozygotes showed the highest point estimate for genetic risk. Increased susceptibility to LADA was conferred by genotypes shown below (*Table 8*)

Genotypes conferring susceptibility to LADA
DRB1*0301/DRB1*0401;
DRB1*0301/DRB1*0301;
DRB1*0301/DRB1*0701;
DQB1*0201/DQB1*0302;
DQB1*0201/DQB1*0201;
DQB1*0201/DQB1*0202;
DQB1*0201/DQB1*0501
DQB1*0302/DQB1*0302

Table 8: Genotypes conferring susceptibility to LADA(184)

Protective effects of LADA were conferred by the following genotypes: DRB1*0701/DRB1*0501-06 and DQB1*0301/DQB1*0602 (263) As for the European population, in LADA China study, analysis of HLA-DQ gene showed significantly higher frequency of diabetes-susceptibility haplotypes in patients with LADA (63 %) compared to subjects with DM2 (47.1%) and controls (43.2%). Frequency of diabetes-protective haplotypes was significantly lower in LADA (22.8%) than DM2 (33.3%) and control (32.7%) subjects. Diabetes susceptibility and protective haplotypes are shown below (*Table 9*)

Total susceptibility haplotypes	Total protective haplotypes
DQA1*03-DQB1*0302,	DQA1*0102-DQB1*0601
DQA1*03-DQB1*0303,	DQA1*0102-DQB1*0602
DQA1*03-DQB1*0401	DQA1*0601-DQB1*0301
DQA1*05- DQB1*0201	

Table 9: Diabetes susceptibility and protective haplotypes in LADA China study(87)

6.2 Association with non-HLA loci

As reported for juvenile DM1, INS (11p15.5), followed by PTPN22 (1p13.2) showed strongest association outside MHC region with adult-onset autoimmune diabetes.(76) Based on these observations it has been suggested that LADA represents a subtype of classic DM-1.(185,186) However, evidence has shown that LADA differs from this subgroup of DM1-A. Patients with LADA have less severe symptom and progression towards insulin dependence is slow (72) Studies have shown the association of strong DM2 susceptibility gene TCF7L2

(10q25.3) variant rs 7903146 C to T polymorphism with LADA.(187,188) TCF7L2 gene has not been found to be associated with DM1.(72,189) TCF7L2 gene variant confers similar effect size in LADA and type 2 diabetes. Due to the overlapping of path mechanisms, LADA is not clearly distinguishable from DM1 and DM2. LADA lies in the middle of the continuous spectrum of the diabetes disease process starting from classic childhood DM1-A and age-related deterioration of glucose tolerance at other end. (Figure 5.) (190)

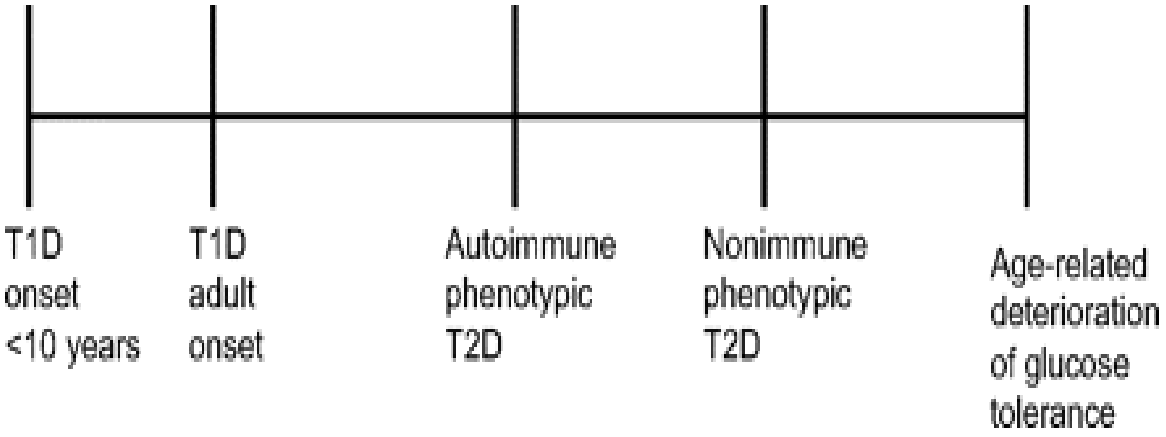

Figure 5: LADA lies in the middle of the continuous spectrum of the diabetes disease.(190) (Reproduced with permission from the American Association for Clinical Chemistry)

7. MONOCYTE GENE EXPRESSION PROFILES IN AUTOIMMUNE DIABETES

Padmos et al. investigated the patterns of inflammatory gene expressions in monocytes of patients with DM1-A (juvenile onset), LADA, DM1 (adult-onset), and as controls, DM2 patients and healthy controls. These genes are involved in the process of inflammation, motility, adhesion, chemokines, cell survival/ apoptosis, mitogen activated protein kinase (MAPK) pathway and metabolism. Two clusters of genes were identified. Cluster 1 included 12 proinflammatory cytokines with putative gene phosphodiesterase 4B (PDE4B) and cluster 2 comprised 10 genes with putative fatty acid-binding protein 5 (FABP5). PDE4B plays crucial role in the

uptake, transport and metabolism.(191) Cluster 1 was found in LADA (60%), adult onset DM1-A (28%), juvenile onset DM1-A (10%) and DM2 (10%) whereas cluster 2 was found in 43% of juvenile onset DM1-A and 33% of LADA patients, and 10% each in adult onset DM1-A and control subjects. The distinct monocyte gene expression profile supports the idea of heterogeneity in the pathogenesis of autoimmune diabetes.(191)

8. IMMUNOGENETIC SIMILARITIES BETWEEN LADA, DM1-A AND DM2

Results of genetic studies in subjects with LADA are consistent with a markedly increased high risk HLA genotype DQB1*0201/DQB1*0302, reduced DQB1*0602, DM2 associated TCF7L2 polymorphism with modest increase in GAD positive patients.(192) LADA shares genetic features with both DM1-A (HLA, INS, VNTR and PTPN22) and DM2 (TCF7L2) which suggests that LADA is a mixture of DM1 and DM2.(185,193) A non HLA MHC class 1 related A (MICA) gene is also associated with LADA. MICA gene encodes stress inducible proteins on cell surface. Sequencing of MICA gene has shown that A trinucleotide repeat (GCT) microsatellite polymorphism MICA 5.1 allele of this gene (with five repetitions of GCT along with an additional nucleotide insertion (GGCT) is significantly increased in LADA and adult onset DM1 (onset > 25 years).(194)

In relation to epitope specificity, GADA in DM1 and LADA are directed towards C-terminal and middle epitopes.(167,195) Both antibody positive DM2 and DM1 patients show similar PBMC reactivity to numerous islet proteins.(167) It has been suggested that Natural Killer (NK) cells play an immunoregulatory role in the prevention of autoimmune disease by down regulation of T-cell responses and by cross talking with dendritic cells.(196) In both DM1 and LADA, the NK cell deficiency

might contribute to the breakdown of self-tolerance that leads to β-cell destruction.(197,198)

9. IMMUNOGENETIC DIFFERENCES BETWEEN LADA, DM1 AND DM2

DM1 associated high risk genes HLA DR3-DR4 and their alleles DQB1*0302 and DQB1* 0201 have been linked as susceptible genes to LADA. Compared to young onset DM1, adult onset patients with DM1 show lower frequency of these genes. TNF2- allele associated with high amount of TNF alpha production is significantly lower in LADA. DR2 and DQB1*0602 HLA alleles are relatively more common in LADA. These alleles are strongly protective against childhood DM1 but offer less protection in LADA.(199) As compared to DM2, the HLA DQ B1, PTPN22 risk genotype had increased frequency in LADA. However, their presence was much less common than in DM1 diagnosed after 35 years of age. Patients with LADA have decreased frequency of HLA-DQB1 protective genotypes as compared to patients with DM2. Cytotoxic T lymphocyte antigen – 4 (CTLA 4) and INS genes were associated only with DM1.(200)

Appearance of varied antibodies in different clinical subtypes of autoimmune diabetes reflect slow or rapid progression of autoimmune process.(113) A Japanese study showed the presence of a unique epitope at N-terminal of GAD-65 in slowly progressive type (Japanese equivalent of LADA) different from classic DM1.(195) PBMC response to islet protein in antibody negative DM2 is very limited as compared to antibody positive DM2 and DM1-A(190) In comparison to adult onset DM1, more frequently, patients with LADA have single islet cell-specific autoantibody positivity. Patients with LADA present more anti GAD autoantibody and less often ICA whereas, clustering of more than one antibodies characterize adult

DM1.(72,127) Different antibody isotypes in IgG subclass in adults with LADA have been observed. Compared to DM1 patients, IgG4 was commonly found in LADA whereas IgG1 was most common subtype in both conditions.(35,201) The slower disease progression in LADA has been attributed to restricted antigen spreading than in DM1.

10. AUTOIMMUNE DIABETES SPECTRUM

Categorization of the type of diabetes cannot be solely based on the presence of diabetes associated autoantibodies. Patients progressing towards insulin deficiency are also characterized by younger age at diagnosis, low BMI, lower endogenous insulin secretion and high HbA1c at diagnosis as shown in below (figure 8).(202)

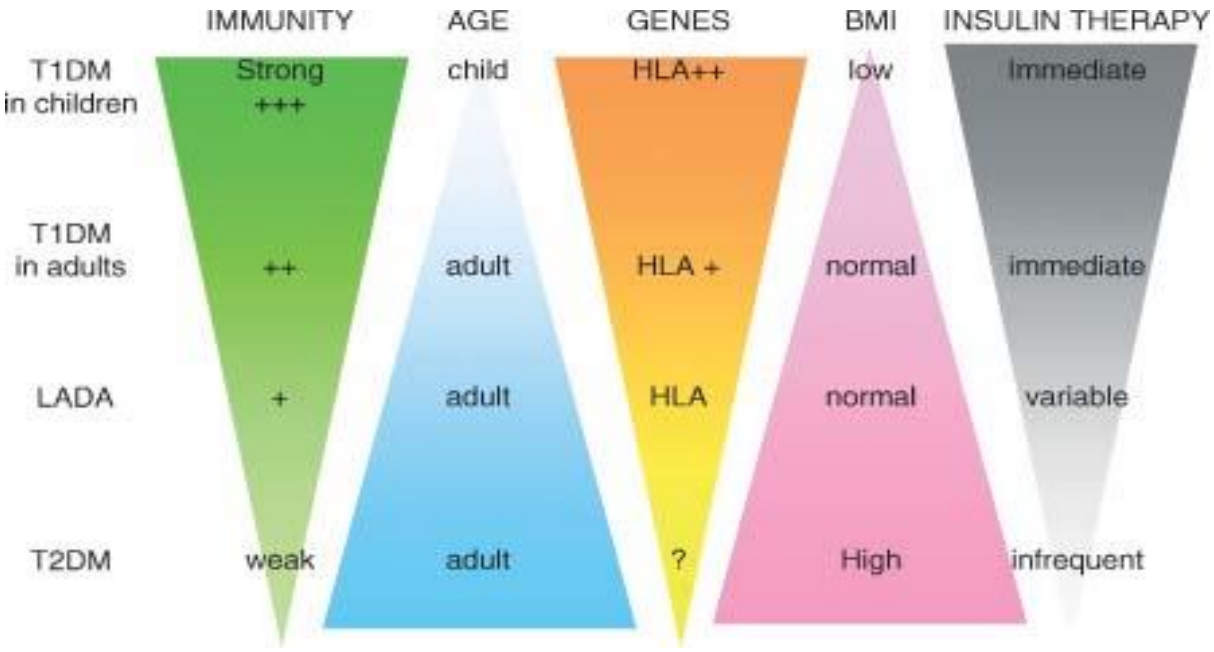

Figure 8: The spectrum of diabetes includes variable risk according to type of diabetes for immunity, age at presentation, HLA genetic susceptibility, BMI and insulin therapy. Reproduced from Leslie et al. Diabetes classification: grey zones, sound and smoke: Action LADA 1. Diabetes Metab Res Rev. 2008;24(7):511-519.(202)

11. TREATMENT STRATEGIES WITH FOCUS ON PREVENTION AND REGENERATION OF β-CELLS IN LADA

Considering the autoimmune nature of LADA with impairment of β-cell function at diagnosis currently insulin therapy is the treatment of choice. Glibenclamide should be avoided as it might further aggravate the autoimmune process.(203) Treatment with sulfonylureas (SUs) leads to earlier insulin dependence and poor metabolic control if SUs are prescribed for patients with LADA compared to insulin.(204) Insulin does not seem to have immunomodulating effect and cannot be considered a preventive treatment for autoimmune diabetes. Unspecific effect of insulin on glucose toxicity helps in improvement of β-cell function.(37,205,206) Vitamin D may protect beta cells in LADA(207) although not much information is available in this regard. DiaPep277, a heat-shock protein peptide was found to preserve endogenous insulin perhaps through induction of a shift from T-helper 1 (interferon-γ production reduced) to a T-helper 2 (interleukin-9 and -13 increased) which are produced by autoimmune T-cells. Further studies are needed to clarify the effect on β-cell function in autoimmune diabetes.(208)

In comparison to insulin alone, treatment with DPP4 inhibitor sitagliptin and insulin maintained β-cell function in LADA patients.(209) Subcutaneous GAD65 in LADA increased fasting C peptide levels after 24 weeks in those treated with moderate doses of 20 µg. Interestingly, lower (4 µg) or higher (50 or 100 µg) doses did not show any difference. This has been the first safe report of immunomodulation in LADA.(210)

Most recent evidence supports Dulaglutide, a weekly GLP-1 receptor agonist as an effective antihyperglycemic treatment for patients with LADA.(211) Potential therapeutic development should focus on beta cell preservation and regeneration.

RESEARCH OBJECTIVES

1. JUSTIFICATION

Adult-onset autoimmune diabetes is heterogeneous consisting of various groups. This observation has been intensively investigated in Europe and needs a comprehensive search in India. For almost two decades, LADA had been continually an area of interest for researchers and clinicians worldwide. Epidemiological studies have reported varied prevalence of LADA ranging from 1.4% in Korean population to approximately 10% in European individuals. In South Asia, data on LADA are sparse. Significant discrepancy in the frequency of LADA has been observed in earlier studies on Indian population ranging from 2.6% to 58%. Such variation in the results may be attributed to local differences and conflicting methodology adopted by various authors. Diagnosing LADA early in the disease process is important as it may have therapeutic implications.

2. OBJECTIVES

2.1 General objective

In view of the wide variation seen in the results of past studies on Indian subjects, we conducted a study to find the prevalence and characterize patients with LADA in an area of India, adopting the criteria suggested by the Immunology of Diabetes Society (IDS) and The European Action LADA group. using a validated method.

2.2 Specific objective

To investigate the prevalence, phenotype characteristics, biochemical and immunological features of adult-onset autoimmune diabetes in an area of National

Capital Region (NCR) of Northern India and their comparison with other populations in the world; in particular, from Asian and Western countries.

MATERIAL AND METHODS

SUBJECTS

This study was conducted in the National Capital Region (NCR) of North India and included diabetic patients over 30 years of age consecutively attended at the Diabetes Clinic. Majority of the population investigated were inhabitants of the urban areas of NCR surrounding the capital city of New Delhi. All participants were natives of Northern states of India, mainly Uttar Pradesh, Uttarakhand, Punjab, Haryana, Bihar and Delhi. The socio-economic status of most of the study participants ranged from lower middle to upper middle. From November 2015 until 2016 a total of 139 subjects were voluntarily recruited according to the inclusion requirements of the research protocol.

STUDY DESIGN

This was a cross-sectional investigation. The Sample size was calculated using Granmo software (1998),(212) accepting the confidence interval of 95% (0.95) for a precision of +/- 0.05 units in two sided test for an estimation proportion 0.1. Inclusion criteria were: a) diagnosis of diabetes by standard criteria; b) age at diagnosis of diabetes, 30 – 70 years; c) duration of diabetes between 6 months to 5 years. Any subject not fulfilling the inclusion criteria or not willing to participate were excluded.

In this population, subjects with glutamic acid decarboxylase autoantibody (GADA) in whom insulin was started at diagnosis or within one month of diagnoses were defined as DM1.(81) All antibody negative subjects were diagnosed as DM2.(20) LADA patients were defined as patients aged 30-70 years at the time of diabetes diagnosis who did not require insulin for at least 6 months after diagnosis and

depicted GADA at their sera. The Study was conducted in accordance with Declaration of Helsinki. Ethical committee approval was obtained and written informed consent document was signed by all subjects.

1. METHODOLOGY

1.1 Clinical history

Clinical history of all subjects was reviewed with the aim of collecting data on age, sex, date of diagnosis, family history, and treatment of Diabetes, hypertension and dyslipidemia. Time to start of insulin was calculated as the time between the date of diagnosis and the date of the first insulin treatment.

1.2 Data collection and creation of the database

The data were collected at the time of the study visit. Case report forms (CRF) were completed with the information obtained from medical history. All reports were entered into Microsoft Excel worksheets to create a database. Data were later imported to the Statistical Package for the Social Sciences SPSS (SPSS Inc.) for subsequent analysis.

1.3 Anthropometric and blood pressure measurement

Anthropometric measurement included measurement of body weight in kilograms (Kg), height and Abdominal circumference (AC) in centimeters, Body Mass Index (BMI) was calculated as weight divided by height squared in meters (kg/m^2).

AC was measured without clothes midway between the lower rib margin and iliac crest using non stretchable tape in concordance with technique defined by National Institutes of Health (NIH).(213) Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in sitting position; the average of both readings was noted for analysis.

2. DIAGNOSIS OF METABOLIC SYNDROME

Metabolic syndrome was assessed according to the revised National Cholesterol Education Program (NCEP): Adult Treatment Panel III criteria.(214) All subjects fulfilled the criteria of hyperglycemia. Additionally, two of the following criteria were required for the diagnosis:

1) Waist circumference ≥ 90 cm (Asian male) or ≥ 80 cm (Asian female)

2) Serum triglyceride ≥ 150 mg/dl or use of drug treatment for dyslipidaemia

3) Serum HDL cholesterol ≤ 40 mg/dl (male) or ≤ 50 mg/dl (female)

4) SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg or use of antihypertensive medication

3. BIOCHEMICAL ANALYSIS AND ANTIBODY ASSAY

A fasting (12 hours) blood sample was collected and analyzed locally, using standardized assays to measure glucose, HbA1c, lipid profile, creatinine, C-peptide

and GAD-65 antibody. Specific methodology used to measure each parameter is described below in detail.

3.1 Glucose

Glucose was measured by hexokinase method(215) using Siemens *ADVIA® 1800* autoanalyzer. For this purpose, 3.0 ml venous whole blood was collected in vacuum tube containing the glycolytic inhibitor sodium fluoride. Specimens were centrifuged immediately at 1500 RCF (Relative Centrifugal force) for 15 minutes. 1ml plasma was transferred to clean plastic screw cap vial labelled with barcode; samples were refrigerated and processed in the same day.

3.2 Glycosylated haemoglobin (HbA1C)

HbA1c was performed using Bio-Rad Laboratories Inc. *VARIANT™* II Dual (A2/F/A1c) instrument by ion-exchange high performance liquid chromatography (HPLC), a National Glycohemoglobin Standardization Program (NGSP) certified method. (216,217)

3.3 Lipid profile

The lipid profile included the measurement of Total cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein-cholesterol (LDL-C) and High Density Lipoprotein cholesterol (HDL-C). TC was measured by enzymatic method; cholesterol oxidase-Peroxidase (CHOD-POD) based on the principle first described by Stadtman et al. and later adopted by Rautela and Liedtke.(218,219). TG was measured by

enzymatic method; glycerol-3-phosphate Oxidase-Peroxidase (GPO-POD).(220) LDL-C cholesterol was measured using automated direct assay referenced to the beta-quantification method.(221) and HDL-C cholesterol was measured by direct enzymatic method.(222) 4ml venous whole blood was collected in plastic serum separator tubes. Immediate mixing was done by inverting the tube gently 4-5 times. Blood was allowed to clot in an upright position for at least 30 minutes before centrifugation. Specimen was centrifuged for at least 15 minutes at 3000-3500 rpm within one hour of collection. Around 2.5 ml serum was transferred to properly labelled plastic-screw cap vial and transported refrigerated and processed same day. All methods were performed on Siemens *ADVIA® 1800* chemical autoanalyzer using Siemens Dimension® Flex® reagent cartridges.

3.4 Creatinine

Serum creatinine was measured by colorimetric Jeff's kinetic method performed on Siemens *ADVIA® 1800* chemical autoanalyzer.(223) The normal reference range was 0.80 - 1.30 mg/dL.

3.5 C-Peptide

C-peptide was measured to assess insulin secretion. It was analyzed using Siemens *ADVIA* Centaur XP® immunoassay system by direct chemiluminescent technology (CLIA) with coefficient of variation (CV) <10% and normal reference range, 0.48 to 5.05 ng/mL(224) Serum was separated as per the standard procedure mentioned above. Volume of 0.5 ml serum was required for analysis. Samples were transported refrigerated and processed same day or were frozen at or below -20°C if the sample

was not assayed within 24 hours. All frozen samples were processed within 2-5 days of storage.

3.6 GAD-65 Antibody

GAD-65 antibody was analyzed using RSR- ELISA kits (RSR Limited, Cardiff, UK) with 98% specificity and 92% sensitivity in the Diabetes Antibody Standardization Program (DASP) 2005.(225) 50 µl serum was required for one assay. Serum was stored in aliquots at -20 degree Celsius till analysis.

Assay cut off of < 5 U/mL was considered negative and ≥ 5 U/mL was considered positive.(226) Our results did not show a bimodal distribution of GADA titer. hence, to analyze the characteristics of LADA subjects according to GADA titer, based on the median value of GADA titer, LADA subjects were stratified into two subgroups: GAD-high titer (>13.6 U/mL) and GADA-low titer (≤13.6 U/mL). The interassay precision of the kit has been shown to have a CV of 5.7% with a sample of 97 U/mL, 5.2% for a sample of 21.0 U/mL and 6.4% for a sample of 5.7% (n=20). The intra-assay precision was 7.3% for a sample of 97.2 U/mL and 8.5% for a sample of 20.0 U/mL, and 3.5% for a sample of 7.0 (n=25).

The wells of the ELISA plate were GAD-65 coated. Any antibodies directed against any GAD-65 antigens present in the serum were bound to immobilised GAD-65 on the plate. After 1-hour wash step, GAD65-Biotin was added in 2nd incubation step, a bridge was formed between the GAD-65 immobilised on the plate and the GAD65-biotin. In the 3rd incubation step, the amount of GAD65-Biotin bound was determined by adding Streptavidin Peroxidase, that specifically binds to Biotin.

Unbound Streptavidin Peroxidase was washed away and addition of chromogenic substrate 3,3′, 5,5′-tetramethylbenzidine (TMP) yielded a blue color. The reaction was stopped by addition of stop solution turning well contents to yellow color. The absorbance of yellow mixture was measured using ELISA plate reader ERBA LISA SCAN EM TRANSASIA®

6. STATISTICAL ANALYSIS

Quantitative variables were described using the means and standard deviations or medians and interquartile ranges. Categorical variables were described as n (%). The comparison between mean values was estimated by Independent t test/Mann-Whitney U test. The comparison of categorical variables was analyzed using chi-square/ Fisher exact test. For the purpose of C-peptide secretion analysis, disease duration (in months) was calculated as the period between the date of diagnosis and the date of the study assessment. The disease duration was stratified into 2 periods (< 36 months and >36 months) for the two categories analyzed; i.e., LADA and T2DM. For all analyses, IBM SPSS statistics for windows software (version 21.0; Armonk NY, USA) was used and an α value of 0.05 for statistical significance was considered for all analyses.

RESULTS

1- Demographics of study participants

The 139 study subjects consisted of 80 (57.6%) males and 59 (42.5%) females. Overall, mean (sd) age was 46.7 (9.3) years and age at diagnosis was 43.9 (9.5) years. The mean disease duration was 34.5 (17.9) months, waist circumference was 96.3 (9.9) cms and BMI was 28.1 (4.8) kg/$m^{2.}$ The demographics and biochemical characteristics of studied participants are shown in *Table 10.*

Table 10: Baseline demographics and biochemical characteristics of all 139 subjects

Characteristics	**Mean ± SD, Median [IQR] or n (%)**
Males, n (%)	80 (57.6)
Females, n (%)	59 (42.4)
Age (years)	46.7 ± 9.3
Age at diagnosis (years)	43.9 ± 9.5
Duration of disease (months)	34.5 ± 17.9
Weight (kg)	74.7 ± 13.2
BMI (kg/m^2)	28.1 ± 4.8
Waist circumference (cms)	96.3 ± 9.9
Family history of diabetes, n (%)	90 (64.7)
SBP (mmHg)	125.8 ± 14.4
DBP(mmHg)	80.0 [75-84]
Antihypertensive treatment, n (%)	46 (33.1)
Treatment for dyslipidemia, n (%)	5 (3.6)
FBS	137 [120-169]
HbA1c	7.1 [6.6-8.7]
Cholesterol total (mg/dl)	167.3 ± 37.8
Triglycerides (mg/dl)	148 [107-206]
LDL cholesterol (mg/dl)	103.3 ± 31.3
HDL cholesterol (mg/dl)	43.4 ± 10.6
C-Peptide (ng/ml)	2.2 ± 0.9
GADA titer	
Positive (≥ 5 U/ml, n (%)	9 (6.5%)
Negative (< 5U/ml, n (%)	130 (93.5%)
Metabolic syndrome, n (%)	120 (86.3)
Treatment	
Diet, n (%)	10 (7.2)
OHA, n (%)	121 (87.1)
Insulin with or without OHA, n (%)	8(5.8)

Data are expressed as means (SD), median [IQR] or n (%)

2- Prevalence of adult-onset autoimmune diabetes

Within the entire cohort of 139 subjects with adult-onset diabetes, 9 (6.5%) were positive for GADA. All antibody positive subjects did not require insulin within first six months after diagnosis of diabetes and were classified as LADA. Based on diagnostic criteria adopted no subject was diagnosed with DM1-A.

GADA negative (n=130) subjects were diagnosed with DM2. The prevalence of LADA was 6.5% (6.3% in men and 6.8% in women); 95% confidence interval (CI): 3.29-12.0% among adult onset diabetic patients. Prevalence of LADA seemed to gradually decline with increasing age. However, the prevalence of DM2 continually increased with age. Age and gender wise prevalence of LADA and DM2 is shown in *Table 11 and Fig. 9.*

Table 11: Age and Gender wise prevalence of LADA and DM2 among all study subjects n=139

Age (years)	**LADA**			**DM2**		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
< 40	4/26 (15.4)	1/10 (10)	5/36 (13.9)	22/26(84.6)	9/10 (90)	31/36 (86.1)
40-49	1/33 (3)	2/16 (12.5)	3/49 (6.1)	32/33 (97)	14/16 (87.5)	46/49 (93.9)
50-59	0/18 (0)	1/22 (4.5)	1/40 (2.5)	18/18 (100)	21/22 (95.5)	39/40 (97.5)
≥ 60	0/3 (0)	0/11 (0)	0/14 (0)	3/3 (100)	11/11 (100)	14/14 (100)
Total	5/80 (6.3)	4/59 (6.8)	9/139 (6.5)	75/80(93.8)	55/59 (93.2)	130/139(93.5)

Data expressed as n (%)

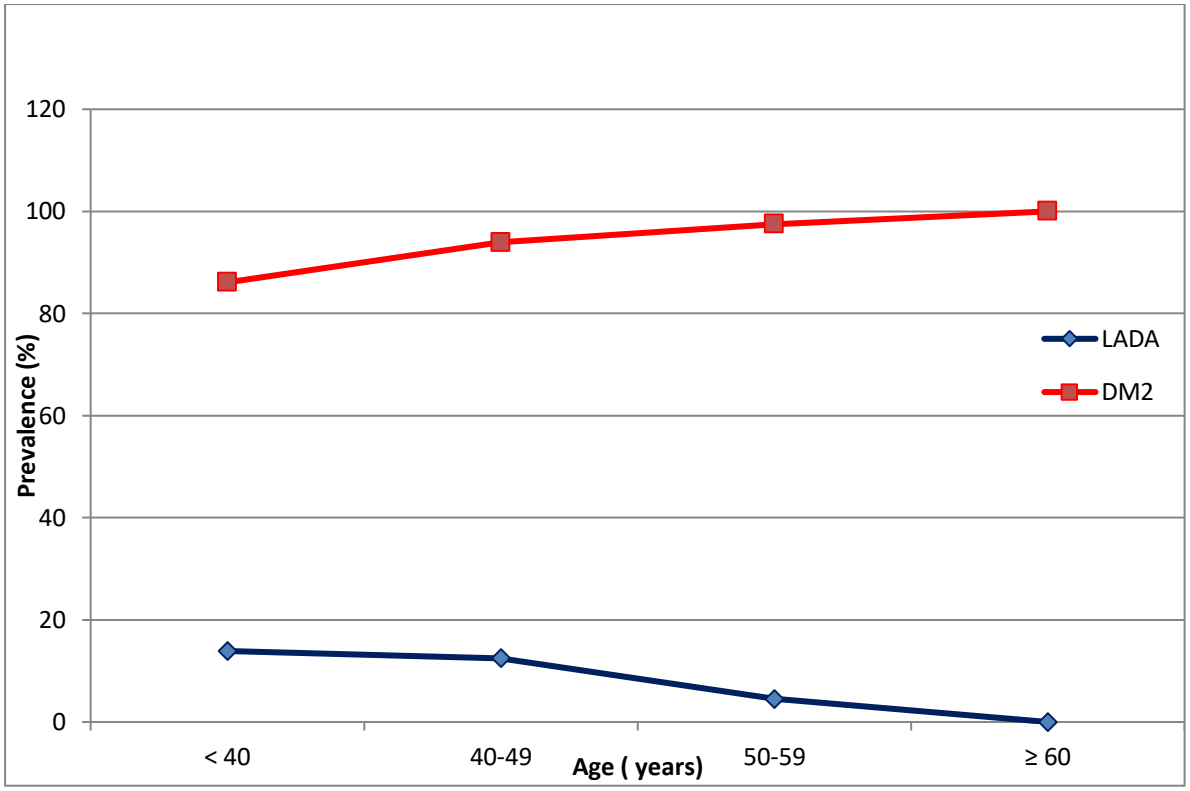

Figure 9: – Age wise prevalence of LADA and DM2 among all study subjects.

3- Comparison of characteristics of LADA and DM2 subjects

LADA (n=9) and DM2 (n=130) patients were compared. LADA patients were younger (40.8 ± 7.6 vs. 47.2 ± 9.3 years; p = 0.045), had lower age at onset of diabetes (37.1 ± 7.4 vs. 44.4 ± 9.4 years; p = 0.025), abdominal circumference (88.9 ± 8.3 vs. 96.8 ± 9.8 cms; p = 0.021), systolic blood pressure (SBP) (115.3 ± 16.4 vs. 126.5 ± 14.0 mmHg; p = 0.033), triglycerides (107 (92-141) vs. 151 (112.3-210) mg/dl; p = 0.033), fasting C-peptide (1.5 ± 0.9 vs. 2.3 ± 0.8 ng/ml; p = 0.009) and prevalence of metabolic syndrome (MS) (44.4 % vs. 88.5 %; p = 0.003). LADA patients had also longer duration of diabetes (46 ± 12.7 vs. 33.7 ± 17.9; p = 0.045). Frequency of positive family history of diabetes was lower in LADA patients; however, the difference was not statistically significant (44.4% vs. 66.2; p = 0.278). Higher

proportion of LADA subjects were on insulin treatment (22.2% vs. 4.6%; p = 0.085); the difference between the group of LADA and DM2 did not reach statistical significance. There were no significant differences in BMI, FPG, HbA1C, Cholesterol-total, LDL-C, HDL-C, and DBP between two groups. The characteristics of LADA and DM2 patients are summarized in *Table 12.*

Table 12: Characteristics of LADA subjects *vs.* DM2 subjects

Cases	LADA (n=9)	DM2 (n=130)	P-value
Females, n (%)	4 (44.4%)	55 (42.3%)	
Males, n (%)	5 (55.6%)	75 (57.7%)	1.000
Age (years)	40.8 ± 7.6	47.2 ± 9.3	**0.045**
Age at diagnosis (years)	37.1 ± 7.4	44.4 ± 9.4	**0.025**
Disease Duration (months)	46 ± 12.7	33.7 ± 17.9	**0.045**
BMI (Kg/m^2)	25.6 ± 4.2	28.3 ± 4.8	0.108
Waist circumference (cms)	88.9 ± 8.3	96.8 ± 9.8	**0.021**
SBP (mmHg)	115.3 ± 16.4	126.5 ± 14.0	**0.023**
DBP(mmHg)	80.0 [65.5-82.0]	80.0 [75.0- 84.3]	0.330
Antihypertensive treatment n (%)	2 (22.2%)	44 (33.8%)	0.718
Family history of Diabetes, n (%)	4 (44.4%)	86 (66.2)	0.278
Triglycerides (mg/dl)	107.0 [92.0- 141.0]	151.0 [112.3-210]	**0.033**
Total cholesterol (mg/dl)	160.4 ± 35.7	167.8 ± 38.1	0.577
HDL Cholesterol (mg/dl)	46.1 ± 7.5	43.2 ± 10.8	0.425
LDL Cholesterol (mg/dl)	104 ± 33.9	103.3 ± 31.2	0.948
Metabolic syndrome, n (%)	4 (44.4%)	115(88.5%)	**0.003**
Fasting plasma glucose(mg/dl)	139 [117.5- 162.5]	137 [120-169.3]	0.898
HbA1C (%)	8.1 [7.0- 10.6]	7.1[6.6-8.5]	0.096
C-Peptide fasting (ng/ml)	1.5 ± 0.9	2.3 ± 0.8	**0.009**
Insulin treatment, n (%)	2 (22.2%)	6 (4.6%)	0.085
Time to insulin (months)	25.0 ± 26.8	47.2 ± 5.3	0.449

Data are means ± SD or n (%)

4- Comparison of characteristics of LADA GADA- high subjects *vs.* GADA- low subjects

To further analyze characteristics of LADA subjects according to GADA titer, based on the median value of GADA titer, LADA subjects were divided into 2 sub-groups:

GADA-high titer *(> 13.6 U/ml)* and GADA-low titer *(≤13.6 U/ml)*. Patients with GADA-at high titer (GADA-High, n = 4) were compared with GADA at- low titer (GADA-Low, n = 5) group. All GADA-high patients were male (0% vs. 80%; p = 0.048), had lower BMI (22.6 ± 4.1 vs. 28.1 ± 2.4 Kg/m^2; p = 0.040), abdominal circumference (82.6 ± 7.4 vs. 94.0 ± 4.8 cms; p = 0.026), fasting C-peptide (0.8 ± 0.7 vs. 2.1 ± 0.6 ng/ml; p = 0.025) and prevalence of MS (0% vs. 80%; p = 0.048). No statistical significance was observed between two groups although, LADA GADA-high patients required insulin more frequently (50% vs. 0%; p = 0.167). Characteristics of GADA-High vs. GADA-Low titer are shown in *Table 13*.

Table 13: Characteristics of GADA-high *vs.* GADA-low LADA subjects

Cases	**GADA high (n=4)**	**GADA low (n=5)**	**P-value**
Female, n (%)	0 (0%)	4 (80%)	
Male, n (%)	4 (100%)	1 (20%)	0.048
Age (years)	37.3 ± 3.6	43.6 ± 9.2	0.236
Age at diagnosis (years)	33.3 ± 3.0	40.2 ± 8.8	0.178
Disease Duration (months)	50 ± 11.2	42.8 ± 14.1	0.433
BMI (Kg/m^2)	22.6 ± 4.1	28.1 ± 2.4	**0.040**
Waist (cms)	82.6 ± 7.4	94.0 ± 4.8	**0.026**
SBP (mmHg)	105.5 ± 9.0	123.2 ± 17.3	0.109
DBP(mmHg)*	70.5 ± 9.5	79.2 ± 12.5	0.288
Antihypertensive treatment n (%)	0 (0%)	2 (40%)	0.444
Triglycerides (mg/dl)	96.8 ± 12.7	131.2 ± 34.7	0.092
Total cholesterol (mg/dl)	157.5 ± 37.2	163 ± 38.6	0.841
HDL Cholesterol (mg/dl)	47.8 ± 6	44.8 ± 9.0	0.590
LDL Cholesterol (mg/dl)	100 ± 39.4	107.2 ± 33.5	0.775
Metabolic syndrome (n, %)	0 (0%)	4 (80%)	0.048
Fasting plasma glucose (mg/dl)	140± 29.9	154.8 ± 57.8	0.659
HbA1C (%)	8.9 ± 3.2	8.8 ± 1.7	0.953
Family history of Diabetes, n (%)	1(25%)	3 (60%)	0.524
C-Peptide fasting (ng/ml)	0.8 ± 0.7	2.1 ± 0.6	**0.025**
Insulin treatment	2 (50%)	0 (0%)	0.167

Data are means ± SD or n (%)

5- Comparison of characteristics of LADA subjects with GADA at high titer (GADA-high) *vs.* DM2 subjects

Patients with GADA- at high titer (GADA-high, n = 4) were compared with DM2, n = 130) group. GADA-high patients were younger (37.3 ± 3.6 vs. 47.2 ± 9.3 years; p = 0.035), had lower age at onset (33.3 ± 3.0 vs. 44.4 ± 9.4 years; p = 0.020), BMI (22.6 ± 4.1 vs. 28.3 ± 4.8 Kg/m^2; p = 0.040), abdominal circumference (82.6 ± 7.4 vs. 96.8 ± 9.8 cms; p = 0.005), SBP (105.5 ± 9.0 vs. 126.5 ± 14.0 mmHg; p = 0.003), triglycerides (99.5 [83.8 - 107.0] vs. 151[210 - 112.3] mg/dl; p = 0.026), fasting C-peptide (0.8 ± 0.7 vs. 2.3 ± 0.8 ng/ml; p = 0.001) and prevalence of MS (0% vs. 88.5%; p < 0.001). The rate of patients on insulin was higher in GADA-high compared to DM2 (50% vs. 4.6%; p = 0.018). The characteristics of GADA-high versus DM2 subjects are shown in *Table 14.*

Table 14: Characteristics of GADA-high LADA subjects *vs.* DM2 subjects

Cases	GADA- high (n = 4)	DM2 (n = 130)	P-value
Females, n (%)	0 (0%)	55 (42.3%)	
Males, n (%)	4 (100%)	75 (57.7%)	0.144
Age (years)	37.3 ± 3.6	47.2 ± 9.3	**0.035**
Age at diagnosis (years)	33.3 ± 3.0	44.4 ± 9.4	**0.020**
Disease duration (months)	50 ± 11.2	33.7 ± 17.9	0.074
BMI (Kg/m^2)	22.6 ± 4.1	28.3 ± 4.8	**0.020**
Waist (cms)	82.6 ± 7.4	96.8 ± 9.8	**0.005**
SBP (mmHg)	105.5 ± 9.0	126.5 ± 14.0	**0.003**
DBP(mmHg)*	69.5 [62.3-79.8]	80 [75.0-84.3]	0.075
Antihypertensive treatment n (%)	0 (0%)	44 (33.8%)	0.302
Triglycerides (mg/dl) *	99.5 [83.8-107.0]	151[112.3-210]	0.026
Total cholesterol (mg/dl)	157.5 ± 37.2	167.8 ± 38.1	0.596
HDL cholesterol (mg/dl)	47.8 ± 6	43.2 ± 10.8	0.403
LDL cholesterol (mg/dl)	100 ± 39.4	103.3 ± 31.2	0.837
Metabolic syndrome (n, %)	0 (0%)	115(88.5%)	**< 0.001**
Fasting plasma glucose (mg/dl) *	143 [109.8 - 167.3]	137 [120-169.3]	0.927
HbA1C (%)*	8.4 [6.2 - 12.2]	7.1 [6.6-8.5]	0.596
Family history of Diabetes, n (%)	1(25%)	86 (66.2%)	0.124
C-Peptide fasting (ng/ml)	0.8 ± 0.7	2.3 ± 0.8	**0.001**
Insulin treatment	2 (50%)	6 (4.6%)	**0.018**

Data expressed as means ± SD, medians [IQR] or n (%)

6- Comparison of characteristics of GADA-low LADA subjects *vs.* DM2 subjects

Patients with GADA- at low titer (GADA-low, n=4) were compared with DM2 patients (n=130) group. There were no significant differences between characteristics of DM2 and GADA-low patients. The characteristics of GADA-low versus DM2 patients are presented in *Table 15.*

Table 15: Characteristics of GADA-low LADA subjects *vs.* DM2 subjects

Cases	GADA- low (n = 5)	DM2 (n = 130)	P-value
Females, n (%)	4 (80%)	55 (42.3%)	0.167
Males, n (%)	1 (20%)	75 (57.7%)	0.400
Age (years)	43.6 ± 9.2	47.2 ± 9.3	0.329
Age at diagnosis (years)	40.2 ± 8.8	44.4 ± 9.4	0.265
Disease Duration (months)	42.8 ± 14.1	33.7 ± 17.9	0.922
BMI (Kg/m^2)	28.1 ± 2.4	28.3 ± 4.8	0.531
Waist (cms)	94.0 ± 4.8	96.8 ± 9.8	0.605
SBP (mmHg)	123.2 ± 17.3	126.5 ± 14.0	0.792
DBP(mmHg)	80 [70-88]	80 [75.0-84.3]	1.000
Antihypertensive treatment n (%)	2 (40%)	44 (33.8%)	0.354
Triglycerides (mg/dl)	122 [100-167]	151 [112.3-210]	0.775
Total cholesterol (mg/dl)	163 ± 38.6	167.8 ± 38.1	0.741
HDL Cholesterol (mg/dl)	44.8 ± 9.0	43.2 ± 10.8	0.784
LDL Cholesterol (mg/dl)	107.2 ± 33.5	103.3 ± 31.2	0.473
Metabolic syndrome (n, %)	4 (80%)	115(88.5%)	0.798
Fasting plasma glucose (mg/dl)	139 [117-201]	137 [120-169.3]	0.073
HbA1C (%)	8.1 [7.5-11.5]	7.1 [6.6-8.5]	1.000
Family history of Diabetes, n (%)	3 (60%)	86 (66.2%)	0.568
C- Peptide fasting (ng/ml)	2.1 ± 0.6	2.3 ± 0.8	1.000
Insulin treatment	0 (0%)	6 (4.6%)	0.167

Data are means ± SD, medians [IQR] or n (%)

7- Analysis of C-Peptide secretion according to duration of diabetes

Above observations in table 3 showed that subjects with LADA had lower fasting C-peptide levels compared with DM2 subjects. For the purpose of assessment of C-peptide secretions according to disease duration, LADA and DM2 groups were stratified into two periods (< 36 months and > 36 months). In comparison to DM2 subjects, LADA subjects displayed significantly lower fasting C-peptide concentrations in those with less than 36 months duration (1.73 ± 0.10 *vs.* 2.29 ± 0.88 ng/ml; p = < 0.001; 95% CI: - 0.81 — - 0.31) and the difference remained in patients with more than 36 months of disease duration (1.39 ± 1.10 *vs.* 2.25 ± 0.77 ng/ml; p = 0.016; 95% CI: - 1.54 — - 0.17 *(Table 16a, Table 16b and Fig. 10).*

Table 16a: C- Peptide levels in subjects with < 36 months duration of diabetes

Cases	**LADA (n = 3)**	**DM2 (n = 71)**	**P- value**	**95% CI**
C-peptide (ng/ml)	1.73 ± 0.10	2.29 ± 0.88	**< 0.001**	- 0.81 — - 0.31

Data are mean ± SD

Table 16b: C-Peptide levels in subjects with > 36 months duration of diabetes

Cases	**LADA (n = 6)**	**DM2 (n = 59)**	**P- value**	**95% CI**
C-peptide(ng/ml)	1.39 ± 1.10	2.25 ± 0.77	**0.016**	- 1.54 — - 0.17

Data are mean ± SD

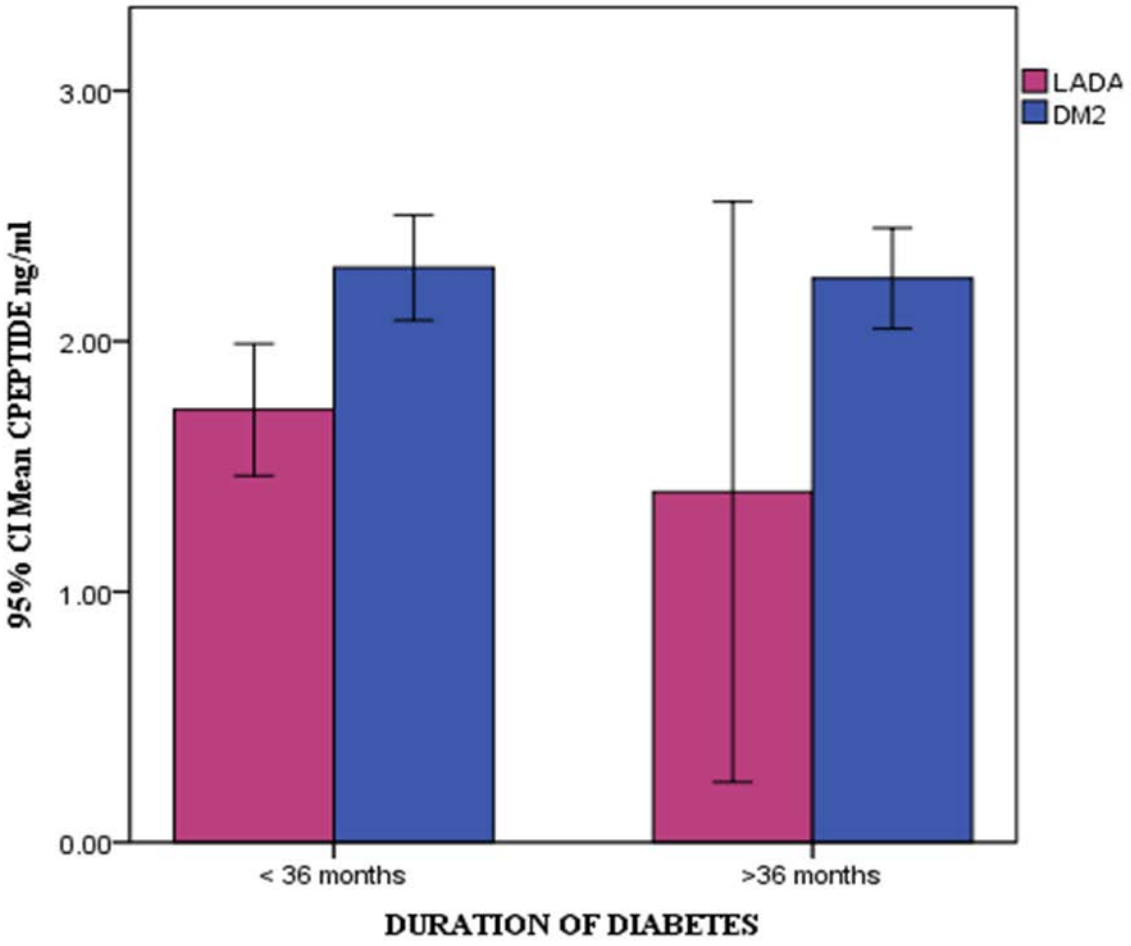

Figure 10: C- Peptide concentrations of LADA and DM2 divided in two categories according to duration of diabetes: < 36 months (3 and 71 subjects, respectively) and over 36 months (6 and 59 subjects, respectively). Error bars indicate the 95% confidence intervals (CI).

8- Assessment of insulin treated *vs.* non- insulin treated groups within LADA and DM2 patients

With the objective of evaluating differences in characteristics, insulin treated LADA patients (n = 2) and DM2 patients (n = 6) were compared with non-insulin treated LADA patients (n = 7) and DM2 (n = 124) patients respectively. Among LADA patients, those who were taking insulin treatment had significantly longer duration of diabetes (58.5 ± 0.7 *vs*. 42.4 ± 12.1 months; p = 0.012) lower abdominal circumference (76.4 ± 3.3 *vs.* 92.5 ± 4.71 cms; p = 0.003) and lower fasting C-Peptide levels (0.4 ± 0.4 *vs*. 1.8 ± 0.7 ng/ml; p = 0.027) than those who were not taking insulin treatment. Although, the proportion of subjects with high-GADA titer was higher in insulin treated LADA subjects (2/2 (100%) *vs.* 2/7 (40%); p = 0.167) the difference was not significant statistically. Similarly, although not statistically different, non-insulin treated LADA subjects had higher proportion of MS than insulin treated LADA subjects (0/2 (0%) *vs.* 4/7 (57.1%); p = 0.444) There was no significant difference in gender, age, age at diagnosis, BMI, SBP, DBP and HbA1c in the two groups of subjects (*Table 17a)*

In DM2 patients, longer diabetes duration (58.2 ± 2.0 *vs.*32.5 ± 17.5 months; p = <. 001), lower BMI (23.2 ± 4.1 *vs.* 28.5 ± 4kg/m^2; p = 0.007), abdominal circumference (85.0 ± 12.2 *vs*.97.4 ± 9.4 cms; p = 0.002) SBP (119.3 ± 5.1 *vs*. 126.9 ± 14.2 mmHg; p = 0.013) and fasting C-Peptide (1.2 ± 0.3 *vs.* 2.3 ± 0.8 ng/ml; p = < 0.001) were associated with insulin therapy. Frequency of MS was significantly lower in insulin treated DM2 subjects than non-insulin treated subjects (3/6 (50%) *vs.* 112/124 (90.2%); p = 0.020). There was no significant association observed between gender, age, age at diabetes onset, DBP and HbA1c with insulin treatment *(Table 17b).*

Table 17*a*: Insulin treated vs. non–insulin treated LADA subjects

	LADA (n = 9)		
Treatment	Insulin *n, %* (2, 22.2%)	No insulin *n, %* (7, 77.7%)	P-value
Females, n (%)	0 (0)	4 (57.1)	
Males, n (%)	2 (100)	3(42.9)	0.444
Age (years)	38.0 ± 5.7	41.6 ± 8.3	0.593
Age at diagnosis (years)	33.5 ± 5.0	38.1 ± 8.0	0.474
Disease duration(months)	58.5 ± 0.7	42.4 ± 12.1	**0.012**
BMI(kg/m$^{2)}$	22.3 ± 7.0	26.6 ± 3.2	0.214
Waist circumference(cms)	76.4 ± 3.3	92.5 ± 4.7	**0.003**
SBP (mmHg)	101 ± 1.4	119.4 ± 16.4	0.174
DBP(mmHg)	69.5 ± 0.7	77.0 ± 12.7	0.453
HbA1c (%)	11.4 ± 2.1	8.1 ± 1.8	0.064
MS (%)	0 (0)	4 (57.1)	0.444
C-Peptide (ng/ml)	0.4 ± 0.4	1.8 ± 0.7	**0.027**
High-GADA titer, n (%)	2 (100)	2 (40)	0.167

Data are expressed as mean ± SD and n (%).

Table 17 *b*: Insulin treated vs. non-insulin treated in DM2 subjects

	DM2 (n = 130)		
Treatment	Insulin *n, %*(6, 4.6%)	No insulin *n, %* (124,95.4%)	P-value
Females, n (%)	2 (33.3)	53(42.7)	
Males, n (%)	4 (66.6)	71(57.2)	1.000
Age (years)	43.5 ± 5.3	47.3 ± 9.4	0.323
Age at diagnosis (years)	38.6 ± 5.2	44.7 ± 9.5	0.128
Disease duration(months)	58.2 ± 2.0	32.5 ± 17.5	**<0.001**
BMI(kg/m$^{2)}$	23.2 ± 4.12	28.5 ± 4.7	**0.007**
Waist circumference(cms)	85.0 ± 12.2	97.4 ± 9.4	**0.002**
SBP (mmHg)	119.3 ± 5.1	126.8 ± 14.2	**0.013**
DBP(mmHg)	94.0 [71.3 – 129]	80.0 [75.0 – 85.0]	0.267
HbA1c (%)	9.2 [6.9 – 13.2]	7.0 [6.6 – 8.3]	0.051
MS (%)	3 (50%)	112 (90.3)	**0.020**
C-Peptide (ng/ml)	1.2 ± 0.3	2.3 ± 0.8	**< 0.001**

Data are expressed as mean ± SD, median [IQR] and as n (%).

9. Assessment of insulin treated LADA patients *vs.* insulin treated DM2 patients

With the aim to assess the factors associated with insulin treatment in LADA and DM2 patients, insulin treated LADA patients (n =2) were compared with insulin treated DM2 patients (n=6). Insulin treated LADA subjects had significantly lower

SBP (101 ± 1.4 *vs.* 119.3 ± 5.1; p = 0.003 mmHg) and fasting C-Peptide (0.4 ± 0.4 *vs.*1.2 ± 0.3 ng/ml; p = 0.021) than insulin treated DM2 subjects. *(Table 18)* There was no significant difference observed in other variables between two groups.

Table 18: Insulin treated LADA subjects vs. insulin treated DM2 subjects

	Insulin treated LADA (n=2)	Insulin treated DM2 (n=6)	P-value
Females, n (%)	0 (0)	2 (33.3)	
Males, n (%)	2 (100)	4 (66.6)	1.000
Age (years)	38.0 ± 5.7	43.5 ± 5.3	0.257
Age at diagnosis (years)	33.5 ± 5.0	38.6 ± 5.2	0.266
Disease duration (months)	58.5 ± 0.7	58.2 ± 2.0	0.836
BMI (kg/m^2)	22.3 ± 7.0	23.2 ± 4.12	0.814
Waist circumference (cms)	76.4 ± 3.3	85.0 ± 12.2	0.170
SBP (mmHg)	101 ± 1.4	119.3 ± 5.1	**0.003**
DBP(mmHg)	69.5 ± 0.7	94.0 [71.3 – 129]	0.372
HbA1c (%)	11.4 ± 2.1	9.2 [6.9 – 13.2]	0.557
MS (%)	0 (0)	3 (50)	0.464
C-Peptide (ng/ml)	0.4 ± 0.4	1.2 ± 0.3	**0.021**

Data are means ± SD, medians [IQR] or n (%)

10- Comparison of time to insulin in LADA and DM2 subjects

Time to first insulin treatment since diagnosis of diabetes was calculated in LADA and DM2 subjects. 22.2% of patients in LADA group (n=9) and 4.6% of patients in DM2 group (n=130) required insulin. When compared to DM2 group, LADA group showed earlier requirement of insulin treatment although, the difference was not significant. (p = 0.112). This difference corresponded with hazard ratio of 4.37 (p = 0.144; 95% CI 0.6-31.4) for insulin treatment in LADA compared to DM2 patients.

11- Analysis of non-constituent variables of Metabolic Syndrome (MS) in LADA and DM2 subjects with and without MS

To observe association of non-constituent variables of MS in LADA and DM2, subjects were subdivided into two subgroups with and without MS and compared.

Compared with LADA patients without MS, all LADA patients with MS were female (100% *vs*. 0%; p = 0.008). Age at recruitment, age at diagnosis and duration of diabetes were not significantly different between groups. *(Table 19a)* DM2 patients with MS had higher proportion of females (47% *vs.* 7%; p = 0.004), higher age (47.9 ± 9.3 *vs*. 41.4 ± 6.2 years; p = 0.002) and age at diagnosis (45.2 ± 9.6 *vs*. 38.3 ± 5.4 years; p = < 0.001) compared to the group of DM2 patients not depicting MS. *(Table 19b)*

Table 19 a: Non- constituent variables of MS in LADA subjects with and without MS

	LADA with MS n=*4*	LADA without MS *n=5*	P-value
Male, n (%)	0 (0%)	5 (100%)	-
Female, n (%)	4 (100%)	0(0%)	**0.008**
Age (years)	45.8 ± 9.0	36.8 ± 3.3	0.075
Age at diagnosis (years)	42.0 ± 9.0	33.2 ± 2.58	0.073
Duration of Diabetes (months)	47.0 ± 12.11	45.2 ± 14.4	0.848

Data are means ± SD, medians [IQR] or n (%)

Table 19 b: Non- constituent variables of MS in DM2 subjects with and without MS

	DM2 with MS *n=115*	DM2 without MS *n=15*	P-value
Male, n (%)	61 (53%)	14 (93%)	-
Female, n (%)	54 (47%)	1 (7%)	**0.004**
Age (years)	47.9 ± 9.3	41.4 ± 6.2	**0.002**
Age at diagnosis (years)	45.2 ± 9.6	38.3 ± 5.4	**0.000**
Duration of Diabetes (months)	33.2 ± 17.5	37.7 ± 21.3	0.443

Data are means ± SD, medians [IQR] or n (%)

DISCUSSION

A- LADA AS A DISTINCT CLINICAL ENTITY

In the past, DM1-A was invariably considered to be the only form of autoimmune diabetes in both children and adults. However, in the last few decades growing number of publications have supported the idea of another subtype of adult-onset autoimmune diabetes called LADA, presenting slow clinical onset when compared to DM1-A, and a faster progression to insulinopenia when compared to DM2. For many years, the concept of LADA as a distinct identity has faced many controversies.(154) and the adoption of age and insulin criteria's in defining LADA has been criticized by various authors.(113,155)

In spite of all controversies, the concept about LADA, eponym coined by Zimmet et al.(14) is undeniable today. It may be known by any other name but clearly there is a sub-group of adult patients, clinically diagnosed as DM2 who are initially non-insulin requiring and depict autoimmune markers in serum. To some extent, neither age cut-off nor the time to insulin treatment means so much. In order to assess the prevalence and to characterize LADA in various populations, a standardised diagnostic criterion is required. The adoption of standard criterion and valid methods to define LADA would facilitate the rational comparison of the disease between different populations and a more efficient management for all health providers.

B-COMPARISON OF LADA BETWEEN ASIAN AND WESTERN POPULATIONS.

B.1. Prevalence of LADA

Differences in the prevalence of LADA have been observed worldwide probably, due

in part, to the heterogeneity of this disease. Past studies involving Indian subjects have resulted in inconclusive heterogeneous information which can be attributed to the discrepancy in methodology adopted by various investigators to diagnose LADA.(92,93,103)

This thesis investigated the prevalence of adult-onset autoimmune diabetes and characterizes the clinical, metabolic and immunological profile of patients diagnosed with LADA in Asian Indians.

In the current study, LADA emerged as the most prevalent form of adult-onset autoimmune diabetes. Similar observations of LADA being more prevalent than DM-1A were reported by a multicenter European Action-LADA study and a study from UAE involving a large cohort of adult-onset diabetic subjects.(81,99) As per IDS and Action-LADA group diagnostic criteria, based on GADA measurement, the prevalence of LADA in our cohort was 6.5% which was surprisingly, exactly similar to the LADA prevalence reported in the northern region of China in a large multicenter LADA China study.(87) The overall prevalence of LADA in China, based on GADA determination, was 5.9%.(87)

The prevalence of LADA in our study was lower than 9.7% (81) and 11.6% (80) observed in European subjects but higher than in a cohort from UAE 2.6%,(99) South Korea 1.7%, and 4.4%,(44,227), Japan 3.8% (EHIME study),(88) and North America 4.2% (ADOPT study).(96) To ascertain prevalence of adult-onset autoimmune diabetes, similar to our study, some studies used only GADA(87,88,96) whereas additionally, IA- 2/ICA and/or ZnT8 autoantibodies were used by others.(81,99)

GADA are most frequent and persistent autoantibodies.(228) Among GADA negative patients, only small proportion of patients are positive for other diabetes associated

autoantibodies. Thus, majority of autoimmune diabetes in adults can be detected by using only GADA measurement and GADA only can be used for screening LADA(228) specifically, in resource constraint settings in the developing world. The expected difference in LADA prevalence using only GADA or GADA along with other diabetes autoantibody would not be significant.(81) In the past, low frequency of IA-2 autoantibody has been reported among adults of North Indian origin (100) and Indo-Aryan children with DM1.(229)

Prevalence of LADA in our subjects was highest 13.9 % in individuals aged < 40 years, this is in contrast to the highest prevalence of 13.9% in patients aged 50-59 years reported by Xiuying et al. in a small Chinese cohort.(230) However, consistent with our results, decreasing trend of LADA prevalence with increasing age was evident in large Chinese and European studies.(81)

DM-1A was not prevalent in our cohort. There are no prevalence data available for adult-onset (> 30 years) DM-1A in North Indians but earlier studies have shown the prevalence of DM1-A to be lower among children and adolescents in North Indian populations than in Caucasians. (229,231,232)

B.2. GAD autoantibodies and other immunogenetic features in LADA.

Frequency of GADA positivity in our cohort was considerably higher than 1.5% and 1.6% reported in two large studies involving adult north Indian subjects.(100,102) The participants of these two studies had higher mean age and longer duration of diabetes than in our study. This can be the possible explanation for the discrepancy in results as diabetes autoantibodies in sera tend to disappear with increasing age and duration of diabetes.(81,228) Moreover, sensitivity of the assay used for GADA measurement in one of these studies was lower than used in our analyses.(102)

Prevalence of GADA positivity and LADA in the current study was much lower than demonstrated in Southern India.(92,101) This is contrary to the findings observed in China and Europe, where compared to north, the frequency of LADA was lower in the southern region.(92,101) Interestingly, lower frequency of LADA 2.6% was noted in an island nation Srilanka near southern coast of India.(97) It needs to be assessed if geographical, climatic and cultural differences within a country or continent directly contribute towards regional discrepancies in the prevalence of LADA by conducting large scale studies.

There is not a great discrepancy in the GADA positivity rate in both eastern and western populations.(228,233) Islet antigen-reactive T-cell response in LADA reflects stronger autoimmune reaction than DM2 but similar to DM1 in both populations.(234) Compared to positivity rates for other islet autoantibodies namely IAA, IA-2A and ZnT8, Chinese population has been found to have lower positive rates.(228,233)

In agreement with previous reports, our data also indicate that based on GADA titer different subgroups can be identified within LADA.(81,85,87,188) Beside distinct clinical and phenotypic features, GADA titer is important in defining the severity of disease process and may help in the selection of appropriate therapeutic choices. It has been shown that high-GADA titer patients have tendency of greater HLA association for autoimmune diabetes.(85,131,235) Data from both west and east has shown that high risk HLA haplotypes are associated with younger age, lower β-cell function, leaner body shape, and presence of multiple islet autoantibodies.(83,86–88,236) In Asian Chinese, most common susceptible HLA-DQ genes are moderate risk haplotypes DQA1*03-DQB1*0303, DQA1*03-DQB1*0401, and DQA1*05-DQB1*0201and protective haplotype DQA1*0102-DQB1*0602(126,234) whereas Caucasian patients with LADA show increased frequency of high risk susceptible

DRB1*03/DRB1*04-DQB1*0302 haplotype. β- cell damage in Asians may be less severe as in comparison to Caucasians, Chinese LADA subjects have moderate immune-genotype.(83,237)

Most recently, in Chinese population, discrepancy between LADA and DM1 has been noted in the risk conferred by HLA-DRB1-DQA1-DQB1 loci. Conferred risk significantly differs from those patients with Caucasian roots. This observation may provide further insight in understanding ethiopathogenesis of patients with LADA in Asia.(236) Both Asian and Caucasian LADA patients have inflammatory cytokines and/or chemokines including interleukin (IL)-6, lipocalin 2, and TNF-α similar to those in DM1 subjects.(238,239) LADA and DM1 patients have similar adhesion molecules (soluble (s) E-selectin, soluble intercellular adhesion molecule (ICAM)-1, and soluble vascular cell adhesion molecule (VCAM)-1) and chemokines (CCL2, CCL3, and CCL4).(240)

B.3. Serum C-Peptide levels in LADA.

Compared to DM2 patients, serum C-peptide concentration in the current study were lower in LADA patients. This difference was evident up to 36 months from diagnosis and contrary to Spanish data (124) the difference remained even after 36 months of duration. In The Spanish LADA Study, it was observed that fasting C-peptide concentration in LADA patients was higher than in DM1 subjects but was lower than in DM2 patients ($p < 0.01$). However, this difference in C-peptide was seen only during first 36 months of the disease. Thereafter, overlapping of C-peptide concentrations in LADA patients with that of DM1 and DM2 subjects was noticed. In comparison to patients with DM2, faster progression to insulin treatment from the diagnosis of diabetes was observed in LADA patients. Hazard ratio for insulin

treatment in LADA compared with DM2 was quite elevated (mean value, 8.34; p<0.001).(124)

Analysis of stimulated C-peptide secretion using the mixed-meal tolerance test revealed that patients with LADA have a lower stimulated C-peptide response than the DM2 group and a higher response than the DM1 group.(128) In adults, fasting C-peptide and C-peptide secretion after oral glucose are decreased in GADA positive patients.(80,82,83)

An inverse relation between GADA titres and C-peptide has been reported in LADA patients with rapid progression to insulin therapy.(82,83,124) This observation might explain the higher incidence of insulin treatment in patients with higher GADA titres. Evidence of insulitis in LADA patients was confirmed by increased pancreatic (99m) Tc-IL-2 uptake during pancreatic scintigraphy and MRI. An inverse association between (99m) Tc-IL-2 uptake in LADA patients and duration of disease was found.(241) Better glycaemic control and less requirement of insulin have been observed in patients with low GAD65Ab titers. However, no correlation was seen in IA-2 index with HbA1c.(242) The Wadena City Health Study demonstrated that in non–diabetic adults, insulin secretion is undiminished with age in response to orally administered mixed meal. (243)

B.4. Insulin resistance and metabolic syndrome in LADA

Insulin resistance may vary from low in DM1 to high in DM2. It is known that insulin resistance (IR) does not have any significant role in the disease process of autoimmune DM1.

However, there are controversies related to the contribution of IR in the pathophysiology of LADA. It has been hypothesized that insulin resistance may play a unique role in LADA. In contrast to DM1 and DM2, in LADA, anti-islet autoimmunity, non-autoimmune β-cell dysfunction and increased IR are three mechanism that are supposed to contribute in the disease process.(129) In an ongoing autoimmune destructive process of islet β-cells, diabetes occurs when insulin secretion is not able to meet the high demands rendered by IR that varies from low in DM1 to high in DM2.(129)

Reports have revealed that LADA patients have lower degree of IR than in DM2 but comparable to DM1. Compared to normal population, higher degree of insulin resistance was observed in LADA and DM2 patients.(244) Among Chinese population, Li et al showed a close association between LADA and MS. (245) Positive correlation has been seen between BMI with insulin resistance (IR) both in LADA and DM2.(96)142 Lower BMI has been noted in patients with LADA as compared to DM2 patients.(96,246) However, some studies failed to show any significant difference.(247)

In Action LADA 3 Study, significantly, higher prevalence of metabolic syndrome has been reported in patients with DM2 than in patients with LADA or adult DM1. Significant difference was noted in the frequency of MS in DM1 (31.9%) and LADA (41.9%) ($p=0.015$). Frequency in both groups was less than in DM2 patients (88.8%) ($p<0.0001$, for each). The same study concluded that metabolic syndrome does not characterize autoimmune diabetes. No significant difference in the prevalence of metabolic syndrome was reported in control subjects and autoimmune diabetes if glucose was eliminated as a variable.(131)

In The Spanish LADA Study, the prevalence of MS was higher among LADA patients

than in patients with DM1, but lower than in patients with DM2.(124) Similarly, LADA China study exhibited the presence of MS in LADA, although the prevalence was lower than in DM2 (62% vs 75.5%).(87) As a result of IR in LADA the risk of MS and cardiovascular complications may necessitate it to be considered as a therapeutic target.(129)

Similar to European and Chinese cohorts, current study showed that MS is more common in DM2 subjects than in LADA subjects. Frequency of MS among LADA subjects in our study was 44.4%, slightly higher than 41.9% in European LADA subjects.(131) but much lower than 62% reported in Chinese LADA subjects.(87) Our data further indicates that MS is not prevalent in LADA patients with high-GADA titer. Compared with LADA patients without MS, all LADA patients with MS tended to be female. The current study is the first to describe MS in Indian LADA subjects.

B.5. Clinical features and therapeutic considerations in LADA.

Characteristics of LADA among European, Chinese and Arab populations are remarkably different from GADA-negative DM2 subjects.(81,87,99) In accordance, patients with LADA in our cohort, compared with DM2 patients, were younger at recruitment and onset of diabetes, had lower abdominal circumference, triglycerides, fasting C-peptide (FCP) and frequency of MS. As in Caucasians and Chinese,(81,87) characteristics of LADA patients with high-GADA titer in our study were significantly different than low-GADA titer LADA patients. Patients with high-GADA titer, compared with low-GADA titer, were more likely to be male, leaner and on insulin treatment with lower frequency of MS.

Fasting C-Peptide levels were lower in high- GADA titer patients. Age difference between high-GADA and low-GADA patients was not significant. Contrary to our findings, compared to low-GADA patients, European high-GADA patients tended to be female and younger. Other observations were nearly similar to those reported in large European Action LADA-7 and LADA China study. Further, our data show that compared to LADA high-GADA titer subjects, DM2 subjects had significantly higher systolic blood pressure (SBP) and BMI. Interestingly, this difference was not evident on comparison of total LADA patients with DM2 patients.

In our analysis, LADA patients with low-GADA titer did not significantly differ from DM2 patients. This observation is in accordance with Asian Chinese data (228,248) but contradictory to European data that showed difference between low-GADA titer and DM2 subjects.

We also looked at common and differentiating features associated with insulin requirements among LADA and DM2 subjects. Both insulin treated LADA and DM2 subjects had longer duration of diabetes, lower abdominal circumference and lower fasting C-Peptide levels than noninsulin treated subjects. Insulin treated DM2 subjects, additionally, had a significantly lower BMI and SBP compared with non-insulin treated DM2 subjects. Our results are in contrast to those of Radke et al.(82) who reported that insulin treated DM2 patients were more obese than non-insulin treated DM2 patients.(82)

Our findings show that longer disease duration and lean phenotype is associated with insulin treatment in both LADA and DM2 subjects. Further, comparison of insulin-treated patients with LADA and insulin treated DM2 patients revealed significantly lower SBP and fasting C- peptide in insulin-treated LADA patients. Possibly, the factors necessitating insulin initiation, in LADA and DM2, are not

exactly the same.

In comparison to insulin-treated LADA subjects, higher SBP among insulin-treated DM2 subjects in our study, probably indicate towards a possible association between insulin treatment and insulin resistance along with insulin deficiency. Although, other features of insulin resistance like BMI, waist circumference, DBP did not attain statistical significance but were higher in insulin treated DM2 patients than insulin treated LADA patients.

Above, we have shown that higher proportion of high-GADA titer LADA subjects were treated with insulin compared to low-GADA titer LADA subjects. In insulin treated LADA subjects, insulin treatment was linked to β-cell failure and autoimmunity. Low number of insulin treated patients in our study limits the interpretation but these observations are somewhat in line with those reported in HUNT study by Radke et al.(82)

Although, not statistically significant but similar to Spanish data, our results also showed faster progression to first insulin treatment in LADA patients compared with DM2 patients.(124) Unlike other reports, the proportion of LADA and DM2 subjects on insulin therapy was relatively less in our cohort.(81,82,87,124) This can be probably explained by the fact that generally, compared to others, in India, there is a resistance to insulin therapy among patients and physicians delaying the insulin initiation longer.(249)

Even though, inconclusive, but our research data presents some apparently new information. In our cohort, GADA titers in LADA patients showed some association with gender. Patients with high-GADA were all male whereas low-GADA patients were mostly female. MS was absent in LADA patient with high-GADA titre probably indicating no role of IR in the pathogenesis of high-GADA titer LADA. Among LADA

patients MS was predominant in female sex. These observations probably suggest likeliness of presence of higher IR among female LADA patients as compared to male LADA patients.

There was no significant difference between LADA patients with low GADA titers and DM2. This finding probably hints towards a possibility that LADA patients with low-GADA titer are somewhat similar to DM2 patients as far as metabolic features and β-cell function is concerned and may necessitate development of different therapeutic strategies in LADA based on GADA titers. As this was in concordance with Chinese population(87) but contrary to European data(81) the role of ethnicity in this regard also needs to be investigated.

Presumably, one standard treatment strategy will not be suitable for all LADA patients. Further, our results show that the difference in C-Peptide levels between LADA and DM2 patients persisted even after 36 months of duration possibly, because of better β-cell function in South Asians DM2 patients as compared to Caucasians.(250)

Our results indicate that LADA represents 6.5 % of cases among adult-onset diabetes in a region of north India. LADA is a prevalent form of adult-onset autoimmune diabetes and not rare. LADA patient have distinct phenotypic and metabolic features with lower residual β-cell function than DM2 patients. These patients are generally misdiagnosed with DM2 and generally receive sulphonylurea therapy for years, which, further exhausts β-cells and aggravates the autoimmune process.(203)

It becomes increasingly important to screen LADA patients early to avoid use of β-cell damaging therapies and achieve optimal metabolic control in these patients. At times, due to resource and economic constraints in the developing world, routine

screening with diabetes autoantibodies is not always performed. Not necessarily, but different phenotypic and metabolic features of LADA may help treating physicians identify patients for potential antibody screening.

Though, there are no optimal management strategies for LADA in place but insulin treatment alone or along with therapies that tend to preserve β-cells may help in achieving tight metabolic control in majority of LADA patients. Tight glycemic control in LADA may slow down the rapid decline in β-cell function.(251)

As antibody titers define the severity of disease, LADA patients with high-GADA titers, needs to be closely monitored to assess deterioration in glycemic control and β-cell function. Probably, due to similarities with DM2, LADA patients with low-GADA titers may additionally require therapies targeting insulin resistance and thus reducing cardiovascular complications.

With the ultimate aim of improving the management of LADA patients, in future, large scale studies using validated methods are required to have an improved understanding on various aspects of LADA and establish optimal preventive and treatment strategies for LADA. Diagnosing LADA solely on the basis of autoantibody detection poses a challenge as titer of auto antibodies may fluctuate and disappear with time.(228)

Due to overlapping of certain features, in addition to antibody assays, other novel markers should be identified to differentiate LADA and DM2 at an early stage. Global collaboration using standardised assays and diagnostic criteria is required to obtain insights into ethnic differences in LADA.

CONCLUSIONS

1- LADA represents 6.5% of cases among all adult-onset diabetes in a region of Northern India, considerably a higher frequency than reported in two previous studies. In diabetic subjects diagnosed at 31-40 years of age, the frequency detected of LADA was 13.9%. A decreasing trend of LADA with increasing age was suggested, similar to reports from Chinese and European publications.

2- In our study, LADA was the prevalent subtype of adult-onset autoimmune diabetes, in agreement with earlier reports showing lower prevalence of DM-1A among children and adolescents in Northern India. In this investigated population, the prevalence of LADA was much lower than previously reported in Southern India.

3- The group of subjects with diagnosis of LADA were younger, and presented lower abdominal circumference, serum C-peptide and triglycerides levels at fasting than the group of subjects with DM2 from the same area of Northern India.

4- In our study, LADA patients depicting high titers of GADA in their sera at the time of diagnosis, were more likely to be male, leaner, and insulin- treated, and less likely to display systolic hypertension and the metabolic syndrome.

5- In the same investigated population, LADA patients depicting low titers of GADA in their sera were mostly females, and they did not show phenotypic differences than DM-2 patients, similar to data reported for Asian Chinese population, in contradiction to the data reported for European population.

6- In comparison to subjects with DM2, serum C-peptide levels at fasting at the time of diagnosis were lower in LADA patients in the current study. This difference remained after 36 months, contrary to data of Spanish LADA Study.

Concluding remarks.

A- We acknowledge that due to small number of subjects in LADA group, our results cannot be generalized. Furthermore, due to the inclusion of patients with disease duration up to five years after diagnosis, LADA cases might have been underestimated as GADA titer may fluctuate and decrease over time.(228)

B- Nevertheless, to best of our knowledge, this is the first study in the region of Northern India providing original insights into clinical, metabolic and immunological characteristics of patients with LAD

FUTURE OUTLOOK

Most patients with adult-onset autoimmune diabetes are non-insulin requiring at least, in the first few months after diagnosis. However, as discussed earlier the criteria of age at onset and insulin free period are arbitrary. In clinical practice, substantial number of patients who initially present as DM2 may actually have decline in β-cell function due to autoimmune mediated damage of beta cells. Due to the slow progression, if not diagnosed early in the disease process, these patients may eventually be managed with OHA's especially SU's for years particularly in the developing world. The suboptimal glycaemic control will lead to more long term complications of diabetes.

Because of ongoing autoimmune process in LADA, merely achieving the glycaemic control does not seem to be an adequate treatment strategy. The emphasis should be to explore all possible treatment options that may ultimately help in β-cell preservation and regeneration as preserved insulin secretion capacity has shown better clinical results.(252) In comparison to DM1-A with rapid disease progression, treatment strategies with potential to preserve and regenerate β-cells may prove to be beneficial in LADA due to its slower progression. It has been shown that drugs in this regard currently approved for the treatment of DM2 may be used for LADA treatment.(252) Current data supports only a possible protective effect of incretin hormones on human β-cells, reducing apoptosis however, in animal models glucagon-like peptide 1 (GLP1) has shown to induce β-cell self-renewal, reduces β-cell apoptosis and promotes β-cell neogenesis from ductal cell precursors. LADA patients are less likely to benefit from incretins than patients with DM2 in terms of reductions in levels of glycaemia however this observation does not rule out the possibility of GLP1 receptor agonists in terms of β-cell protection suggested by 17% reduction in insulin doses when used with insulin even in those with low C-

Peptide levels.(253) Dipeptidyl peptidase 4 (DPP4) inhibitors like sitagliptin, linagliptin and saxagliptin have also shown some beneficial effects in terms of β-cell protection.(209,254,255) Most recently, a Japanese pilot trial demonstrated that compared to insulin, treatment with sitagliptin may be more effective in preserving β-cell function for at least 4 years possibly through immune modulatory effects of DPP4 inhibitors.(256) Large scale clinical trials with longer duration are required to get a further clarity in this matter.

The role of insulin sensitizers like metformin in the treatment of LADA needs to be further looked into considering the evidence of presence of IR in LADA. Currently, there are no controlled trials available on effect of metformin alone in LADA. Use of metformin alone or in combination with other available drugs (OHAs and insulin) is a matter of uncertainty. So far no reports either favouring or discouraging its use are available.(74) Rosiglitazone, insulin sensitizer of thiazolidinedione class has shown encouraging results when used along with insulin. β-cell function remained stable in those who were receiving rosiglitazone with insulin.(252,257) However, concerns about harmful cardiovascular effects of rosiglitazone should be kept in mind.(258) In spite of heterogeneity seen in the prevalence, clinical features and diagnostic criteria's used in various studies with different ethnic population it is known that a condition presently known by name LADA exits and should be diagnosed early in the disease process. Although, this condition may be known by any other names as mentioned earlier but it is important to classify this condition as it may have therapeutic implications.

Because of the clinical, immunogenetic and metabolic heterogeneity of LADA seen in various populations, the results of studies done in one particular population cannot be extrapolated to other ethnic groups. More studies are required from

populations with different ethnic backgrounds to correctly classify this sub-group and further investigate possibilities of future treatment options.

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WILEY

REVIEW ARTICLE

Latent autoimmune diabetes in adults (LADA) in Asian and European populations

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Summary

Diabetes mellitus is a chronic disorder caused by relative or absolute insulin deficiency and characterized by chronic hyperglycaemia. It is expected that by year 2025, 80% of all type 2 diabetic patients will be living in developing or low- and middle-income countries. Among Asians, there has been an overall increase in abdominal obesity; however, the risk of diabetes in these populations starts at much lower body mass index as compared to Caucasians. A significant proportion of diabetic patients with adult-onset, initially nonrequiring insulin treatment, have diabetes-associated autoantibodies in their sera. A new subclass of diabetes with the designation of latent autoimmune diabetes of adult-onset (LADA) has been proposed for this category of subjects. Studies have demonstrated that patients with autoimmune diabetes, characterized by the presence of glutamic decarboxylase autoantibodies display a different clinical phenotype from classical type 2 diabetes without glutamic decarboxylase autoantibodies. This subset of phenotypic type 2 diabetes subjects with islet autoantibodies tend to have sulphonylurea failure and need insulin treatment earlier in the disease process. Diagnosing LADA at an initial stage will be important so that insulin can be initiated earlier, facilitating improved glycemic control sooner as well as the preservation of residual beta-cell function in adult-onset autoimmune diabetes. Because of differences in dietary habits, environmental factors, and phenotypic characteristics between European and Asian populations, there may be heterogeneity in the prevalence and other characteristics of LADA in these two populations.

KEYWORDS

adults, Asians, autoimmune, diabetes, Europeans, latent

1 | INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder caused by relative or absolute insulin deficiency and characterized by chronic hyperglycaemia.[1] According to the data released by International Diabetes Federation an estimated 415 million people have diabetes. Its incidence is increasing rapidly, and by 2040, this number is expected to rise to 642 million.[2] It is expected that by the year 2025, 80% of all type 2 diabetic patients will be living in developing or low-and middle-income countries. Among Asians, there has been an overall increase in abdominal obesity and the risk of diabetes in these populations starts at much lower body mass index (BMI) as compared to Caucasians. South Asians are more insulin resistant than Europeans despite low BMI.[3]

A significant proportion of diabetic patients with adult-onset, initially nonrequiring insulin treatment has autoantibodies against pancreatic antigens in their sera. A new subclass of diabetes, latent autoimmune diabetes in adult-onset (LADA) has been proposed for this category of subjects.[4,5] Preliminary studies have demonstrated that patients with autoimmune diabetes, characterized by the presence of glutamic decarboxylase autoantibodies (GADA) display a different clinical phenotype from classical type 2 diabetes (DM2) without GADAs and tend to have sulphonylurea failure requiring early insulin treatment[6–8] Diagnosing LADA at an initial stage is important to facilitate improved glycemic control as well as the preservation of residual beta-cell function. Ethnic variation and correct diagnosis of LADA also have relevant therapeutic implications.[3] Because of differences in dietary habits, environmental factors, and phenotypic characteristics between European and Asian populations, there may be heterogeneity in the prevalence and characteristics of LADA in these 2 populations. The aim of this review is to describe main

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characteristics of LADA and highlight the differences and/or similarities of features LADA between European and Asian people. We reviewed the literature using Medline, Pubmed, Pubmed central, Google scholar, Science Direct, and Cochraine library database. Additionally, data were collected through select reputable internet websites and conference abstract books. Search terms, "latent," "autoimmune," "diabetes," "adults," "Asia," and "Europe" were included.

2 | AUTOIMMUNE DIABETES IN ADULTHOOD

Adult-onset autoimmune diabetes consists of several subgroups. In the recent decades, it has been demonstrated that approximately 30% of cases of classical type 1 diabetes (DM-1A) are diagnosed after 30 years of age (late-onset type 1 diabetes).[9,10]

It has been recognized that around 10% of adult subjects initially classified as DM2 have autoantibodies to pancreatic autoantigens in their sera.[11,12]

Mostly Classic DM-1A has been considered to be a childhood disease. However, increasingly autoimmune diabetes is being observed in adults. Depending upon the need of initial insulin treatment, clinicians usually classify those who require immediate insulin as classical DM-1A and the other group not requiring insulin at least in the first 6 months after diagnosis as LADA. Other parameters used in clinical practice to distinguish DM-1A from DM2 are phenotypic characteristics like age, obesity, and presence of other autoimmune disorders. However, this clinical distinction is not straight forward and not always correct.[13]

Hawa et al defined classic DM-1A as subjects with diabetes and associated autoantibodies in whom insulin was started immediately at diagnosis or within 1 month of diagnosis.[14] Characteristically, patients with LADA progress slowly towards insulin requirement (within 5-6 years) and older patients with LADA show even a slower progression.[5,7] Further, LADA is discussed in greater detail below describing phenotypic, clinical, immunogenetic characteristics, and treatment options with focus on specific difference and similarities between European and Asian population.

2.1 | Missing points in the present classification of diabetes in adults

In adult patients with autoimmune diabetes, usually the presence of residual β-cell function makes the clinical presentation similar to DM2. Additionally, many patients with DM2 remain undiagnosed for years and present with severe hyperglycaemia requiring immediate insulin therapy. Diabetes is more heterogeneous than assumed. The present ADA classification does not include many patients with hybrid form of diabetes having genetic predisposition to both DM-1A and DM2 with pancreatic antibodies.[15] This form of diabetes is commonly called LADA or type 1.5 diabetes. Usually, these patients initially diagnosed as DM2 do not require insulin therapy at least within first 6 months but progress to insulin early as compared to antibody negative patients.[16] Such uncertainties mandate a revised and improved classification of diabetes to include new emerging types of diabetes.

3 | DEFINITION, DIAGNOSIS, AND CHRACTERISTICS OF LADA

3.1 | Definition

Latent autoimmune diabetes in adult is defined as initially noninsulin requiring diabetes with associated autoantibodies like GAD, protein tyrosine phosphatase-like protein autoantibody (IA-2), insulin, or Zinc T8 transporter antibody (ZnT8). Patients with LADA are at high risk of progression to insulin dependency. Clinically, such patients are initially diagnosed with DM2.[16,17]

3.2 | Diagnosis

Action LADA group and the Immunology of Diabetes Society have proposed the following specific criteria to diagnose LADA:

1. At diagnosis patient should be at least 30-70 years of age
2. Presence of at least 1 of the 4 islet cell autoantibodies, ie, ICA, autoantibodies to GAD65, IA-2, and insulin) in serum
3. At least, 6 months of noninsulin requiring diabetes[11,18]

Presence of circulating islet autoantibodies distinguishes LADA from DM2 and insulin independence at diagnosis distinguishes LADA from classic DM-1A.[18]

However, the criteria of insulin treatment with insulin within the first 6 months are meant to distinguish LADA and classic DM-1A diagnosed after 30 years of age and is subjective.[18] Initiation of insulin treatment is dependent on the judgment of the treating physician and should not be used alone to define patients with LADA.[19]

It has been argued that to define LADA, criteria of age >30 years is incorrect and arbitrary as there is a subset of obese children who are positive for pancreatic autoantibodies, but are not prone to ketosis.[20,21] As discussed earlier, the disease process in classic DM-1A and LADA is autoimmune in nature whereas the classic DM2 is not autoimmune.[22–24]

4 | COMPARISION OF LADA BETWEEN ASIAN AND WESTERN POPULATION

4.1 | Epidemiology

Around 3-14 % of patients clinically diagnosed as DM2 have islet autoantibodies in their sera. High frequency of 7%-14% GADA-positive DM2 has been reported in northern Europe.[7,25–27] Lower frequency of 4%-6% GADA positivity has been observed in studies from southern Europe, Northern America, and Asia.[28–32] This varied frequency of GADA could be attributed to biases like selection criteria, age at onset of diabetes, disease duration, and assays used.[33]

In Asia and particularly in India, data from studies assessing the prevalence of LADA are sparse. Two studies from China reported 9% and 5.9% prevalence of LADA, respectively.[30,34] In Japan, the prevalence of GAD autoantibodies in adults with DM2 is 3%-4% (Table 1, Figure 1).[31] Prevalence of adult onset DM-1A in Japan is twice

TABLE 1 Prevalence of LADA in Asian and European population

Author	n	LADA (%)	Region	Age at Diagnosis	Duration of DM (years)	Other Specific Inclusion Criteria
Zhou et al[30] LADA China	4880	5.9	China	>30	<1	
Xiuying et al[34] China	498	9.2	China	≥35	Any	
Katulanda et al[35]	992	2.6	Srilanka	16-40	Not specified	Age <45 y at the time of study
Kunungo et al[36]	214	42	India	>20	Any	
Shrivastav et al[37]	300	44.67	India	>20	Not specified	Age 25-40 y, BMI <25 kg/m^2, SU failure
Chandni R et al[38]	31	58	India	>30	<3	BMI <23 kg/m^2
Brahamkshatriya et al[39]	80	5	India			
Takeda H et al[31] (Ehime study)	4098	3.8	Japan	>20	Any	
Park Y et al[40] KNDP study group	884	4.4	Korea	35	<5	Age 35-70 y
Maddaloni et al[41]	17 072	2.6	UAE	30-70	Any	
Buzzetti, R[28] NIRAD	4250	4.5	Italy	>20	6 m-5y	
Radtke et al[25] HUNT	1261	10	Norway			
Tuomi T et al[26] BOTNIA	1122	10.1	Finland	>35	Any	
Turner R et al[7] UKPDS	3672	12	UK	25-65	<1	
Hawa et al[14] Action LADA	6156	9.7	Europe	30-70	<5	

Abbreviations: BMI, body mass index; DM, diabetes mellitus; LADA, latent autoimmune diabetes in adult

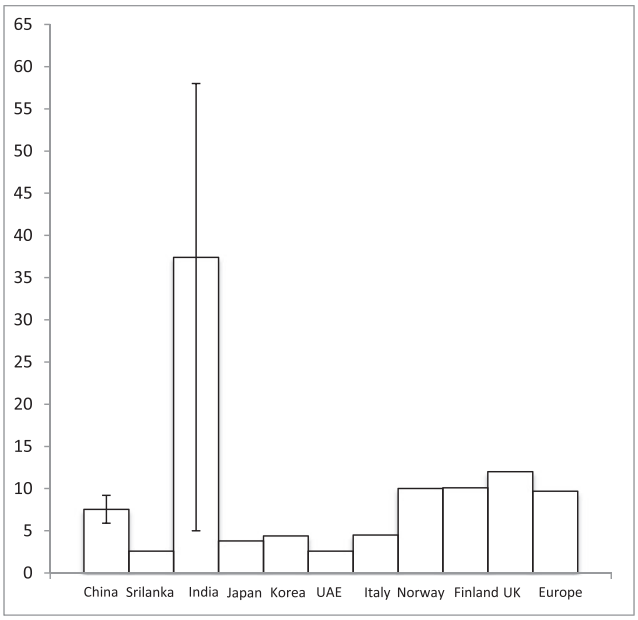

FIGURE 1 Prevalence of latent autoimmune diabetes in adult in different countries of Europe and Asia

compared with childhood DM-1A. The incidence rate of childhood DM-1A in Japan is the lowest in the world.[42] Interestingly, similar prevalence of 4.4% in both Korea and Italy was reported in a collaborative project between both countries.[43] Recently, Maddaloni et al reported a prevalence of 2.6% in the United Arab Emirates (Table 1, Figure 1).[41] Prevalence of LADA among different population is shown below (Table 1).& (Figure 1).

With reference to the prevalence of LADA in India, studies have revealed controversial results[36–39,44,45] Such variation in results may be attributed to the differential aspects such as age at onset, demographics of the studied subjects, and methods used to measure GAD/Protein tyrosine phosphatase-like protein (IA-2) antibodies. Considerably, high prevalence of LADA was reported in few studies.[36,37,46] These studies included specific subgroup of subjects who were young, nonobese, and had early onset of diabetes with higher probability of LADA.[37,38] Such results may not represent the true prevalence of LADA in Indian population. It is assumed that by applying the criteria suggested by Immunology of Diabetes Society and European Action LADA, the prevalence of LADA will be much lower.

Additionally, these studies do not specify the sensitivity and specificity as validated by Diabetes Antibody Standardization Program of the methods used to measure GAD/IA-2 antibodies.[37,39,44,46] Sensitivity and specificity of the methods used may not be ideal.

4.2 | Similarities

Latent autoimmune diabetes in adult is prevalent among both Asians and Europeans. Both Caucasians and Asian Chinese population have shown difference in the prevalence of LADA between northern and southern regions. Studies have reported higher prevalence of LADA in Northern region of both Europe (7%-14%) and China (6.5%).[14,30] Frequency of LADA in Asians is somewhat similar to the prevalence reported in Southern Europe. Amazingly, similar prevalence of 4.4% was noticed in Korean and Italian populations.[40,47]

4.3 | Differences

Possibly, because of different lifestyle, diet, and phenotypic characteristics, there are differences in the prevalence of LADA in both

populations. Compared to Caucasians, results of large studies have shown lower prevalence of LADA in Asian population ranging from 2.6 % in the United Arab Emirates to 5.7% in China. Two large studies[45,48] on Indian subjects also revealed low prevalence of LADA 2.6% and 3.2%. There seems to be a clear difference between the prevalence of LADA in Northern Europe and Asia.

5 | CLINICAL, BIOCHEMICAL, AND METABOLIC FEATURES

Generally, LADA patients are older than 30 years, nonobese, and noninsulin requiring at diagnosis. However, presence of obesity does not exclude LADA. These patients are initially noninsulin requiring but progress towards insulin dependency within a short period ranging from few months to years. As compared to classic DM2 subjects (antibody negative), LADA patients are believed to have rapid progression to insulin dependence. Such patients show extremely diminished C-peptide reserve. As compared to patient with DM2, LADA patients have lower rate of hypertension, lower BMI, waist-hip ratio, total cholesterol, and higher high-density lipoprotein (HDL) cholesterol.[49,50]

An inverse relation between GADA titres and C-peptide has been reported in LADA patients with rapid progression to insulin therapy.[26,50] This observation might explain the higher incidence of insulin treatment in patients with higher-GADA titres. Conversely, patients with low-GADA titre need less insulin and have better glycaemic control.

Insulin resistance (IR) may vary from low in DM-1A to high in DM2. It is known that IR does not have any significant role in the disease process of autoimmune DM-1A. However, there are controversies related to the contribution of IR in the pathophysiology of LADA. It has been hypothesized that IR may play a unique role in LADA.

In contrast to DM-1A and DM2, in LADA, anti-islet autoimmunity, nonautoimmune β-cell dysfunction and increased IR are 3 mechanisms that are supposed to contribute in the disease process. In an ongoing autoimmune destructive process of islet β cells, diabetes occurs when insulin secretion is not able to meet the high demands rendered by IR that varies from low in DM-1A to high in DM2.[51] Reports have revealed that LADA patients have lower degree of IR than in DM2 but comparable to DM-1A. Compared to the normal population, higher degree of IR was observed in LADA and DM2 patients.[52]

In the LADA 3 study, significantly, higher prevalence of metabolic syndrome (MS) has been reported in patients with DM2 than in patients with LADA or adult DM-1A. Significant difference was noted in the frequency of MS in DM-1A (31.9%) and LADA (41.9%) ($P = .015$). Frequency in both groups was less than in DM2 patients (88.8%) ($P < .0001$ for each). As a result of IR in LADA, the risk of MS and cardiovascular complications may necessitate it to be considered as a therapeutic target.[51]

5.1 | Similarities

Among both Caucasians and Asians, LADA is the most prevalent type of adult-onset autoimmune diabetes. As compared to classic DM-1A, LADA was far more frequent (OR 3.3) in the multicentred European Action LADA 7 study.[14] In Europe, prevalence of adult-onset autoimmune diabetes including LADA is greater than childhood DM-1A. Similar observations have been documented in China where interestingly, childhood-onset DM-1A is rare.[30]

Both Caucasian and Asian patients have nearly the same mean age at diagnosis (50.3 years ±12 versus 49.6 years ±8.3, respectively) and hemoglobin A_{1c} values (7.5% ± 1.7 versus 7.4% ± 1.5, respectively).[47] Both Asian and Caucasian LADA patients have inflammatory cytokines and/or chemokines including interleukin 6, lipocalin 2, and tumor necrosis factor-α similar to those in DM1 subjects.[53,54] Various studies from Asia and Western countries demonstrated that IR in LADA is similar to that of DM2.[55–57]

5.2 | Differences

As compared to the Western Caucasian population, lower-mean BMI has been observed in Chinese Asians 28.7 kg/m^2 vs 23.9 kg/m^2.[21,26] Similar findings were observed in another collaborative project between Italy and Korea in which compared to Koreans, Caucasians had significantly higher BMI (27 ± 5.1 *vs.* 25.3 ± 3.3 kg/m^2)[43,47]

In Europeans, the frequency of MS in LADA is similar to that in DM-1A and less than in DM2.[58] Whereas, Asians have a similar frequency compared to that in DM2.[56] Asian data has shown similar high-sensitivity C-reactive protein (hs-CRP), an inflammatory marker in individuals with LADA and DM2.[53] Metabolic syndrome in Asians LADA patients is similar to DM2 whereas in European LADA patients it is similar to DM-1A.[59] Asian patients with LADA may have higher-postprandial glucose values. A study has demonstrated that with the same hemoglobin A_{1c} levels, fasting blood glucose are significantly higher in Caucasians as compared to Asian LADA patients.

Some relevant differences have been noted in the lipid profile of these 2 populations. Triglycerides levels are significantly higher (201 mg⁄dl ± 169 mg⁄dl versus 144 mg⁄dl ± 104

mg⁄dl, respectively, $P < .01$), ($p < .01$) in Asians whereas higher–high-density lipoprotein cholesterol levels (but not total) has been reported in Caucasians (50 mg⁄dl ±3 mg⁄dl versus 44.7 mg⁄dl ± 11 mg⁄dl, respectively, $P < .03$) ($p < .03$). This difference may be attributed to the different dietary pattern in these 2 populations.[43]

Ehime study showed that Japanese LADA patients required less insulin treatment than Caucasians.[54] In addition to immunological factors, variation in environmental and genetic factors among different races might be responsible for such observation.

In Europeans, LADA and DM1 patients had similar level of serum adhesion molecules [soluble (s) E-selectin, soluble intercellular adhesion molecule (ICAM)-1, and soluble vascular cell adhesion molecule (VCAM)-1] and chemokines (CCL2, CCL3, and CCL4).[60] Compared to DM1 such markers were higher in Chinese LADA[53]

6 | IMMUNOLOGICAL FEATURES

Diabetes related antibodies are present in majority of subjects with autoimmune diabetes. There are 4 most described islet autoantibodies, namely, islet cell autoantibodies, to native insulin (IAAs), to GAD (GADA), and to tyrosine phosphatase (insulinoma-associated antigens

IA-2A and IA-2ß).[61–65] It was envisaged that these autoantibodies played a direct role in the destruction of islet β cells. However, this hypothesis has been questioned. It is still not clear if these antibodies are only one of the markers of an autoimmune process or have direct contribution in β-cell damage.[66] IA-2A and IAA do not provide much information in adults[67,68] and finally, it is clear that in adult-onset diabetes, GAD 65 autoantibodies are the most common autoantibody. Antibody clustering characterizes classic childhood DM-1A.[11,33] Changes in the autoantibody status have been observed in longitudinal studies.[7,69] Evidence shows that during the disease process new antibodies may develop and existing autoantibodies may get lost.[70]

It would be imperative to add that autoimmunity in DM2 is not restricted to the presence of autoantibodies. Self reactive T cells (Tregs) have been detected in autoantibody-negative DM2 patients.[71,72] T cells represent a strong link between inflammatory and autoimmune alterations. It has been demonstrated that islet reactive T cells can be present in phenotypic DM2 patients, and their presence is associated with more severe β-cell lesion and lower–residual insulin secretion.[71,72]

6.1 | Similarities

In both Eastern and Western populations, GADA has similar rates of positivity.[73,74] To predict β-cell function, GADA is considered the most important antibody in both populations.[75] Studies in both populations have shown bimodality of GADA titre.[28] In both, high-GADA titre is associated with autoimmune thyroid antibodies. Islet antigen-reactive T cell response in LADA reflects stronger autoimmune reaction than DM2 but similar to DM1 in both populations.[59]

6.2 | Differences

Compared to positivity rates for other islet antibodies, namely, IAA, IA-2A, and Znt8 antibodies have been found to be lower in Chinese populations.[73,74] Decrement in the frequency of GADA with age >30 years has been seen among Europeans.[7] No such effect was noticed among Chinese population.[30] Compared to Italian population, less number of patients had high-titre GADA in China.[28,30] Prevalence of anti-IA2, only positive LADA subjects from the United Arab Emirates was higher than Europeans.[41]

7 | GENETIC FEATURES

Study by Desai et al analysed association of *HLA-DRB1* AND *HLA-DQB1* genotype with LADA in a European population and showed the difference in the distribution of *HLA-DRB1* and *HLA-DQB1* genotype between LADA and control subjects.

HLA-DRB1 in the DRB1*0301/DRB1*0401 heterozygotes conferred the highest risk for LADA. Protective effects of LADA were conferred by the following genotypes: DRB1*0701/DRB1*0501-06 and DQB1*0301/DQB1*0602 [77]

Howson et al investigated 21 human leukocyte antigen (HLA) diabetes related loci and 24 non-HLA loci in autoantibody positive adult diabetic subjects. The analysed data revealed strong association with highly DM1 predisposing alleles, *HLA-DRB1**03 (DR3) and *HLA-DRB1**04 (DR4), without evidence of synergistic effects (OR 5.36 for DR3; OR 4.98 for DR4).[76]

As for the European population, in LADA China study, analysis of *HLA-DQ* gene showed significantly higher frequency of 63% diabetes-susceptibility haplotypes in patients with LADA compared to subjects with DM2 (47.1%) and control (43.2%). Frequency of diabetes-protective haplotypes was significantly lower in LADA (22.8%) than DM2 (33.3%) and control subjects (32.7%).[30] Common and different diabetes susceptibility haplotypes in Europeans and Asians are shown below (Table 2).

Insulin (INS) (11p15.5), followed by protein tyrosine phosphatase nonreceptor type 22 (PTPN22) (1p13.2) showed strongest association outside major histocompatibility complex region. In the past, it has been demonstrated that DM-1A specific genes like HLA, *PTPN22*, and INS increases the risk of LADA. On the basis of these observations, it has been suggested that LADA represents a subtype of classic DM-1.[78,79] However, evidence has shown that LADA differs from this subgroup of DM-1A. Patients with LADA have less severe symptom and progression towards insulin dependence is slow similar to DM2.[11]

Later, various studies have shown the association of strong DM2 susceptibility gene transcription factor 7-like 2 (TCF7L2) gene (10q25.3) variant rs 7903146 C to T polymorphism with LADA.[80,81] This finding raised the possibility of *TCF7L2* gene contributing to the risk of LADA. *TCF7L2* gene has not been found to be associated with DM-1A.[11,82] TCF7L2 gene variant confers similar effect size in LADA and DM2.

Because of the overlapping of path mechanisms, LADA is not clearly distinguishable from DM1 and DM2. LADA lies in the middle of the continuous spectrum of the diabetes disease process starting from classic childhood DM-1A and age-related deterioration of glucose tolerance at other end.[83]

Padmos et al investigated the differential patterns of inflammatory gene expressions in monocytes of patients with DM-1A (juvenile onset), LADA, DM-1A (adult onset), and as controls, DM2 patients and healthy controls. These genes are involved in the process of inflammation, motility, adhesion, chemokines, cell survival/ apoptosis,

TABLE 2 Common and different diabetes susceptibility haplotypes in European and Asians[30,77]

Common	Chinese Asians	Europeans
DQA1*03-DQB1*0302,	DQA1*03-DQB1*0303,	DQB1*0201/DQB1*0202;
DQA1*03-DQB1*0401		DRB1*0301/DRB1*0301;
DQA1*05- DQB1*0201		DQB1*0302/DQB1*0302
		DQB1*0201/DQB1*0501
		DRB1*0301/DRB1*0701;

mitogen activated protein kinase pathway and metabolism. Two clusters of genes were identified. Cluster 1 included 12 proinflammatory cytokines with putative gene phosphodiesterase 4B (PDE4B) and cluster 2 comprised 10 genes with putative fatty acid-binding protein 5 (FABP5). *PDE4B* plays crucial role in the cytokine production of monocytes [144] and *FABP5* may play a role in fatty acid uptake, transport, and metabolism.[84]

Overall, cluster 1 was found in LADA (60%), adult-onset DM1-A (28%), juvenile-onset DM-1A (10%), and DM2 (10%) whereas cluster 2 was found in 43% of juvenile-onset DM1-A and 33% of LADA patients less and than 10% each in adult-onset DM1-A and control subjects. In monocytes of DM2 subjects, many inflammatory genes showed upregulation but mostly (83%-100%) had normal expression of *PDE4B* and *FABP5*, the key genes. The distinct monocyte gene expression profile supports the idea of heterogeneity in the pathogenesis of autoimmune diabetes.[84]

7.1 | Similarities

Data from both Western and Eastern countries have shown that in general, compared to DM2, HLA diabetes susceptibility haplotypes are more frequent in LADA and DM1 whereas protective haplotypes are less frequent.[28,85] High-risk HLA haplotypes are associated with younger age, lower–β-cell function, leaner body shape, and presence of multiple islet autoantibodies.[26,29–31,86]

7.2 | Difference

In Asian Chinese, most common susceptible *HLA-DQ* genes are moderate risk haplotypes DQA1**03-DQB1*0303*, *DQA1*03-DQB1*0401*, and *DQA1*05-DQB1*0201* and protective haplotype *DQA1*0102-DQB1*0602*.[30,87] whereas Caucasian patients with LADA show increased frequency of high-risk susceptible *DRB1*03/DRB1*04-DQB1*0302* haplotype. β- cell damage in Asians may be less severe as in comparison to Caucasians, Chinese LADA subjects have moderate immunogenotype.[26,86]

DM2 associated gene *TCF7L2* is also associated with LADA. Single nucleotide polymorphism of *TCF7L2* in Chinese is rs290487 allele different from Europeans with rs7903146T allele.[88,89]

8 | TREATMENT STRATEGIES

Considering the autoimmune nature of LADA with impairment of β cell function at diagnosis, insulin therapy is the treatment of choice. Initial treatment with glibenclamide should be avoided as it might further aggravate the autoimmune process.[90] Metformin can be useful in obese patients with LADA. However, the treatment of choice is insulin.

Insulin does not seem to have immunomodulating effect and cannot be considered a preventive treatment for autoimmune diabetes. Unspecific effect of insulin on glucose toxicity helps in improvement of β-cell function.[91–93] DiaPep277, a heat-shock protein peptide was found to preserve endogenous insulin perhaps through induction of a shift from T-helper 1 (interferon-γ production reduced) to a T-helper 2 (interleukin 9 and -13 increased), which are produced by autoimmune T cells. Further studies are needed to clarify the effect on β-cell function in autoimmune diabetes.[94]

Subcutaneous GAD65 in LADA increased fasting C peptide levels after 24 weeks in those treated with moderate doses of 20 μg. Interestingly, lower (4 μg) or higher (50 or 100 μg) doses did not show any difference. This is first safe report of immunomodulation in LADA.[95]

In comparison to insulin alone, treatment with DPP4 inhibitor sitagliptin and insulin maintained β-cell function in LADA patients.[96] Exenatide, a Glucagon-like peptide (GLP-1) agonist as adjunctive agent to insulin therapy shows beneficial effects on postprandial glucose and insulin sensitivity in DM-1A patients and in future, along with insulin, as an adjunct therapy, incretins may play a vital role in the management of these patients.[97] Considering these results, it can be hypothesised that these agents might have a potential therapeutic role in the management of LADA patients in future.

Recently, keeping in mind the pit falls of earlier therapeutic approaches used in DM1, recently, a novel trial using the immunosuppressant agent cyclosporine A, a proton pump inhibitor, lansprazole that markedly increases the plasma gastrin levels was started.[98]

Data from Cohraine database have reported that treatment with Sulphonylurea (SU) leads to earlier insulin dependence. Vitamin D with insulin may protect pancreatic beta cells in LADA. Novel treatments such as GAD65 in certain doses (20 μg) have been suggested to maintain fasting and stimulated C-peptide levels. Presently, no significant evidence exists to justify in favour of or against available treatment of LADA. However, in view of above data SUs, most frequently used oral hypoglycaemic agents (OHA) should be avoided in patients with LADA.[99]

Future strategies should be based on the development of pharmacological therapies not only to cure autoimmune diabetes but most importantly for prevention. As targeting autoimmunity is a difficult drug target, unless potential drugs are used for prevention, the benefits will reach only to the limited population. Potential therapeutic development should focus on pancreatic preservation and regeneration.

8.1 | Similarities

In spite of the fact that LADA is a prevalent subtype of diabetes, no treatment guidelines for LADA are available. In any population, general treatment strategy for LADA should target glycaemia, immunomodulation, and long-term β-cell preservation. Studies in both Asians and Europeans clearly show the contraindication of SU as potential therapy in LADA from both physiopathology and clinical observations.[42,90,100] Insulin does not seem to have immunomodulating effect and cannot be considered a preventive treatment for autoimmune diabetes. However, treatment with insulin has shown better preservation of beta-cell function in both populations probably by reducing glucose toxicity.[91–93,101]

Use of DPP4 inhibitors in both Asian Chinese and Caucasians have shown to improve C-peptide levels.[96,102,103] However, more studies are needed to assess the long-term clinical effects.

Further, potential treatment like subcutaneous GAD65 in LADA focusing on immunomodulation and long-term beta-cell preservation

have shown positive results in Western population and are likely to be tested in Asian Chinese population in the near future.[95]

8.2 | Differences

To date, there are no evidences to suggest different treatment strategies for LADA in Asian and European people. However, it is hypothesised that due to differences in ethnicity, lifestyle, and eating habits, efficacy of any particular treatment in LADA may differ between 2 populations in a similar way as in Asian DM2 patients in whom incretin-based therapies and alpha-glucosidase inhibitors show better efficacy than in Caucasians.[104,105]

A pilot trial using rosiglitazone plus insulin in Chinese Asians showed preserved β-cell function as compared to Insulin alone.[106] Interestingly, Chinese herb extract tripterygium an oral immunosuppressive agent maintained stimulated C-peptide levels in Chinese LADA subjects.[107,108] Similarly, in one study, 1-α-hydroxyvitamin D3 exhibited protective effects on residual β-cell function in Asian Chinese LADA subjects.[109] No such data is available in European population

9 | CONCLUDING REMARKS AND FUTURE OUTLOOK

Most patients with adult-onset autoimmune diabetes are noninsulin requiring at least, in the first few months after diagnosis. However, as discussed earlier, the criteria of age at onset and the insulin free period are arbitrary. In clinical practice, substantial number of patients who initially present as DM2 may actually have decline in β-cell function because of autoimmune-mediated damage of beta cells. Because of the slow progression, if not diagnosed early in the disease process, these patients may eventually be managed with OHA's for years particularly in the developing world. The suboptimal glycaemic control will lead to more long-term complications of diabetes.

In spite of heterogeneity seen in the prevalence, clinical features and diagnostic criteria's used in various studies with different ethnic population, it is known that a condition presently known by the name LADA exists and should be diagnosed early in the disease process. Although, this condition may be known by any other names as mentioned earlier but it is important to classify this condition as it may have therapeutic implications. Because of the immunegenetic and clinical heterogeneity of LADA seen in various populations, the results of studies done in various populations cannot be extrapolated to other ethnic groups. More studies are required from populations with different ethnic backgrounds to correctly classify this subgroup and further investigate possibilities of future treatment option. Future therapeutic strategies have been discussed above.

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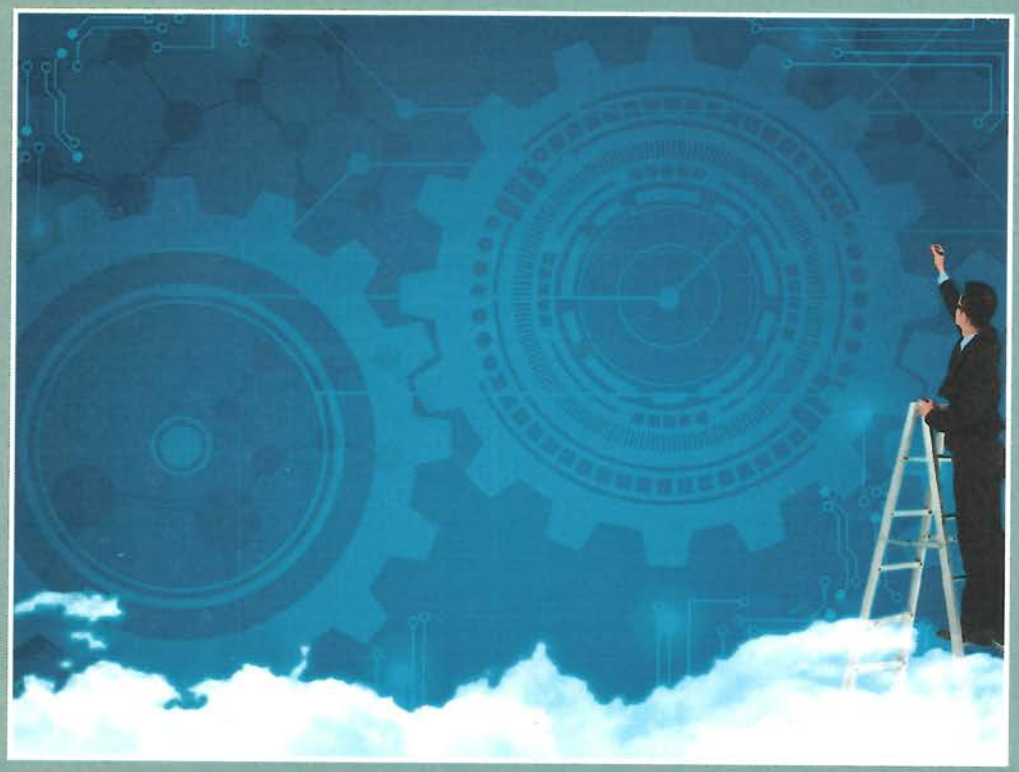
APRIL 2017 VOLUME 23 SUPPLEMENT 3

ENDOCRINE PRACTICE

CLINICAL AND INVESTIGATIVE ENDOCRINOLOGY AND DIABETES



2017 AACE ANNUAL SCIENTIFIC & CLINICAL CONGRESS ABSTRACTS

ABSTRACT CATEGORIES

1	Adrenal Disorders	121	Obesity
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of type 2 diabetes mellitus,hence the need for postpartum screening and follow up. This study revealed that majority of women with GDM did not have postpartum screening as recommended. This is similar to other studies that found most women with GDM do not have standard re-testing at the end of postpartum period. Mothers who had insulin therapy comprised majority of subjects with postpartum diabetes mellitus.The drawback to follow up among GDM mothers may have been due to lack of communication after patients discharge. This can be improved with use of text messages and telephone calls which were not used for the subjects.

Conclusion: Women with GDM that required insulin during pregnancy are at higher risk of having diabetes mellitus in the postpartum period.Women with GDM should have a long term management plan from pregnancy period to prevent being lost during follow up

Abstract #235

PREVALENCE AND CHARACTERISTICS OF LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA) IN A REGION OF INDIA

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Objective: LADA is a subtype of diabetes defined by the presence of islet auto antibodies in the sera and lack of insulin requirement in the first 6 months of diagnosis. Due to heterogeneity of LADA, observations from one population cannot be assumed to be valid in others. We aimed to study the prevalence and characteristics of adult-onset autoimmune diabetes in a region of North India.

Methods: A total of 139 patients aged 30–70 years at diagnosis of diabetes with disease duration 6 months to 5 years were examined cross-sectionally. Clinical data were collected and glucose, HbA1c, lipid profile, creatinine, C-peptide and GAD-65 antibody (GADA) were measured in a fasting blood sample.

Results: Prevalence of LADA was 6.5% (9/139). GADA negative patients were diagnosed with type 2 diabetes (DM2). No patient was diagnosed with type 1 diabetes. LADA (n=9) and DM2 (n=130) patients were compared. LADA patients were younger (p = 0.045), had lower age at onset (p= 0.025), waist (p=0.021), systolic blood pressure (SBP) (p=0.033), triglycerides (p=0.033), fasting C-peptide (FCP) (p=0.009) and prevalence of metabolic syndrome (MS) (p=0.003). LADA patients had also longer duration of diabetes (p=0.045). Patients with GADA at-high titer (GADA-Hi, n=4) were compared with GADA at- low titer (GADA-Lo, n=5) group. Compared to GADA-Lo, all GADA-Hi patients were male (p=0.048), had lower BMI (p=0.040), waist (p=0.026), FCP (p=0.025) and MS (p=0.048).Compared to DM2 patients, GADA-Hi patients were younger (p=0.035), had lower age at onset (p=0.020), BMI (p=0.040), waist (p=0.005), SBP (p=0.003), triglycerides (p=0.026), FCP (p=0.001) and MS (p<0.001). The rate of patients on insulin was higher in GADA-Hi compared to DM2 (p=0.018). No difference was observed between DM2 and GADA-Lo patients.

Discussion: This study shows different clinical and metabolic profile of LADA patients compared to DM2 patients. GADA titer is an important parameter in defining the severity of the disease as patients with high GADA titer tend to have significant β-cell impairment. Earlier studies on Indians have shown varied results probably due to the different methodologies used. We adopted criteria suggested by Immunology of Diabetes Society and Action LADA group using a Diabetes Antibody Standardization Program validated method to measure GADA.

Conclusion: Our results indicate that LADA is prevalent form of adult-onset autoimmune diabetes and is not rare. Diagnosis of LADA should not be delayed as it may have therapeutic implications. In developing world, sometimes routine antibody testing is not cost effective. Thus, clinical and phenotypic features of LADA may help clinicians recognize patients for potential antibody screening.

Abstract #236

FERRITIN AND SERUM IRON AS SURROGATE MARKERS OF POOR GLYCEMIC CONTROL AND MICROVASCULAR COMPLICATIONS IN TYPE-2 DIABETES MELLITUS

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Objective: This study was designed to find the correlation of Iron indices with HbA1c levels and microvascular complications among patients with Type 2 DM

Methods: 100 T-2DM were studied. The mean Age of study group was 58.57 ± 3.17 years; whereas the mean age of Control group was 53.95 ± 4.43. The mean HbA1c of Study group was 9.46 ± 1.31; whereas the mean HbA1c of Control group was 6.42 ± 0.28. The duration of diabetes in Study group was 9.69 ± 2.69 years; whereas it is 5.26 ± 2.81 years in Control group. The mean Serum iron level in Study group was 155.08 ± 22.13 µg/dl; whereas it is 88.81 ± 38.04 µg/dl in Control group. The mean Serum ferritin level in Study group was 284.79 ± 50.06 ng/ml; whereas it is 181.31 ± 54.08 ng/ml in Control group. The mean

PREVALENCE AND CHARACTERISTICS OF LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA) IN A REGION OF INDIA

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INTRODUCTION

LADA is defined as a slow onset, initially noninsulin requiring diabetes with islet auto antibodies. Patients with LADA are at high risk of progression to insulin dependency early in the disease process.[3,5] LADA is generally misdiagnosed as type 2 diabetes (DM2)and the correct diagnosis is delayed by years[1–4] Diagnosing LADA at an initial stage is important to facilitate improved glycemic control as well as the preservation of residual beta cell function. Glutamic acid decarboxylase antibodies (GADA) are considered to be the ideal marker for screening of LADA.[5] In Western and Chinese populations, with in LADA heterogeneity has been observed in the features of low GADA titer LADA patients and high GADA titer LADA patients.[6] Prevalence of LADA varies from 0% in an ethnic group of Papua New Guinea to approximately 10% in Europeans [8,9,10] Many similarities and differences exist between Asian and European population with LADA.[11] Significant discrepancy in the frequency of LADA has been observed in Indian population ranging from 2.6% to 58 % [7] probably, due to regional diversity and different methodology. In view of these variations, we investigated the prevalence and characteristics of patients with LADA. To diagnose LADA, we adopted diagnostic criteria as suggested by Immunology of Diabetes society (IDS) using a Diabetes antibody standardization program (DASP) validated method for antibody measurement. [8]

OBJECTIVES

Due to heterogeneity of LADA, observations from one population cannot be assumed to be valid in others. Aim of our study was: : 1) To study the prevalence of adult-onset autoimmune diabetes, characterise LADA and compare with DM2. 2) To study within LADA heterogeneity based on GADA titer.

MATERIALS AND METHODS

This was a cross-sectional investigation included 139 diabetic patients. Inclusion criteria were 1) age at diagnosis between 30 – 70 years 2) Disease duration between 6 months to 5 years. All antibody negative subjects were diagnosed as DM2. [9] LADA was defined as patients aged 30-70 years at the time of diabetes diagnosis who did not require insulin for at least 6 months after diagnosis, with GADA depicted at their sera. All subjects were screened for GADA using RSR- ELISA kits (RSR Limited, Cardiff, UK) with 98% specificity and 92% sensitivity in the DASP 2005.[10,8] Assay cut off of < 5 U/mL was considered negative and ≥ 5 U/mL was considered positive. Based on the median value of GADA titer, LADA subjects were divided into 2 sub groups: GADA-high titer *(> 13.6 U/ml)* and GADA-low titer *(≤13.6 U/ml)*. Metabolic syndrome was assessed according to the revised National Cholesterol Education Program (NCEP): Adult Treatment Panel III criteria [12]

RESULTS

Prevalence of LADA was 6.5% (9/139) 95% Confidence interval (CI): 3.29 - 12.0% among patients with adult-onset diabetes. All GADA negative patients were diagnosed with type 2 diabetes (DM2). Prevalence of LADA seemed to gradually decline with increasing age. However, the prevalence of DM2 continually increased with age. No patient was diagnosed with type 1 diabetes. LADA patients were younger , had lower age at onset, waist , systolic blood pressure(SBP) triglycerides, fasting C-peptide and prevalence of metabolic syndrome (MS) (Table 1). Compared to GADA-Low LADA, all GADA-High patients were male, had lower BMI, waist, FCP and MS (Table 2). Compared to DM2 patients, GADA-High patients were younger had lower age at onset, BMI, waist, SBP, triglycerides , FCP and MS (Table 3). The rate of patients on insulin was higher in GADA-High compared to DM2 . No difference was observed between DM2 and GADA-Low patients.

Table 1: LADA subjects vs. DM2 subjects

Cases	LADA (n=9)	DM2 (n=130)	P-value
Females, n (%)	4 (44.4%)	55 (42.3%)	
Males, n (%)	5 (55.6%)	75 (57.7%)	1.000
Age (years)	40.8 ± 7.6	47.2 ± 9.3	**0.045**
Age at diagnosis (years)	37.1 ± 7.4	44.4 ± 9.4	**0.025**
Disease Duration (months)	46 ± 12.7	33.7 ± 17.9	0.045
BMI (Kg/m²)	25.6 ± 4.2	28.3 ± 4.8	0.108
Waist circumference (cms)	88.9 ± 8.3	96.8 ± 9.8	**0.021**
SBP (mmHg)	115.3 ± 16.4	126.5 ± 14.0	**0.023**
DBP(mmHg)	80.0 [65.5-82.0]	80.0 [75.0- 84.3]	0.330
Antihypertensive treatment n (%)	2 (22.2%)	44 (33.8%)	0.718
Family history of Diabetes, n (%)	4 (44.4%)	86 (66.2)	0.278
Triglycerides (mg/dl)	107.0 [92.0- 141.0]	151.0 [112.3-210]	**0.033**
Total cholesterol (mg/dl)	160.4 ± 35.7	167.8 ± 38.1	0.577
HDL Cholesterol (mg/dl)	46.1 ± 7.5	43.2 ± 10.8	0.425
LDL Cholesterol (mg/dl)	104 ± 33.9	103.3 ± 31.2	0.948
Metabolic syndrome, n (%)	4 (44.4%)	115(88.5%)	**0.003**
Fasting plasma glucose(mg/dl)	139 [117.5- 162.5]	137 [120-169.3]	0.898
HbA1C (%)	8.1 [7.0- 10.6]	7.1[6.6-8.5]	0.096
C-Peptide fasting (ng/ml)	1.5 ± 0.9	2.3 ± 0.8	**0.009**
Insulin treatment, n (%)	2 (22.2%)	6 (4.6%)	0.085
Time to insulin (months)	25.0 ± 26.8	47.2 ± 5.3	0.449

Table 2: GADA-high vs. GADA-low

Cases	GADA high (n=4)	GADA low (n=5)	P-value
Female, n (%)	0 (0%)	4 (80%)	
Male, n (%)	4 (100%)	1 (20%)	**0.048**
Age (years)	37.3 ± 3.6	43.6 ± 9.2	0.236
Age at diagnosis (years)	33.3 ± 3.0	40.2 ± 8.8	0.178
Disease Duration (months)	50 ± 11.2	42.8 ± 14.1	0.433
BMI (Kg/m²)	22.6 ± 4.1	28.1 ± 2.4	**0.040**
Waist (cms)	82.6 ± 7.4	94.0 ± 4.8	**0.026**
SBP (mmHg)	105.5 ± 9.0	123.2 ± 17.3	0.109
DBP(mmHg)*	70.5 ± 9.5	79.2 ± 12.5	0.288
Antihypertensive treatment n (%)	0 (0%)	2 (40%)	0.444
Triglycerides (mg/dl)*	96.8 ± 12.7	131.2 ± 34.7	0.092
Total cholesterol (mg/dl)	157.5 ± 37.2	163 ± 38.6	0.841
HDL Cholesterol (mg/dl)	47.8 ± 6	44.8 ± 9.0	0.590
LDL Cholesterol (mg/dl)	100 ± 39.4	107.2 ± 33.5	0.775
Metabolic syndrome (n, %)	0 (0%)	4 (80%)	**0.048**
Fasting plasma glucose (mg/dl)*	140± 29.9	154.8 ± 57.8	0.659
HbA1C (%)*	8.9 ± 3.2	8.8 ± 1.7	0.953
Family history of Diabetes, n (%)	1(25%)	3 (60%)	0.524
C-Peptide fasting (ng/ml)	0.8 ± 0.7	2.1 ± 0.6	**0.025**
Insulin treatment	2 (50%)	0 (0%)	0.167

Table 3: GADA-high subjects vs. DM2

Cases	GADA- high (n = 4)	DM2 (n = 130)	P-value
Females, n (%)	0 (0%)	55 (42.3%)	
Males, n (%)	4 (100%)	75 (57.7%)	0.144
Age (years)	37.3 ± 3.6	47.2 ± 9.3	**0.035**
Age at diagnosis (years)	33.3 ± 3.0	44.4 ± 9.4	**0.020**
Disease duration (months)	50 ± 11.2	33.7 ± 17.9	0.074
BMI (Kg/m²)	22.6 ± 4.1	28.3 ± 4.8	**0.020**
Waist (cms)	82.6 ± 7.4	96.8 ± 9.8	**0.005**
SBP (mmHg)	105.5 ± 9.0	126.5 ± 14.0	**0.003**
DBP(mmHg)*	69.5 [62.3-79.8]	80 [75.0-84.3]	0.075
Antihypertensive treatment n (%)	0 (0%)	44 (33.8%)	0.302
Triglycerides (mg/dl)*	99.5 [83.8-107.0]	151[112.3-210]	**0.026**
Total cholesterol (mg/dl)	157.5 ± 37.2	167.8 ± 38.1	0.596
HDL cholesterol (mg/dl)	47.8 ± 6	43.2 ± 10.8	0.403
LDL cholesterol (mg/dl)	100 ± 39.4	103.3 ± 31.2	0.837
Metabolic syndrome (n, %)	0 (0%)	115(88.5%)	**< 0.001**
Fasting plasma glucose (mg/dl)*	143 [109.8 - 167.3]	137 [120-169.3]	0.927
HbA1C (%)*	8.4 [6.2 - 12.2]	7.1 [6.6-8.5]	0.596
Family history of Diabetes, n (%)	1(25%)	86 (66.2%)	0.124
C-Peptide fasting (ng/ml)	0.8 ± 0.7	2.3 ± 0.8	**0.001**
Insulin treatment	2 (50%)	6 (4.6%)	**0.018**

Data are means ± SD, median [IQR], or n (%)

DISCUSSION

This study shows different clinical and metabolic profile of LADA patients compared to DM2 patients. GADA titer is an important parameter in defining the severity of the disease as patients with high GADA titer tend to have significant β-cell impairment. Earlier studies on Indians have shown varied results probably due to the different methodologies used. We adopted criteria suggested by Immunology of Diabetes Society and Action LADA group using a Diabetes Antibody Standardization Program (DASP) validated method to measure GADA.

CONCLUSION

- Our results indicate that LADA is a prevalent form of adult-onset autoimmune diabetes and is not rare.
- Diagnosis of LADA should not be delayed as it may have therapeutic implications.
- In the developing world, sometimes routine antibody testing is not cost effective. Thus, clinical and phenotypic features of LADA may help clinicians recognize patients for potential antibody screening.

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Latent Autoimmune Diabetes in Adults (LADA): The Prevalent Form of Adult-Onset Autoimmune Diabetes in a Region of India

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Abstract

Aims: We undertook a study to investigate the prevalence and general characteristics of patients with Latent autoimmune diabetes in adults (LADA).

Methods: This was a cross-sectional investigation conducted in the National Capital Region of India. Total of 139 subjects were recruited in the study. All subjects were screened for Glutamic acid decarboxylase autoantibody (GADA). GADA was analysed using ELISA kits with 98% specificity and 92% sensitivity.

Results: The prevalence of LADA was 6.5% (6.3% in men and 6.8% in women); 95% Confidence interval (CI): 3.29 - 12.0% among adult-onset diabetic patients. Prevalence of LADA seemed to gradually decline with increasing age. LADA (n=9) and DM2 (n=130) patients were compared. LADA patients were younger (p = 0.045), had lower age at onset of diabetes (p = 0.025), waist circumference (p = 0.021). LADA patients had also longer duration of diabetes (p = 0.045). There was no significant difference in BMI between two groups.

Conclusions: Our results indicate that LADA represents 6.5 % of cases among adult-onset diabetes in a region of north India. LADA is a prevalent form of adult-onset autoimmune diabetes and not rare. This study shows different phenotypic features of LADA patients compared to DM2 patients. In the developing world, sometimes routine antibody testing may be considered not to be cost-effective. Thus, clinical and phenotypic features of LADA may help clinicians recognize patients for potential antibody screening.

Keywords: *Adult; Autoimmune; Diabetes; latent*

Abbreviations: BMI : Body mass index; DASP : Diabetes antibody standardization program; DM1 : Type 1 diabetes; DM-1A : Classic Type 1 Diabetes; DM2 : Type 2 diabetes; ELISA : Enzyme-linked immunosorbent assay; GADA : Glutamic decarboxylase autoantibodies; HLA : Human leukocyte antigen; IA-2A : Insulinoma-associated Antigen; ICA : Islet cell antibodies; IDS : Immunology of Diabetes society; LADA : Latent autoimmune diabetes in adults; TCF7L2 : Transcription factor 7-like 2; ZMIZ1 : Zinc finger MIZ-type containing 1; ZnT8A : zinc transporter 8 autoantibodies

Introduction

Previously, autoimmune diabetes was generally considered to be a disease of childhood and adolescence, however, these days this concept does not hold true. It has been observed that adult-onset autoimmune diabetes is not as rare as once considered. Almost, for last two decades, Latent autoimmune diabetes in adults (LADA) had been continually an area of interest and concern for researchers and clinicians worldwide. As the name suggests LADA is autoimmune mediated diabetes of adult onset defined by the presence of at least one diabetes associated pancreatic auto antibodies in their sera [1,2].

Glutamic decarboxylase autoantibodies (GADA) are the most prevalent and persistent autoantibodies in majority of patients with LADA. Although, the fluctuating titer of GADA in serum may pose challenges in the diagnosis of LADA in some patients [3,4]. Insulinoma-Associated Antigen (IA-2A) antibodies alone are not used to screen patients due to very low diagnostic sensitivity for LADA [5].

Preliminary data has shown that patients with autoimmune diabetes, characterized by the presence of GADA, display a different clinical phenotype from classical type 2 diabetes (DM2) without GADA [2,6,7].

Over the years, LADA has been extensively studied by various investigators but many controversial issues related to its etiopathogenesis and potential treatment options still prevail. Due to resemblance of phenotypic characteristics, slow onset and non requirement of insulin at diagnosis, LADA is generally misdiagnosed as DM2 and the correct diagnosis is delayed by years.

LADA has been described as a subtype of T cell-mediated DM-1 [3,8]. However, the association of DM-2 associated Transcription factor 7-like 2 (TCF7L2) and Zinc finger MIZ-type

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containing 1 (ZMIZ1) risk gene variants with LADA is important and against the arguments denying LADA as a distinct entity; also, the differences in human leukocyte antigen (HLA) haplotypes for both risk and protection complement the idea of LADA as a separate identity [9-13].

Both classic DM-1A and LADA are autoimmune in nature but lack of necessity of insulin treatment at diagnosis or at least within first six months after diagnosis clinically, differentiates LADA from classic DM1-A [14,15]. Mostly, only one pancreatic autoantibody is present in LADA patients whereas, DM-1A patients typically are positive for more than one pancreatic auto antibodies [16]. Epidemiological studies from different parts of the world have shown diverse prevalence of LADA ranging from 0% in an ethnic group of Papua New Guinea to approximately 10% in European individuals [15,17].

In European Action LADA study, within the entire cohort of patients with autoimmune diabetes, 90.5% were positive for GADA. A significant difference was noted in the characteristics of autoantibodies-positive patients compared with autoantibodies-negative patients. Action LADA experience has shown that nearly 10% of adult-onset diabetic patients have autoimmune diabetes, and LADA is more prevalent than classic type 1 diabetes [15].

Similarly, based on GADA positive results in a large LADA China study, 5.9% patients were classified as LADA [18]. In concordance with European population, a clear difference in phenotypic and biochemical characteristics was observed in GADA-positive patients. In comparison with GADA-negative type 2 diabetic subjects, GADA positive subjects were leaner, had lower insulin secretion and less metabolic syndrome.

Data from South Asian region on LADA are sparse. Significant discrepancy in the frequency of LADA has been observed in earlier studies on Indian population ranging from 2.6% to 58 % [19]. In part, such heterogeneity in the results could be attributed to the regional diversity and different methodology adopted by authors. Some of these studies included only specific subgroups like subjects with low BMI, young age (< 25 years) and sulfonylurea failure with likelihood of high prevalence of LADA [19].

LADA may have ethnic variations and results from particular population cannot be extrapolated to other population. Diagnosing LADA early in the disease process is important as it may have therapeutic implications [19]. Many similarities and differences exist between Asian and European population with LADA [19]. To have a better understanding of LADA more research needs be carried out, especially in the developing economies with approximately 2/3 of world's diabetic population [20]. In view of the extreme variations seen in the results of past studies on Indian subjects, we undertook a study to investigate the prevalence of LADA and also assess general characteristics of patients with LADA. To diagnose LADA, we adopted diagnostic criteria as suggested by Immunology of Diabetes society (IDS) and European Action LADA group using a Diabetes antibody standardization program (DASP) validated method.

Methods

Subjects

This study was conducted in the National Capital Region (NCR) of Northern India and included diabetic patients over 30 years of age consecutively attended at the Diabetes Clinic. Majority of the population investigated were inhabitants of the urban areas of NCR surrounding the capital city of New Delhi. All participants were natives of Northern states of India mainly Uttar Pradesh, Uttarakhand, Punjab, Haryana, Bihar and Delhi. The socio-economic status of most of the study participants ranged from lower middle to upper middle.

Study Design

This study was a cross-sectional investigation. The Sample size was calculated by accepting the confidence interval of 95% (0.95) for a precision of +/- 0.05 units in two sided test for an estimation proportion 0.1, from November 2015 until June 2016 total of 139 subjects were recruited in the study. Inclusion criteria were 1) age at diagnosis between 30 – 70 years 2) Duration of disease between 6 months to 5 years. Any subject not fulfilling the inclusion criteria or not willing to participate were excluded.

In this population, subjects with glutamic acid decarboxylase autoantibody (GADA) in whom insulin was started at diagnosis or within 1 month after diagnosis were to be defined as DM1-A. [15] All antibody negative subjects were diagnosed as DM2. [21] LADA was defined as patients aged 30-70 years at the time of diabetes diagnosis who did not require insulin for at least 6 months after diagnosis, with GADA depicted at their sera.

Methodology

We reviewed detailed medical history of all subjects with the aim of collecting clinical and demographic data. All subjects were screened for GADA. GADA was analysed using RSR- ELISA kits (RSR Limited, Cardiff, UK) with 98% specificity and 92% sensitivity in the DASP 2005 [22]. Venous whole blood was collected in plastic serum separator tubes. Specimens were centrifuged for at least 15 minutes within one hour of collection. Thereafter, serum was transferred to properly labelled plastic-screw cap vial and frozen at -20 degree Celsius. All frozen samples were processed within a week of storage. Assay cut off of < 5 U/ml was considered negative and ≥ 5 U/ml was considered positive.

Statistical analysis

Quantitative variables were described as means ± standard deviations or medians and inter quartile ranges. Categorical variables described were described as n (%) Mean values between groups were compared using Independent t-Test/Mann-Whitney U test. The comparison of categorical variables was analyzed using chi-square /fisher exact test.

For all analyses, IBM SPSS statistics for windows software (version 21.0; Armonk NY, USA) and an alpha value of 0.05 for statistical significance were used.

Results

Demographics of study participants

The 139 study subjects consisted of 80 (57.6%) male and 59 (42.5%) female. Overall, mean (SD) age was 46.7 (9.3) years and age at diagnosis was 43.9 (9.5) years. The mean disease duration was 34.5 (17.9), waist circumference was 96.3 (9.9) cms and BMI was 28.1 (4.8) kg/m2. The demographics of studied participants are shown in (Table 1).

Table1: Baseline demographics of all 139 study subjects

Characteristics	Mean ± SD, Median [IQR] or n (%)
Males, n (%)	80 (57.6)
Females, n (%)	59 (42.4)
Age (years)	46.7 ± 9.3
Age at diagnosis (years)	43.9 ± 9.5
Duration of disease (months)	34.5 ± 17.9
Weight(kg)	74.7 ± 13.2
BMI(kg/m2)	28.1 ± 4.8
Waist circumference (cms)	96.3 ± 9.9
Family history of diabetes, n (%)	90 (64.7)

Data are expressed as means (SD), median [IQR] or n (%)

Prevalence of adult-onset autoimmune diabetes

Within the entire cohort of 139 subjects with adult-onset diabetes, 9 (6.5%) were positive for GADA. All antibody positive subjects did not require insulin within first six months after diagnosis of diabetes and were classified as LADA. As per the diagnostic criteria adopted by us, no subject was diagnosed with DM1-A.

GADA negative 130 subjects were diagnosed with DM2. The prevalence of LADA was 6.5% (6.3% in men and 6.8% in women); 95% Confidence interval (CI): 3.29 - 12.0% among adult-onset diabetic patients. Prevalence of LADA seemed to gradually decline with increasing age. However, the prevalence of DM2 continually increased with age. Age wise prevalence of LADA and DM2 is shown in (Table 2) and (Fig. 1).

Comparison of phenotypic features of LADA and DM2 subjects

LADA (n=9) and DM2 (n=130) patients were compared. LADA patients were younger (40.8 ± 7.6 vs. 47.2 ± 9.3 years; p = 0.045), had lower age at onset of diabetes (37.1 ± 7.4 vs. 44.4 ± 9.4 years; p = 0.025), waist circumference (88.9 ± 8.3 vs. 96.8 ± 9.8 cms; p = 0.021). LADA patients had also longer duration of diabetes (46 ± 12.7 vs. 33.7 ± 17.9; p = 0.045). Frequency of positive family history of diabetes was lower in LADA patients however, the difference was not statistically significant (44.4% vs. 66.2; p = 0.278). There was no significant difference in BMI between two groups. The phenotypic characteristics of LADA and DM2 patients are summarized in (Table 3).

Table 2: Age and Gender wise prevalence of LADA and DM2 among all study subjects n=139

Age (years)	LADA			DM2		
	Male	Female	Total	Male	Female	Total
< 40	4/26 (15.4)	1/10 (10)	5/36 (13.9)	22/26 (84.6)	9/10 (90)	31/36 (86.1)
40-49	1/33 (3)	2/16 (12.5)	3/49 (6.1)	32/33 (97)	14/16 (87.5)	46/49 (93.9)
50-59	0/18 (0)	1/22 (4.5)	1/40 (2.5)	18/18 (100)	21/22 (95.5)	39/40 (97.5)
≥ 60	0/3 (0)	0/11 (0)	0/14 (0)	3/3 (100)	11/11 (100)	14/14 (100)
Total	5/80 (6.3)	4/59 (6.8)	9/139 (6.5)	75/80 (93.8)	55/59 (93.2)	130/139 (93.5)

Data expressed as n (%)

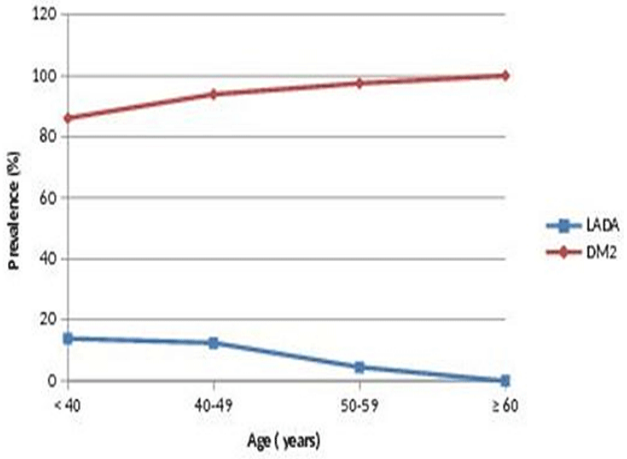

Figure 1: Age wise prevalence of LADA and DM2 among all study subjects.

Table 3: Characteristics of LADA subjects *vs.* DM2 subjects

Cases	LADA (n=9)	DM2 (n=130)	P-value
Females, n (%)	4 (44.4%)	55 (42.3%)	
Males, n (%)	5 (55.6%)	75 (57.7%)	1
Age (years)	40.8 ± 7.6	47.2 ± 9.3	0.045
Age at diagnosis (years)	37.1 ± 7.4	44.4 ± 9.4	0.025
Disease Duration (months)	46 ± 12.7	33.7 ± 17.9	0.045
BMI (Kg/m2)	25.6 ± 4.2	28.3 ± 4.8	0.108
Waist circumference (cms)	88.9 ± 8.3	96.8 ± 9.8	0.021
Family history of Diabetes, n (%)	4 (44.4%)	86 (66.2)	0.278

Data are expressed as means (SD), median [IQR] or n (%)

Discussion

Adult-onset autoimmune diabetes had been continually an area of interest for clinicians and researchers worldwide. In the past, DM1-A was invariably considered to be the only form of autoimmune diabetes in both children and adults. However, in the last few decades growing number of publications have supported the idea of another form of adult- onset autoimmune diabetes called LADA, which in comparison to DM1-A, have slow onset, but generally show faster deterioration of β-cell function when compared to DM2. For many years, the concept of LADA as a distinct identity has faced many controversies [23]. The adaptation of age and insulin criteria's in defining LADA has been criticized [24,25].

In spite of all controversies, the concept about LADA, is undeniable today [2]. It may be known by any other name but clearly there is a sub-group of adult patients, clinically diagnosed as DM2 who are initially non insulin requiring and have evidence of autoimmunity. To some extent, neither age cut-off nor the time to insulin treatment means so much. However, from epidemiological point of view, to establish correct prevalence and characterize LADA in various populations, standardised diagnostic criteria is required. By adopting standardised criteria and valid methods, it would be more appropriate to do rational comparison between different populations.

Differences in the prevalence of LADA have been observed worldwide probably, due to the heterogeneity of type of adult-onset autoimmune diabetes. Past studies involving Indian subjects have resulted in inconclusive heterogeneous information which can be attributed to the discrepancy in methodology adopted by various investigators to diagnose LADA [26,27].

We investigated the prevalence of adult-onset autoimmune diabetes and characterized the phenotypic features of patients diagnosed with LADA in Asian Indians. The features of LADA were compared with antibody-negative patients with DM2.

In the current study, LADA emerged as the most prevalent form of adult-onset autoimmune diabetes. Similar observations of LADA being more prevalent than DM-1A were reported by a multicenter European Action-LADA study and a study from UAE involving a large cohort of adult-onset diabetic subjects [15,28]. As per Immunology of Diabetes society (IDS) and Action-LADA group diagnostic criteria, based on GADA measurement, the prevalence of LADA in our cohort was 6.5% which was surprisingly, exactly similar to the LADA prevalence reported in the northern region of China in a large multicenter LADA China study [18]. The overall prevalence of LADA in China, based on GADA determination, was 5.9% [18].

The prevalence of LADA in our study was lower than 9.7% and 11.6% observed in European subjects but higher than in a cohort from UAE 2.6%, South Korea 1.7% and 4.4%, Japan 3.8% (EHIME study) and North America 4.2% (ADOPT study) [15,7,28,29,30,31,32]. To ascertain prevalence of adult-onset autoimmune diabetes, similar to our study, some studies used only GADA whereas additionally IA-2/ICA and/or znt8 autoantibodies were used by others [15,18,28,31].

GADA are most frequent and persistent autoantibodies [3]. Among GADA negative patients, only small proportion of patients are positive for other diabetes associated autoantibodies. Thus, majority of autoimmune diabetes in adults can be detected by using only GADA measurement and GADA only can be used for screening LADA specifically, in resource constraint settings in the developing world [3]. The expected difference in LADA prevalence using only GADA or GADA along with other diabetes autoantibody would not be significant [15]. In the past, low frequency of IA-2 autoantibody has been reported among adults of North Indian origin and Indo-Aryan children with DM1 [33,34].

Prevalence of LADA in our subjects was highest 13.9 % in individuals aged < 40 years; this is in contrast to the highest prevalence of 13.9% in patients aged 50-59 years in a small Chinese cohort [35]. However, consistent with our results, decreasing trend of LADA prevalence with increasing age was evident in large Chinese and European studies [15].

DM-1A was not prevalent in our cohort. There are no prevalence data available for adult-onset (> 30 years) DM-1A in North Indians but earlier studies have shown the prevalence of DM1-A to be lower among children and adolescents in North Indian populations than in Caucasians [34,36].

Frequency of GADA positivity in our cohort was considerably higher than 1.5% and 1.6% reported in two large studies involving adult north Indian subjects The participants of these two studies had higher mean age and longer duration of diabetes than in our study [33,37]. This can be the possible explanation for the discrepancy in results as diabetes autoantibodies in the sera tend to disappear with increasing age and duration of diabetes [3,15]. Moreover, sensitivity of the assay used for GADA measurement in one of these studies was lower than used in our analyses [37].

Prevalence of GADA positivity and LADA in the current study was much lower than demonstrated in Southern India [27,38]. This is contrary to the findings observed in China and Europe, where compared to north, the frequency of LADA was lower in the southern region [9,18,39–42]. Interestingly, lower frequency of LADA 2.6% was noted in an island nation Srilanka near southern coast of India [43]. It needs to be assessed if geographical, climatic and cultural differences within a country or continent directly contribute towards regional discrepancies in the prevalence of LADA by conducting large scale studies.

Characteristics of LADA among European, Chinese and Arab populations are remarkably different from GADA-negative DM2 subjects [15,18,28]. In accordance, patients with LADA in our cohort, compared with DM2 patients, were younger at recruitment and onset of diabetes, had lower waist circumference.

There are some limitations of our study. We acknowledge that due to small sample size we cannot generalize our results however, to best of our knowledge this is the first study investigating the prevalence and general characteristics of LADA in the National Capital Region of India. Hence, our study paves the way for future research with large sample size. Further, due to

inclusion of patients with disease duration up to five years after diagnosis, LADA cases might have been underestimated as GADA titer may fluctuate and decrease over time.[3]

Conclusion

Our results indicate that LADA represents 6.5 % of cases among adult-onset diabetes in a region of north India. LADA is a prevalent form of adult-onset autoimmune diabetes and not rare. This study shows different phenotypic features of LADA patients compared to DM2 patients. These patients are generally misdiagnosed with DM2 and receive sulphonylurea therapy for years, which, further exhausts β-cells and aggravates the autoimmune process [44]. The knowledge that adult-onset diabetic have GADA should alert the health care providers to the high probability of more rapid progression to insulin therapy than in classic type 2 diabetes. In future, along with insulin, other potential pharmacological therapies like incretins, vitamin D and GAD65, which tend to preserve β-cells, may play an important role in the management of these patients [19].

To avoid use of β-cell damaging therapies and achieve optimal metabolic control in these patients it becomes increasingly important to screen for LADA early. Early insulin treatment in LADA may restore better metabolic control however; no clear potential long term effect has been demonstrated on preservation of beta cell function [45]. Not all, but majority of LADA patients tend to progress towards insulin dependency within a few years after the diagnosis. The risk of developing other organ-specific autoimmune diseases in LADA patients is high [5].

In the developing world, sometimes routine antibody testing may be considered not to be cost-effective. Thus, clinical and phenotypic features of LADA may help clinicians recognize patients for potential antibody screening.

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Human and animal rights

Study was approved by the appropriate ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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Latent Autoimmune Diabetes in Adults in North Indian Region: Assessment of β-Cell Function, Metabolic and Immunological Features

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Abstract

Background: We undertook a study to assess β-cell function, metabolic and immunological features of patients with latent autoimmune diabetes in adults (LADA) and investigate heterogeneity within LADA based on low and high glutamic acid decarboxylase autoantibodies (GADA) titers.
Methods: A total of 139 patients with adult-onset diabetes were examined cross-sectionally in the National capital region of Northern India. Medical history of all subjects was reviewed with the aim of collecting clinical data. Glucose, glycosylated hemoglobin, lipid profile, creatinine, C-peptide, and GADA were measured in 10–12 hrs fasting blood sample.
Results: Assessment of metabolic features revealed lower mean systolic blood pressure in subjects with LADA than in those with type 2 diabetes (DM2). Mean triglyceride levels were lower in LADA subjects compared to DM2 subjects. Compared to DM2 subjects, prevalence of metabolic syndrome (MS) was also lower in LADA subjects. Compared to GADA-low, all GADA-high patients were male, had lower waist circumference, fasting C-peptide (FCP), and prevalence of MS. Compared to DM2 patients, GADA-high patients were younger, had lower age at onset, body mass index, waist circumference, systolic blood pressure, triglycerides, FCP, and prevalence of MS. The rate of patients on insulin was higher in GADA-high compared to DM2. There were no significant differences between characteristics of DM2 and GADA-low patients.
Conclusions: Our results indicate that LADA patients have distinct metabolic features with lower residual β-cell function than DM2 patients. GADA titer is important parameter in defining the severity of the disease as patients with high GADA titer tend to have significant β-cell impairment.

Keywords: latent, autoimmune, diabetes, β-cell, metabolic syndrome

Introduction

In the past, diabetes was considered to be a disease specific to the developed world but in the last few decades, the prevalence of diabetes in developing countries has increased exponentially. Almost three quarter of diabetic population live in low- or middle-income economies. In India, the prevalence of diabetes in adults is 9.3%.[1]

Type 2 diabetes (DM2) is the prevalent form of diabetes among adults; however, adulthood does not exclude autoimmune diabetes.[2] A subset of adults, who are initially presumed to have DM2, show islet autoantibodies in their sera and are termed as latent autoimmune diabetes in adults (LADA).[3] These patients do not require insulin at the diagnosis of diabetes but generally progress toward insulin requirement earlier than DM2 patients.[4,5] Compared to the high prevalence of 9.7% reported by a large European Action LADA study, lower prevalence of LADA was observed in Asians ranging from 2.6% to 5.7%.[6–10] Glutamic acid decarboxylase autoantibodies (GADA) are considered to be the ideal marker for screening of LADA.[11] In the Western and Chinese populations, heterogeneity has been observed in the clinical and metabolic features of low GADA titer LADA patients and high GADA titer LADA patients.[12]

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Although, LADA is autoimmune in nature it also shares some metabolic features with DM2.[2,13] Data about association of metabolic syndrome (MS) with LADA are inconclusive. A Chinese study demonstrated a close association of LADA with MS, which was comparable to DM2 patients.[14] In contrast, a large multicenter European study showed significantly higher prevalence of MS in DM2 patients than in LADA patients and concluded that MS is not a characteristic of LADA.[15] Features of LADA differ between different populations.[16] Data related to β-cell function, MS, and features based on GADA titer in LADA patients are not available in North Indians. Information on various characteristics of LADA may help in better understanding of the disease process and finding appropriate treatment strategies in this population.

Therefore, we aimed to define β-cell function, metabolic and immunological features and assess heterogeneity within LADA based on low and high GADA titers. All features of LADA were compared with DM2 patients.

Materials and Methods

This study was conducted in the National capital region of North India. The study design was cross-sectional and recruited consecutive patients with adult-onset diabetes who attended Diabetes Clinic and fulfilled the inclusion criteria. All subjects were aged 30–70 years and diagnosed within last 5 years. Total of 139 subjects that included 9 LADA and 130 DM2 subjects were studied. LADA and DM2 in the studied subjects were defined on the basis of GADA status and time to insulin initiation. GADA negative subjects were diagnosed as DM2, and subjects with positive GADA who did not require insulin at least in first 6 months after diagnosis were defined as LADA.

Medical history of all subjects was reviewed with the aim of collecting clinical data. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in sitting position. An average of both reading was used for analysis. Glucose, glycosylated hemoglobin (HbA1c), lipid profile, creatinine, C-peptide, and GAD-65 antibody (GADA) were measured from a 10–12 hrs fasting blood sample.

Diagnosis of MS

MS was assessed according to the revised National Cholesterol Education Program (NCEP): Adult Treatment Panel III criteria[17] All subjects fulfilled the criteria of hyperglycemia. In addition, two of the following criteria were required for the diagnosis of MS:

1. Waist circumference ≥90 cm (Asian male) or ≥80 cm (Asian female).
2. Serum triglyceride ≥150 mg/dL or use of drug treatment for dyslipidemia.
3. Serum high-density lipoprotein cholesterol (HDL-C) ≤40 mg/dL (male) or ≤50 mg/dL (female).
4. Systolic blood pressure (SBP) ≥130 mmHg and/or DBP ≥85 mmHg or use of antihypertensive medication.

Biochemical analysis and antibody assay

A fasting blood sample was collected and analyzed locally, using standardized assays to measure glucose, HbA1c, the lipid profile, including the measurement of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and HDL-C, creatinine, C-peptide, and GAD-65 antibody. HbA1c was performed using Bio-Rad Laboratories, Inc. VARIANT™ II Dual (A2/F/A1c) instrument by ion-exchange high performance liquid chromatography, a National glycohemoglobin Standardization program (NGSP) certified method. C-peptide was measured to assess insulin secretion. It was analyzed using Siemens ADVIA Centaur XP® immunoassay system by direct chemiluminescent technology with coefficient of variation <10% and normal reference range of 0.48 to 5.05 ng/mL.[18]

GAD-65 antibody

GAD-65 antibody was analyzed using RSR-ELISA Kits (RSR Limited, Cardiff, UK) with 98% specificity and 92% sensitivity in the Diabetes Antibody Standardization Program (DASP) 2005.[19,20] Assay cutoff of <5 U/mL was considered negative and ≥5 U/mL was considered positive.[21] Our results did not show a bimodal distribution of GADA titer. Hence, to analyze the characteristics of LADA subjects according to GADA titer, based on the median value of GADA titer, LADA subjects were stratified into two subgroups: GADA-high titer (>13.6 U/mL) and GADA-low titer (≤13.6 U/mL). Due to small sample size, LADA subjects were not divided into tertiles, that is, low, medium, and high titer levels. Specimens for C-Peptide and GADA were transported, refrigerated, and processed same day or were frozen at or below –20°C if the sample was not assayed within 24 hrs.

Statistical analysis

Quantitative variables are described as means ± standard deviations or medians and interquartile ranges. Categorical variables were described as *n* (%). Mean values between groups were compared using Independent *t*-test/Mann–Whitney *U* test. The comparison of categorical variables was analyzed using Chi-square/Fisher's exact test.

IBM SPSS statistics for windows software (version 21.0; Armonk, NY) and an α value of 0.05 for statistical significance were used for all analyses.

For the purpose of C-peptide secretion analysis, disease duration (in months) was calculated as the period between the date of diagnosis and the date of the study assessment. The disease duration was stratified into two periods (<36 months and >36 months) for the two categories analyzed, that is, LADA and DM2.

Results

Metabolic characteristics

The metabolic characteristics of LADA and DM2 patients are summarized in Table 1. LADA subjects presented a different metabolic profile than DM2 subjects. Assessment of metabolic features revealed lower mean SBP in subjects with LADA than in those with DM2 (115.3 ± 16.4 vs. 126.5 ± 14.0 mmHg; $P = 0.033$). Mean triglyceride levels (107 (92–141) vs. 151 (112.3–210) mg/dL; $P = 0.033$) were lower in LADA subjects compared to DM2 subjects. Compared to DM2 subjects, prevalence of MS was also lower in LADA subjects (44.4% vs. 88.5%; $P = 0.003$). There was no significant difference in fasting plasma glucose ($P = 0.898$) and HbA1c ($P = 0.096$) between

Table 1. Characteristics of LADA Versus DM2 Subjects

Cases	*LADA* (n = *9*)	*DM2* (n = *130*)	P
SBP (mmHg)	115.3 ± 16.4	126.5 ± 14.0	0.023*
DBP (mmHg)	80.0 [65.5–82.0]	80.0 [75.0–84.3]	0.330
Antihypertensive treatment, *n* (%)	2 (22.2)	44 (33.8)	0.718
Triglycerides (mg/dL)	107.0 [92.0–141.0]	151.0 [112.3–210]	0.033*
Total cholesterol (mg/dL)	160.4 ± 35.7	167.8 ± 38.1	0.577
HDL-C (mg/dL)	46.1 ± 7.5	43.2 ± 10.8	0.425
LDL-C (mg/dL)	104 ± 33.9	103.3 ± 31.2	0.948
MS, *n* (%)	4 (44.4)	115 (88.5)	0.003*
Fasting plasma glucose (mg/dL)	139 [117.5–162.5]	137 [120–169.3]	0.898
HbA1c (%)	8.1 [7.0–10.6]	7.1 [6.6–8.5]	0.096
FCP (ng/mL)	1.5 ± 0.9	2.3 ± 0.8	0.009*
Insulin treatment, *n* (%)	2 (22.2)	6 (4.6)	0.085
Time to insulin (months)	25.0 ± 26.8	47.2 ± 5.3	0.449

Data are means ± SD, median [IQR] or *n* (%), *Significant *P* values.
DBP, diastolic blood pressure; DM2, type 2 diabetes; FCP, fasting C-peptide; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LADA, latent autoimmune diabetes in adults; LDL-C, low-density lipoprotein cholesterol; MS, metabolic syndrome; SBP, systolic blood pressure; SD, standard deviations.

LADA and DM2 subjects, but both parameters were higher in LADA patients than in DM2 patients. Other parameters like cholesterol-total, LDL-C, HDL-C, and DBP did not differ between two groups. Not significantly different but the proportion of those on insulin treatment was higher in LADA subjects than in DM2 subjects (22.2% vs. 4.6%; $P = 0.085$). Similarly, mean time to start insulin was lower in LADA subjects than in DM2 subjects (0.449). The characteristics of LADA and DM2 patients are summarized in Table 1.

Immunologic characteristics

LADA GADA-high subjects versus GADA-low subjects. Patients with GADA at high titer (GADA-high, $n = 4$) were compared with GADA at low titer (GADA-low, $n = 5$) group. Compared to GADA-low patients, all GADA-high patients were male (0% vs. 80%; $P = 0.048$), had lower body mass index (BMI) (22.6 ± 4.1 vs. 28.1 ± 2.4 kg/m^2; $P = 0.040$), waist circumference (82.6 ± 7.4 vs. 94.0 ± 4.8 cm; $P = 0.026$), fasting C-peptide (FCP) (0.8 ± 0.7 vs. 2.1 ± 0.6 ng/mL; $P = 0.025$), and prevalence of MS (0% vs. 80%; $P = 0.048$). No statistical significance was observed between two groups although LADA GADA-high patients required insulin more frequently (50% vs. 0%; $P = 0.167$). The characteristics of GADA-high versus GADA-low titer are presented in Table 2.

LADA subjects with GADA at high titer (GADA-high) versus DM2 subjects. Patients with GADA at high titer (GADA-high, $n = 4$) were compared with DM2 ($n = 130$) group. Compared to DM2 patients, GADA-high patients were younger (37.3 ± 3.6 vs. 47.2 ± 9.3 years; $P = 0.035$), had lower age at onset (33.3 ± 3.0 vs. 44.4 ± 9.4 years; $P = 0.020$), BMI (22.6 ± 4.1 vs. 28.3 ± 4.8 kg/m^2; $P = 0.040$), waist circumference (82.6 ± 7.4 vs. 96.8 ± 9.8 cm; $P = 0.005$), SBP (105.5 ± 9.0 vs. 126.5 ± 14.0 mmHg; $P = 0.003$), triglycerides (99.5 [83.8–107.0] vs. 151 [210–112.3] mg/dL; $P = 0.026$), FCP (0.8 ± 0.7 vs. 2.3 ± 0.8 ng/mL; $P = 0.001$), and prevalence of MS (0% vs. 88.5%; $P < 0.001$). The rate of patients on insulin was higher in GADA high compared to DM2 (50% vs. 4.6%; $P = 0.018$). The characteristics of GADA high versus DM2 subjects are shown in Table 3.

GADA-low LADA subjects versus DM2 subjects. Patients with GADA at low titer (GADA-low, $n = 4$) were compared with DM2 patients ($n = 130$) group. There were no significant differences between characteristics of DM2 and GADA-low patients. The characteristics of GADA low versus DM2 patients are presented in Table 4.

β-cell function. Above observations in Table 1 show that subjects with LADA had lower FCP levels compared with DM2 subjects (1.5 ± 0.9 vs. 2.3 ± 0.8 ng/mL; $P = 0.009$). For the purpose of assessment of C-peptide secretions according to disease duration, the LADA and DM2 groups were

Table 2. Characteristics of GADA-High Versus GADA-Low LADA in Adult Subjects

Cases	*GADA-high* (n = *4*)	*GADA-low* (n = *5*)	P
Female, *n* (%)	0 (0)	4 (80)	
Male, *n* (%)	4 (100)	1 (20)	0.048*
Age (years)	37.3 ± 3.6	43.6 ± 9.2	0.236
Age at diagnosis (years)	33.3 ± 3.0	40.2 ± 8.8	0.178
Disease duration (months)	50 ± 11.2	42.8 ± 14.1	0.433
BMI (kg/m^2)	22.6 ± 4.1	28.1 ± 2.4	0.040*
Waist (cm)	82.6 ± 7.4	94.0 ± 4.8	*0.026*
SBP (mmHg)	105.5 ± 9.0	123.2 ± 17.3	0.109
DBP (mmHg)*	70.5 ± 9.5	79.2 ± 12.5	0.288
Antihypertensive treatment, *n* (%)	0 (0)	2 (40)	0.444
Triglycerides (mg/dL)*	96.8 ± 12.7	131.2 ± 34.7	0.092
Total cholesterol (mg/dL)	157.5 ± 37.2	163 ± 38.6	0.841
HDL-C (mg/dL)	47.8 ± 6	44.8 ± 9.0	0.590
LDL-C (mg/dL)	100 ± 39.4	107.2 ± 33.5	0.775
MS, *n* (%)	0 (0)	4 (80)	0.048*
Fasting plasma glucose (mg/dL)*	140 ± 29.9	154.8 ± 57.8	0.659
HbA1c (%)*	8.9 ± 3.2	8.8 ± 1.7	0.953
Family history of diabetes, *n* (%)	1 (25)	3 (60)	0.524
FCP (ng/mL)	0.8 ± 0.7	2.1 ± 0.6	0.025*
Insulin treatment	2 (50)	0 (0)	0.167

Data are means ± SD or *n* (%), *Significant *P* values.
BMI, body mass index; GADA, glutamic acid decarboxylase autoantibodies.

Table 3. Characteristics of GADA-High LADA Subjects Versus DM2 Subjects

Cases	*GADA-high* (n = *4*)	*DM2* (n = *130*)	P
Females, *n* (%)	0 (0)	55 (42.3)	
Males, *n* (%)	4 (100)	75 (57.7)	0.144
Age (years)	37.3 ± 3.6	47.2 ± 9.3	0.035*
Age at diagnosis (years)	33.3 ± 3.0	44.4 ± 9.4	0.020*
Disease duration (months)	50 ± 11.2	33.7 ± 17.9	0.074
BMI (kg/m^2)	22.6 ± 4.1	28.3 ± 4.8	0.020*
Waist (cm)	82.6 ± 7.4	96.8 ± 9.8	0.005*
SBP (mmHg)	105.5 ± 9.0	126.5 ± 14.0	0.003*
DBP (mmHg)*	69.5 [62.3–79.8]	80 [75.0–84.3]	0.075
Antihypertensive treatment, *n* (%)	0 (0)	44 (33.8)	0.302
Triglycerides (mg/dL)*	99.5 [83.8–107.0]	151 [112.3–210]	0.026
Total cholesterol (mg/dL)	157.5 ± 37.2	167.8 ± 38.1	0.596
HDL-C (mg/dL)	47.8 ± 6	43.2 ± 10.8	0.403
LDL-C (mg/dL)	100 ± 39.4	103.3 ± 31.2	0.837
MS, *n* (%)	0 (0)	115 (88.5)	<0.001*
Fasting plasma glucose (mg/dL)*	143 [109.8–167.3]	137 [120–169.3]	0.927
HbA1c (%)*	8.4 [6.2–12.2]	7.1 [6.6–8.5]	0.596
Family history of diabetes, *n* (%)	1 (25)	86 (66.2)	0.124
FCP (ng/mL)	0.8 ± 0.7	2.3 ± 0.8	0.001*
Insulin treatment	2 (50)	6 (4.6)	0.018*

Data expressed as means ± SD, medians [IQR] or *n* (%), *Significant *P* values.

stratified according to the duration of diabetes into two periods (<36 months and >36 months) and compared. In comparison to DM2 subjects, LADA subjects displayed significantly lower FCP concentrations in those with <36 months duration (1.73 ± 0.10 vs. 2.29 ± 0.88 ng/mL; $P \leq 0.001$; 95% confidence interval [CI]: −0.81 to −0.31) and the difference was also evident in patients with >36 months of disease duration (1.39 ± 1.10 vs. 2.25 ± 0.77 ng/mL; $P = 0.016$; 95% CI: −1.54 to −0.17) (Table 5).

Discussion

It has been observed that characteristics of LADA in European, Chinese, and Arab populations remarkably differ from GADA-negative DM2 subjects.[6,7,10] In accordance, patients with LADA in our cohort showed different features than DM2 patients. LADA patients had lower triglycerides, FCP, and frequency of MS than DM2 subjects. As in Caucasians and Chinese,[7,10] characteristics of LADA patients with high-GADA titer in our study were significantly different than low-GADA titer LADA patients. Patients with high-GADA titer, compared with low-GADA titer, were more likely to be male, leaner, and on insulin treatment with lower frequency of MS. FCP levels were lower in high-GADA titer patients. Age difference between high-GADA and low-GADA patients was not significant. Contrary to our findings, compared to low-GADA patients, European high-GADA patients tended to

Table 4. Characteristics of GADA-Low LADA Subjects Versus DM2 Subjects

Cases	*GADA-low* (n = *5*)	*DM2* (n = *130*)	P
Females, *n* (%)	4 (80)	55 (42.3)	0.167
Males, *n* (%)	1 (20)	75 (57.7)	0.400
Age (years)	43.6 ± 9.2	47.2 ± 9.3	0.329
Age at diagnosis (years)	40.2 ± 8.8	44.4 ± 9.4	0.265
Disease duration (months)	42.8 ± 14.1	33.7 ± 17.9	0.922
BMI (kg/m^2)	28.1 ± 2.4	28.3 ± 4.8	0.531
Waist (cm)	94.0 ± 4.8	96.8 ± 9.8	0.605
SBP (mmHg)	123.2 ± 17.3	126.5 ± 14.0	0.792
DBP (mmHg)	80 [70–88]	80 [75.0–84.3]	1.000
Antihypertensive treatment, *n* (%)	2 (40)	44 (33.8)	0.354
Triglycerides (mg/dL)	122 [100–167]	151 [112.3–210]	0.775
Total cholesterol (mg/dL)	163 ± 38.6	167.8 ± 38.1	0.741
HDL-C (mg/dL)	44.8 ± 9.0	43.2 ± 10.8	0.784
LDL-C (mg/dL)	107.2 ± 33.5	103.3 ± 31.2	0.473
MS, *n* (%)	4 (80)	115 (88.5)	0.798
Fasting plasma glucose (mg/dL)	139 [117–201]	137 [120–169.3]	0.073
HbA1c (%)	8.1 [7.5–11.5]	7.1 [6.6–8.5]	1.000
Family history of diabetes, *n* (%)	3 (60%)	86 (66.2%)	0.568
FCP (ng/mL)	2.1 ± 0.6	2.3 ± 0.8	1.000
Insulin treatment	0 (0)	6 (4.6)	0.167

Data expressed as means ± SD, medians [IQR] or *n* (%).

Table 5. C-Peptide Secretion According to Duration of Diabetes

	<36 months				*>36 months*			
Cases	*LADA (n=3)*	*DM2 (n=71)*	P	*95% CI*	*LADA (n=6)*	*DM2 (n=59)*	P	*95% CI*
C-peptide (ng/mL)	1.73±0.10	2.29±0.88	**<0.001**	−0.81 to −0.31	1.39±1.10	2.25±0.77	**0.016**	−1.54 to −0.17

Data are means±SD.
Significant *P* values are in *bold*.
CI, confidence interval.

be female and younger. Other observations were nearly similar to those reported in large European Action LADA-7 and LADA China study.

Furthermore, our data show that compared to LADA high-GADA titer subjects, DM2 subjects had significantly higher SBP and BMI. Interestingly, this difference was not evident on comparison of total LADA patients with DM2 patients.

Like in a Chinese study,[22,23] LADA patients with low-GADA titer in the present study did not significantly differ from DM2 patients, although this observation was contradictory to the European data that showed a difference between the two groups.[10] Our finding possibly indicates that LADA patients with low-GADA titer are similar to DM2 patients, at least in terms of metabolic features and β-cell function. However, future studies are required to ascertain if LADA patients with low-GADA titer can be actually equated with DM2 patients. Ethnic variation in this regard also needs to be further explored.

In agreement with previous reports, our data also indicate that based on GADA titer, different subgroups can be identified within LADA.[7,10,12,24] Besides distinct clinical and phenotypic features, GADA titer is important in defining the severity of disease process and may help in selection of appropriate therapeutic choices.

Similar to European and Chinese cohorts, current study showed that MS is more common in DM2 subjects than in LADA subjects. Frequency of MS among LADA subjects in our study was 44.4%, slightly higher than 41.9% in European LADA subjects,[15] but much lower than 62% reported in Chinese LADA subjects.[7] Our data further indicate that MS is not prevalent in LADA patients with high-GADA titer. Compared with LADA patients without MS, all LADA patients with MS tended to be female. The current study is the first to describe MS in Indian LADA patients.

Compared to DM2 patients, C-peptide concentration in the current study was lower in LADA patients. This difference was evident up to 36 months from diagnosis and contrary to Spanish data[25] the difference remained even after 36 months of duration.

We acknowledge that due to small number of subjects in LADA group, our results cannot be generalized and future research is needed with larger sample size. Nevertheless, to the best of our knowledge, this is the first study in the region of north India providing insights into clinical, metabolic, and immunological features of LADA.

In conclusion, our results indicate that LADA patients have distinct metabolic features with lower residual β-cell function than DM2 patients. As antibody titers define the severity of disease, LADA patients with high-GADA titers need to be closely monitored to assess deterioration in glycemic control and β-cell function. Due to similarities with DM2, LADA patients with low-GADA titers may additionally require therapies targeting insulin resistance.

With the ultimate aim of improving the management of LADA patients, in future, large-scale studies using validated methods are required to have an improved understanding on various aspects of LADA and establish optimal preventive and treatment strategies for LADA. Diagnosing LADA solely on the basis of autoantibody detection poses a challenge as titer of autoantibodies may fluctuate and disappear with time.[23]

Due to overlapping of certain features, in addition to antibody assays, other novel markers should be identified to differentiate LADA and DM2 at an early stage. Global collaboration using standardized assays and diagnostic criteria is required to obtain insights into ethnic differences in LADA.

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Ethical Approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Author Disclosure Statement

No conflicting financial interests exist.

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