DISSERTATION TITLED

”THE PRESENCE OF GAD AND IA-2 AUTO ANTI-BODIES IN YOUNG ADULTS DIAGNOSED AS TYPE 2 DIABETES MELLITUS

Submitted in partial fulfilment of

requirements for

M.D.DEGREE EXAMINATION BRANCH-I GENERAL MEDICINE

of

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CHENNAI

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MADRAS MEDICAL COLLEGE

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CERTIFICATE

This is to certify that the dissertation entitled "**THE PRESENCE OF GAD AND IA-2 AUTO ANTI-BODIES IN YOUNG ADULTS DIAGNOSED AS TYPE 2 DIABETES MELLITUS** " is a bonafide work done by **DR. V. MAHADEVAN** , Post Graduate Student, Institute of Internal Medicine, Madras Medical College, Chennai-3, in partial fulfillment of the University Rules and Regulations for the award of MD Branch – I General Medicine, under our guidance and supervision, during the academic year 2010 - 2013.

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DECLARATION

I solemnly declare that the dissertation entitled "**THE PRESENCE OF GAD AND IA-2 AUTO ANTI-BODIES IN YOUNG ADULTS DIAGNOSED AS TYPE 2 DIABETES MELLITUS**" is done by me at Madras Medical College, Chennai-3 during May 2012 to November 2012 under the guidance and supervision of Prof . K.S. CHENTHIL, M.D., to be submitted to The Tamilnadu Dr M.G.R Medical University towards the partial fulfillment of requirements for the award of M.D DEGREE IN GENERAL MEDICINE BRANCH-I.

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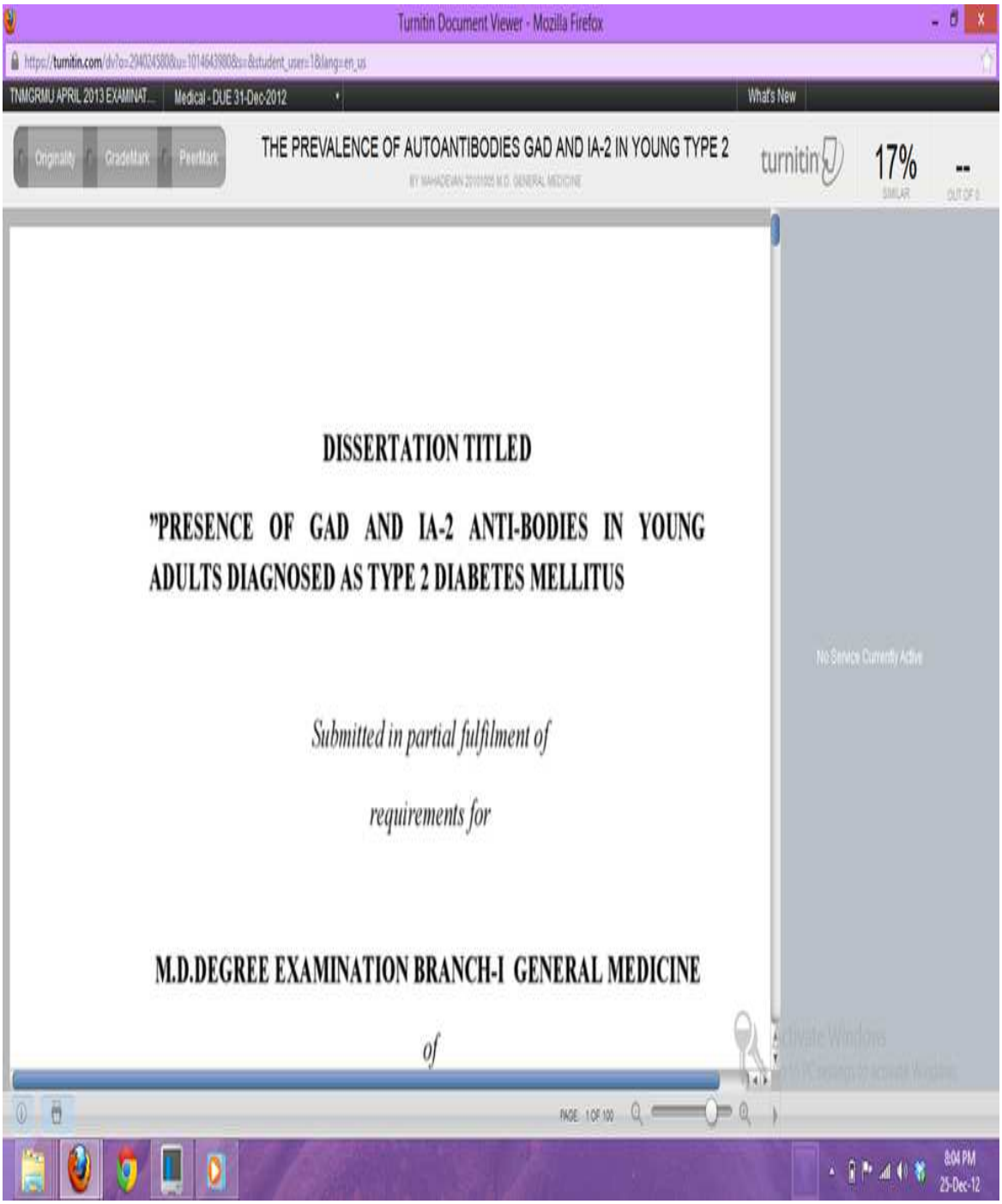
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ABBREVIATIONS

ADA	American Diabetes Association
BMI	Body mass index
Cm	Centimetre
DM	Diabetes Mellitus
DCCT	Diabetes Control and Complication Trail
ELISA	Enzyme Linked Immuno-Sorbent Assay
GAD	Glutamic Acid Decarboxylase
GDM	Gestational Diabetes Mellitus
HDL	High Density Lipoprotein
HLA	Human Leukocyte Antigen
HbA1C	Glycosylated Hemoglobin
Hr	hour
I.P.No.	In Patient No
IAA	Insulin Autoantibody

IA-2	Insulinoma associated protein 2 Autoantibody
ICA	Islet Cell Autoantibody
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGF-1	Insulin like Growth Factor - 1
IGT	Impaired Glucose Tolerance
IU	International units
LDL	Low Density Lipoprotein
Mmol	milli moles
mg/dl	Milligrams per deciliter
OGTT	Oral Glucose Tolerance Test
T1DM	Type 1 Diabetes Mellitus
TGL	Triglycerides
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	Very Low Density Lipoprotein

KEY WORDS

Y	yes
N	no
M	male
F	female
BMI	Body mass index
GDM	Gestational diabetes mellitus
BOH	Bad obstetric history
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
FPG	Fasting plasma Glucose
PPG	Post prandial plasma Glucose
TGL	Triglycerides
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein

VLDL	Very Low Density Lipoprotein
GAD	Glutamic Acid Decarboxylase
IA-2	Insulinoma Associated protein 2

CONTENTS

Introduction

Diabetes Mellitus is a metabolic cum vascular disease, cause of which is mainly due to defect in insulin secretion from the β cell of islet of pancreas or defect in the action of insulin[1,2]. Diabetes mellitus is marked by chronic high glucose concentration in plasma. The resultant hyperglycemia which in long run lead to serious damage and dysfunction of many vital body organs especially the blood vessels and nerves. Symptoms of diabetes recognition dates back to 1550 B.C where diabetes was described as melting[3] down of the flesh and limbs into urine and for the patients never stop making water. Diabetes in Greek means siphon – to explain the liquefaction of the flesh and boned into urine; Mellitus means honeyed urine).

Epidemiology

Diabetes Mellitus is a major threat to the public health in this world. The entire globe is now facing a new hazardous challenge in the form of growing epidemic of diabetes mellitus. Diabetes mellitus is well known for its potentially devastating damage and dysfunction to the organs from head to toe and can occur in all age group from pediatric population to geriatric population. Diabetes which was once thought as a disease of rich people is

now stirring up often in poor rural population. Thus Diabetes has become a common disorder and that too life threatening disease with dramatic increase all over the world. Facts about diabetes that was released by World Health Organization are alarming and urge to take prompt preventive measures.

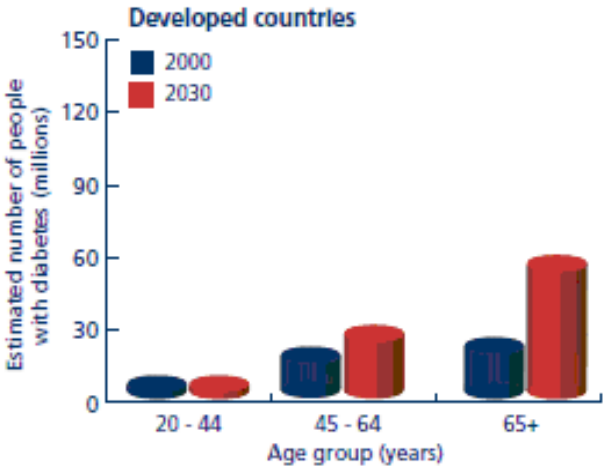
Top 10: Countries/territories of number of people with diabetes (20-79 years), 2011 and 2030

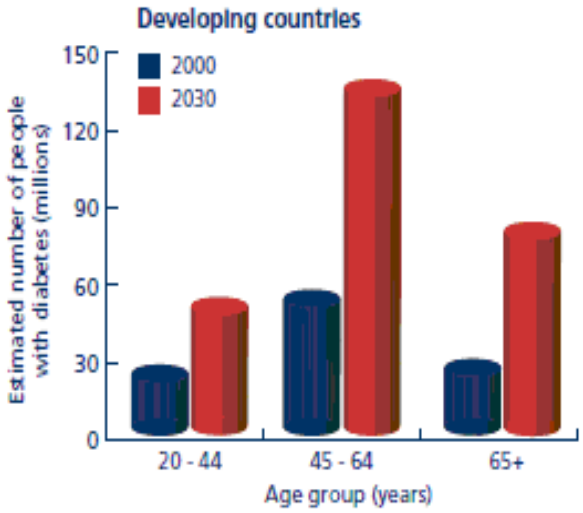
	COUNTRY /TERRITORY	2011 MILLIONS		COUNTRY /TERRITORY	2030 MILLIONS
1	China	90.0	1	China	129.7
2	India	61.3	2	India	101.2
3	United States of America	23.7	3	United States of America	29.6
4	Russian Federation	12.6	4	Brazil	19.6
5	Brazil	12.4	5	Bangladesh	16.8
6	Japan	10.7	6	Mexico	16.4
7	Mexico	10.3	7	Russian Federation	14.1
8	Bangladesh	8.4	8	Egypt	12.4
9	Egypt	7.3	9	Indonesia	11.8
10	Indonesia	7.3	10	Pakistan	11.4

1. Worldwide there are 171 million people living diabetes and this number of people living with diabetes may double by 2030[1,3].
2. During the next 25 years there may be augmentation in diabetic population by 150% especially in developing countries[1,2].

3. There is a gross change in the age of people living with diabetes between developed and developing countries. Diabetic populations in the developed countries are above the age of retirement whereas in developing countries they clump in the working age population especially between the age group between 35 and 60 years.

4. The reason for this escalation in diabetes is due to population ageing, increasing trends towards obesity, sedentary life style. Unhealthy food habits also contribute to the high incidence of diabetes.





5. Diabetes contribution to mortality rate is very high. Every year diabetes attribute to 3.2 million deaths[1,3] which can be simplified as diabetes contribute to one in 20 deaths or in other words six deaths per minute. In days 8,700 deaths occurs due to diabetes per day.

6. As mentioned earlier that diabetes is common in middle aged persons in developing countries, diabetes causes high mortality by being the etiology or part of etiology in one in ten deaths between the age group of 35 to 64 years old.

7. For a diagnosed case of diabetes there is hiding case which has to be explored. In the fact sheet released by IDF there are about 50% diabetes population are living who are not yet diagnosed.

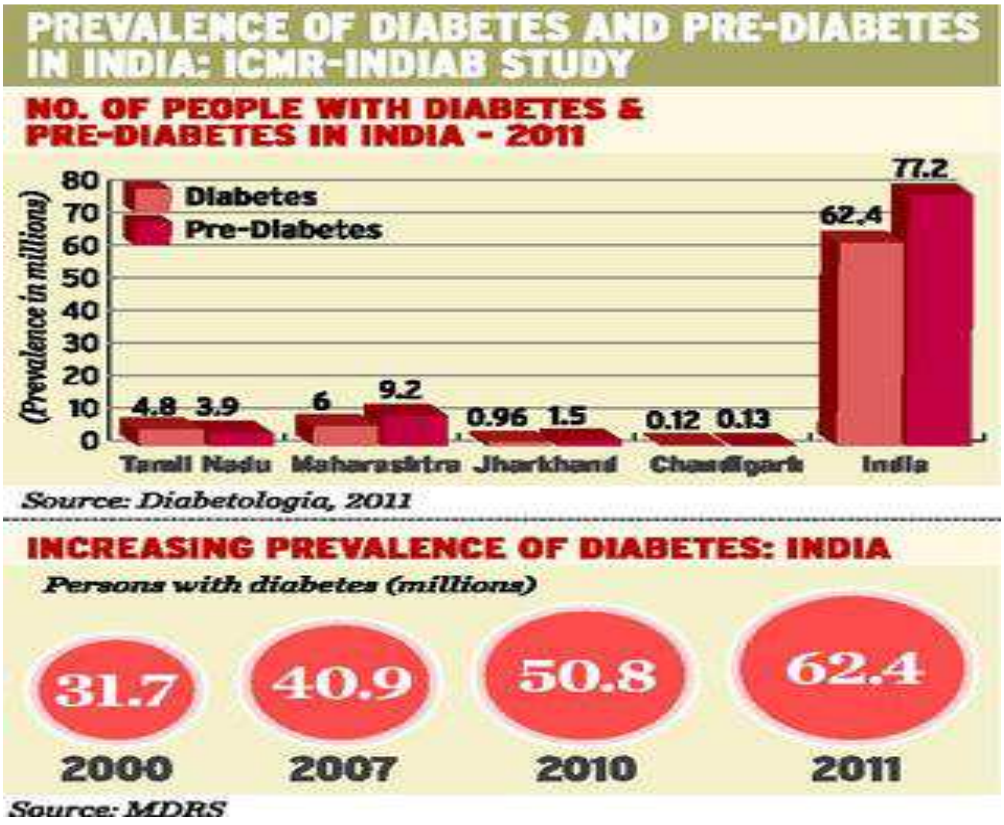
Prevalence of Diabetes in INDIA:

ICMR-INDIAB is the largest recent study conducted exclusive to analyze the impact of Diabetes in different regions of India. Results from the phase I of ICMR-INDIAB study was frightening and the epidemiological data about diabetes also differs in the various regions of India. In India, 62.4 million people are living with diabetes which is very high on compare with the rest of the world. 77.2 million People in India are in pre diabetic stage which should be addressed and needs preventive measures at this juncture.

On dissecting the prevalence of diabetes among the regions of India, Chandigarh scores the high prevalence with 13.6 percent, followed by Tamil Nadu with 10.4 percent. Next in the order is Maharashtra with 8.4 percent followed by Jharkhand with 5.3 percent. This data shows that diabetes is prevalent throughout India without confining to North or Southern part of India and also blooming exponentially.

On converting the percentage into numbers of people living with diabetes in our state, around 4.8 million diabetic populations are living in Tamil Nadu. 3.9 million People are in pre-diabetes stage which has to be taking in hand to reduce the further heave in the impact of diabetes in the state. Though the

number of people with diabetes is rising, the age of onset is decreasing with shift towards young middle age peoples of age group 25-34 years.



In most countries where diabetes is highly prevalent, pre mature illness as well as premature death rate was high. Thus diabetes has emerged as one of the cause for premature death. Diabetes mellitus accelerate the rate of atherosclerosis as well as arteriosclerosis there by contributing to the increased risk of cardiovascular disease. Cardiovascular disease is

responsible for between 50% and 80% of deaths in people with diabetes. Diabetes induced injury doesn't confined to cardiovascular system. Diabetes is the one of the leading cause of visual loss, amputation and chronic kidney disease. Diabetes complications not only affect the individual health. It in turn causes financial burden to the entire family and loss of man power to the society. One positive point at this juncture is that diabetes onset is preventable; diabetes complications are preventable in early stages. It is easy to prevent than to suffer the pain. Promising for healthy life prevents the onset of diabetes, periodic monitoring for emergence of complication prevents the diabetes complications.

Diabetes: Changing Trend

Diabetes is arising as an epidemic especially in young age people which is a worrisome fact. The rise in diabetes mellitus among the young people is bewildering as the rise may be due to earlier age of onset of type 2 diabetes[19,20] or slow onset of type 1 diabetes.

Recent studies show that due to restricted physical activity and consumption of high calorie diet in children, there is high incidence of childhood obesity. Obesity which sets the clock on for insulin resistance in childhood may contribute to the earlier development of type 2 DM at young age. The rise in

the earlier onset type 2 diabetes may be also due to urbanization and parental diabetes. Due to rapid increase in obesity among general population, even people diagnosed as type 1 diabetes are obese or overweight at the time of diagnosis[22].

Diabetes related complication also occurs early than expected if diabetes occurs early. Thus apart from early onset of diabetes mellitus, these young individual are also prone to get vascular complication at an earlier stage which will be a great agony for the entire family. Thus differentiating the type of diabetes on the basis of weight or age of onset becomes baffling and needs further work up to classify the diabetes in young[22,23].

On analyzing the pathogenesis of these two major types of diabetes, autoimmune damage of β- cells of pancreas by various auto antibodies is the major cause for type 1 diabetes. To classify as well as to differentiate type 1 DM from type 2 DM in addition to clinical presentation, identification of auto antibodies[18] to the β- cells of islet of pancreas becomes much essential in youth population. Knowledge about the presence of these autoantibodies to Insulin as well as islets of pancreas especially in overweight and obese young people with diabetes can guide about decisions regarding initiation of insulin therapy at an early stage to achieve euglycemia and avoid diabetes related complication.

Need of the study:

There are several studies such as UKPDS which have demonstrated the presence of auto antibodies like GAD 65 in peoples diagnosed as type 2 diabetes phenotypically. To assess the presence of auto antibodies in young peoples diagnosed as type 2 diabetes phenotypically in our community, I have planned to conduct this study at the Institute of Internal Medicine and Institute of Diabetology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai for a period of 6 months from----------

Aims and Objective

1. To study the presence of GAD and IA-2 antibodies in young adults diagnosed as Type 2 Diabetes phenotypically.

2. To assess the ability of auto antibodies to discriminate between Type 1 diabetes and Type 2 diabetes whose treatment differs.

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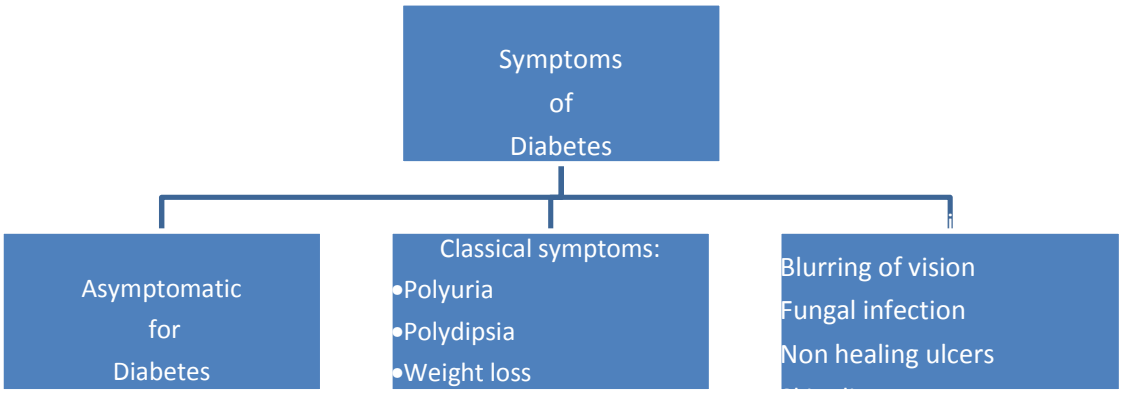
Review of Literature

Diabetes Mellitus is a metabolic cum vascular disease characterized by chronic hyperglycaemia that is due defects in insulin secretion or insulin action or both. Diabetes mellitus is associated with long-term damage of organs especially blood vessels, eyes, kidneys and heart. Thus diabetes leads to deterioration of function of different organs and makes debilitating quality of life of the individual.

Symptoms of diabetes[3,4]:

Half of the patients with diabetes are asymptomatic and detected on investigations done for other purpose or during health screening.

Classical symptoms are frequent urination, excessive thirst, weight loss. But diabetes may be detected first time with presentation of diabetic keto acidosis or even with long term complication like retinopathy or nephropathy. Diabetes may be cause or association with various diseases like fungal infection especially over external genitalia, non-healing ulcers, visual disturbances etc.



Complication of diabetes:

Diabetes complication can be divided as life threatening acute complication which necessitate immediate intervention to save the life of the individual where as long term complication are majority due to advanced glycation end products (AGE) that needs to be prevented than to be treated. Acute complications are metabolic in nature whereas chronic complications are vascular.

Long-term complications of diabetes are grouped as microvascular and macro vascular complication. Microvascular complications include retinopathy, nephropathy and peripheral neuropathy. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes and which if present can aggravate the complication of diabetes more aggressively.

Diagnostic criteria for Diabetes[1,4,5]

On contrast to other endocrine disease where hormone levels were determined for the diagnosis, diabetes is diagnosed by estimating the glucose level in the sera of the patients. Oral glucose tolerance test (OGTT) is the diagnostic test recommended by WHO till date for the diagnosis of diabetes.

OGTT would be administered in the morning after the patients has had at least 3 days of unrestricted diet and usual physical activity. The test should be done overnight fast of at least 8 hr. during which patient may drink water. After collecting the fasting blood sample, the subject is allowed to drink 75gram of anhydrous glucose in 200-300 ml of water over a period of 5

minutes. Blood was drawn at 2 hours of ingestion of glucose. The blood samples are to be immediately centrifuged promptly to separate out the plasma and to be analyzed immediately.

Category	**Venous plasma glucose** **in mgs/dl (mmol/L)**
Diabetes Mellitus Fasting plasma glucose 2hr post glucose load	 ≥ 126mgs/dl (≥ 7.0 mmol/L) ≥ 200mgs/dl (≥ 11.1 mmol/L)
Impaired glucose tolerance (IGT) Fasting plasma glucose 2 hr post glucose load	 < 126 mgs/dl (< 7.0 mmol/L) 140 mgs/dl to 199 mgs/dl (7.8 mmol/L to 11.1 mmol/L)
Impaired fasting glucose(IFG)	

Fasting plasma glucose	110 mgs/dl - 125 mgs/dl (6.1 mmol/L to 6.9 mmol/L)
2 hr post glucose load	< 140mgs/dl (<7.8 mmol/L)

The above diagnostic criteria were adapted from consensus statement of World Health organization (WHO), Geneva and International Diabetes Federation (IDF).

OGTT will not only diagnose diabetes, but also identify two other categories such as Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG) which are forerunner for diabetes mellitus.

Classification of Diabetes Mellitus[1,2]

Earlier classification of Diabetes Mellitus was based on the age such as juvenile onset diabetes mellitus for type 1 diabetes mellitus and adult onset diabetes mellitus for type 2 diabetes mellitus. Subsequently diabetes is classified on basis of the treatment aspect especially on the need of insulin

such as Insulin dependent diabetes (IDDM) for type 1 diabetes mellitus and non-insulin dependent diabetes (NIIDM) for type 2 diabetes mellitus. This classification was confusing as patients with any form of diabetes may require insulin treatment at some stage of their disease.

Hence recent classification of Diabetes Mellitus by WHO and American Diabetic Association was based on the etiopathogenesis which address the pathophysiology involved in the process of the complex disease like diabetes. This classification leads to better understanding about the disease, ease the mode of treatment and paid the way for preventive aspects about the diabetes.

CLASSIFICATION OF DIABETES:

Type 1 Diabetes Mellitus

a-autoimmune

b-idiopathic

Type 2 Diabetes Mellitus

Gestational Diabetes Mellitus

Secondary Diabetes Mellitus

Gestation Diabetes Mellitus –

Defined as any degree of glucose intolerance that is associated with hyperglycemia of variable severity with first time detection during pregnancy

Maturity Onset Diabetes of the Young (MODY).

MODY is a type of diabetes associated with β-cell dysfunction resulting from a specific mutation in MODY related gene. It is due to Genetic defects of β-cell function in the form of impaired insulin secretion which is the prime defect. There may be minimal or no even defects in insulin action. Inheritance pattern of MODY is autosomal dominant. As it is difficult to perform genetic studies in all clinical criteria can be utilized for the diagnosis of MODY.

Obesity is not need to present in patients with MODY mutations and obesity is unlikely to be a consistent feature. MODY is characterized by onset of hyperglycemia at an early age, generally before age 25 years.

Drug or chemical-induced:

Diabetes occurring secondary to drugs or toxins are not common as that of type 2 DM. These drugs on long term can cause diabetes either by producing resistance to the insulin pharmacological action and can have direct injuries effect on β-cell of the pancreas there by producing secretary defect. Few of such drugs are glucocorticoids, thiazide diuretic, nicotinic acid, immunosuppressant like cyclosporine[4] and its followers, pentamidine, diazoxide [3,4]etc.

Diabetes and genetic syndromes:

Diabetes occurs as part of some genetic syndromes like Down syndrome, Turner syndrome, Wolfram syndrome, Klinefelter syndrome

Diabetes in Friedreich's ataxia is common endocrine abnormality which is present in 20% due to both insulin resistance and secretory defect. In Ataxia telangiectasia, there is high incidence of type 1 DM.

Pre diabetes:

Pre diabetes is stage prior to the onset of frank diabetes. People with prediabetes will have blood sugar more than the normal limits but not high

enough to be called as diabetic. It comprises impaired glucose tolerance and impaired fasting glucose. If not intervened with life style changes about 11% will develop full blown type 2 diabetes as per ADA statement. Hence pre diabetes should be used as an opportunity to avoid diabetes as it is a reversible state.

Impaired Glucose Tolerance (IGT)

Impaired Glucose Tolerance (IGT) is a stage in which individuals glucose tolerance is above the conventional normal range but lower than the level considered diagnostic of diabetes. Clinical importance of IGT is high risk for developing frank diabetes and macrovascular complication. IGT is associated with insulin resistance and most of these persons are obese or over weight. IGT can be taken as platform to educate and halt the progression to frank diabetes by life style interventions.

Impaired Fasting glucose (IFG)

Impaired Fasting glucose (IFG) is also a stage of impaired glucose homeostasis. Whose fasting glucose levels were above normal but below those diagnostic for diabetes. This group is also highly vulnerable to get diabetes and can be prevented by life style changes.

Consequences of diabetes

Diabetes is well known disease that produce complication on long term to various organs in the human body in particular to the blood vessels to the heart, brain, kidney, eyes and have a strong predilection towards damaging the nerves.

- Diabetes increases the risk of heart disease as well as cerebrovascular accidents. As well known that major cause of death in diabetic people is due to cardio vascular disease accounting to 50% of people[1,3]. Diabetes is one of the major causes of silent myocardial infarction by causing dysfunction in autonomic nervous system. The risk of coronary artery disease in diabetes population is about 2 to 4 times than the general population. Diabetes people develop coronary artery disease in advance about a decade earlier.

- Diabetes increases the risk of chance of getting a foot ulcer, the gate way to limb amputation. Foot ulcers in diabetic patients are due to combined damage to the nerves of the limbs and occlusion of the arterial tree supplying the limbs. Diabetes is the leading cause for non-traumatic foot amputation and diabetic feet are at

high risk with about 15 – 40[1,3,4] times more likely to have a lower limb amputation compared to the general population

- Worldwide estimation about the blow of diabetes on eye is that more than 2.5 million people's eyes are affected by Diabetes. In industrialized nations, Diabetic retinopathy is the leading cause of vision loss. Pathetic situation in this disease is that the loss of vision due to diabetes is more prevalent in the working age group individuals i.e., in the age of 20 to 65 years.

- Diabetes damages the kidney which occurs after few years of onset of diabetes. Diabetes is one of the leading causes for chronic kidney disease which can be easily prevented by estimation of urine for albumin and reverted by use of pharmacological agents like ACE inhibitors or ARB. It is estimated that mid-way between 10-20% people with diabetes die of kidney disease.

- Damage to nerves by diabetes is the most common long term complication affecting more than 50% of persons with diabetes. Diabetes damages all the nerves like sensory, motor, autonomic in isolation or in combination. Diabetes also affects the cranial nerves

like facial nerve and oculomotor nerve. Diabetes affects the oculomotor nerve with sparing the papillary fibers.

Though diabetic neuropathy can present with different expressions, common clinical presentation are tingling, pain, numbness, or weakness in the feet and hands. On long term, damages to these nerves presents with reduced sensation making these feet for high risk for formation of foot ulcers and eventual amputation.

- Diabetes makes the person to die a decade before than people living without diabetes[3].

Impact of Diabetes in India:

Countries by diabetes cases in SEA

Country	Cases
1. India	63.0 million
2. Bangladesh	5.5 million
3. Sri Lanka	1.1 million
4. Nepal	506,727
5. Mauritius	141,644
6. Bhutan	22,362
7. Maldives	15,908

Global figures for diabetes, 2012 (20-79 years)

Prevalence of diabetes in adults	8.3%
Number of people with diabetes	371 million
Number of undiagnosed cases	187 million
Deaths due to diabetes	4.8 million
Total healthcare expenditures in USD	471.6 billion

Diabetes in India

AT A GLANCE	2012
Total adult population (thousands)	752,631.15
Prevalence (%)	8.37
Number of adults with diabetes (thousands)	63,013.87
Number of adults with undiagnosed diabetes (thousands)	4,437.52
Number of deaths due to diabetes	1,013,057.00
Mean healthcare expenditures per person with diabetes (USD)	67.98

Prevalence of diabetes is increasing in India tremendously on par with the rest of the world**.** As per the diabetes atlas of International Diabetes Federation released in the year 2007, about 40.9 million diabetes people are living in India and this number may increase to 69.9 million[3].

Diabetes patients in Asian Indian differ from that of western population with diabetes in many aspects such as younger age onset, less obesity and genetic factor play a major role.

From the Chennai Urban Rural Epidemiology Study (CURES)[10]following facts about the impact of Diabetes in India were obtained

- Prevalence of Diabetic retinopathy is 17.6% in south Indian population. Diabetic retinopathy can be broadly divided into Non-proliferative diabetic retinopathy and proliferative diabetic retinopathy. NPDR is again sub classified into early, mild, moderate and severe depending upon the presence of microanuerysm, soft and hard exudates, IRMA- intra retinal microvascular abnormalities, dot and flame shaped hemorrhages. Proliferative diabetic retinopathy is characterized by the presence of neovascularization over the optic fundus.

- Prevalence of overt diabetic nephropathy is 2.2%[13] and

Microalbuminuria was present in 26.9%. Microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease which at appropriate intervention can retard or reverse the progress of the disease. Microalbuminuria also severs as a marker for endothelial dysfunction there by a risk factor for cardio vascular disease.

- 3.2% of the diabetes population in India has peripheral vascular[12] disease in India.

- Coronary artery disease prevalence in Indian diabetic is 11% as per the Chennai Urban Population Study (CUPS)[9].

Aetio-pathogenesis:

Regarding etio-pathogenesis, majority of cases of diabetes fall into two broad categories known as type 1 and type 2 diabetes. Diabetes is a complex disease where combinations of multiple genes and environmental factors play a pivotal role leading to loss of β –cell of pancreas thereby causing hyperglycemia. Underlying pathogenesis resulting β-cell loss of pancreas is quite diverse in the subtypes of the disease that ends in progressive cell failure and hyperglycemia as sequel.

Type 1 Diabetes Mellitus[16]:

Type 1 DM most commonly results from a chronic autoimmune destruction of the pancreatic islet β cells. This process would have probably initiated by exposure of a genetically susceptible host to an environmental agent. These patients are prone to ketoacidosis on comparison to type 2 DM.

The pathogenesis in type 1 diabetes mellitus is mostly caused by an autoimmune assault against the β-cells of pancreas inducing progressive cell death. Whereas the pathogenesis in case of type 2 diabetes is more variable.

It involves different degrees of β-cell failure due to interaction between insulin resistances and insulin secretory defect.

Thus diabetes mellitus is etiologically and clinically heterogeneous group of disorders that share hyperglycemia in common. For hyperglycemia to be identified in type 1 diabetes, at least 80-90% of the functional capacities of the β cells should be lost. Exogenous administration of Insulin is the only treatment modality for these patients and its must for their survival

Natural history of type 1 DM:

Natural history of Type 1 DM includes the following major components.

Preclinical autoimmunity:

In this stage, there is an immunological assault on the β cells of the pancreas which leads to progressive loss of the β-cell function. This duration of this stage is variable and may extend from months to years prior to the diagnosis of diabetes by upto 9-13 years. Autoantibodies and T –cells reactive with β-cell antigen can be detected at this stage. Insulin secretion ranges from normal to low with normal glucose in the serum.

Hyperglycemic stage or clinical onset stage:

At this stage 80-90% of the functional capacities of the β-cells are lost. Patients present clinically with polyuria, polydipsia, and weight loss. 20-40% present as diabetic ketoacidosis. Immune markers of type 1 DM such as GAD-65, IA-2 are evident in this stage. Insulin secretion is low.

Remission stage:

This is a transient phase only in the natural history of type 1 DM. There is a transient fall in the requirement of insulin due to an improved β-cell function. Total as well as partial remissions have been reported. Older the age of onset and less severe the initial presentation, absent autoantibodies are consistent with longer remission. In this stage Insulin independence can be seen in 20-70%, blood sugar may be normal or mildly elevated. It is unclear about the mechanism behind the remission. Remission is possible if the offending factor is removed as in the case of type 1 DM occurring due to viral infections or exposure to dietary factors that would have triggered autoimmunity.

Established diabetes:

This stage is long standing diabetes where both acute complications like ketoacidosis and chronic complications like retinopathy, nephropathy, and neuropathy are common. Prevalence of autoantibodies decreases in this

stage and endogenous insulin; c-peptide secretions are progressively lost. These patients require insulin for their survival and periodic monitoring for complications is must.

Most of the type 1 diabetes patients are non-obese. The presence of obesity is not incompatible with the diagnosis. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age. The rate of β-cell destruction is variable especially rapid in children and slower in adults. Genetic, Environmental, dietary factors may have doubtful role in the pathogenesis of type 1 DM.

Genetic factors:

The prevalence of β-cell autoimmunity in first degree relatives of type 1 DM is increased 3-5 times compared with no family history of type 1DM. High risk indicates in individuals heterozygous for HLA DR3/4, DQ2/8.

Environmental factors:[24]

Viruses and components of early childhood diet may cause type 1 DM.

In viruses[21] – rubella, mumps, herpes viruses have been implicated. Increase in the incidence of type 1 DM in patients with congenital rubella syndrome

is obvious and set forth virus infection can be enrolled in the causative agent for type 1 DM.

Regarding dietary factors, breast feeding may be viewed as surrogate for the delay in the introduction of diabetogenic substances present in early childhood diet. Newly diagnosed type 1 DM children have higher levels of serum antibodies against cow's milk – β lactoglobulin. Toxic doses of nitrosamine containing compounds and onset of type 1 DM is recognized.

Type 2 Diabetes Mellitus:

Type 2 diabetes is the most common form of diabetes where vast majority of diagnosed and undiagnosed diabetes fall in this category. Thus type 2 DM represent diabetes in the absence of type 1 DM or other specific type of diabetes. It is characterized by disorders of insulin action and secretion. Etiology of this form of diabetes in not known but auto immune destruction of the β-cells does not occur. Type 2 diabetes patients are overweight or obese. The circulating insulin levels may be normal or elevated yet insufficient to control blood glucose levels because of insulin resistance. Type 2 diabetes occurs more frequently in women with prior gestational diabetes mellitus and in individuals with hypertension or dyslipidemia. Although type 2 diabetes is disease of old aged, now days they are

commonly diagnosed in young, mainly due to obesity epidemic. In Indian population where there is a high prevalence of Type-2 DM, many cases of diabetes occur in young to middle age and adults.

Risk factors for Type-2 DM

- Positive family history –

The empiric risk of family history contributing to Type-2 DM can be due to inherited susceptibility as well as sharing of similar same environment.

- Genetic factors:
  - In contrast to Type-1 DM, genetic factors play a minor role in contributing to susceptibility of Type -2 DM. Type – 2 DM is a complex multi genetic disorder with presence of loci on chromosome 1.
- Obesity:
  - Obesity is a powerful predictor of Type-2 DM. Visceral obesity which can measured by waist circumference or waist hip ratio is the important determinant of risk than body mass index.

- Gestational diabetes mellitus:
    - Women with history of GDM are at risk of developing Type-2 DM later.
- Other risk factors:
    - Physical inactivity,
    - Dietary factors like consumption of high glycemic index with trans-fatty acid diet.
    - Polycystic ovarian syndrome,
    - Metabolic syndrome,
    - Pre diabetes like IGT and IFG.

Pathophysiology in type 2 DM:

Pathophysiology involves both insulin resistance at the peripheral tissue where insulin acts and decline in insulin secretion due loss of function β-cells in the islets of pancreas.

Insulin resistance:

Insulin resistance is a state in which a given concentration of insulin produces a less than normal biologic response. The etiology of insulin resistance at the tissue level especially liver, adipose tissues are not clear. Causes of insulin resistance can be categorized on the basis of etiologic mechanisms like

1. Abnormal β-cell secretory defect
2. Circulating insulin antagonists-
    - excess counter regulatory hormones ,
    - Elevated free fatty acid level
3. Target tissue defect in insulin action- insulin receptor defect

Insulin resistant states share some common somatic features like acanthosis nigricans, hirsutism which may be due to high level of circulating insulin that is capable of binding into IGF-1 receptors stimulating growth and proliferation of various cell types.

Impaired Insulin secretion:

Islet dysfunction in Type -2 DM is not certain whether the defect in insulin secretion is due to loss of β-cell mass or defect in the function of β cells. The reduction of islet cell mass is mainly due to loss of β-cells with normal other cells of islet like α cells. The loss of β-cell mass is due to deposition of amyloid peptide that is co secreted along with insulin. This amyloid peptide deposition leads to islet fibrosis. For the development of fasting hyperglycemia and secretory defect, greater loss of β-cell mass of at least 50% reduction with insulin resistance must.

In initial stages of Type 2 diabetes mellitus, euglycemia can be achieved with help of medical nutrition therapy and oral anti diabetic drugs. Insulin is required for control of blood glucose as the disease progress in addition to pharmacological treatment.

Indication for insulin in Type 2 DM:

- Uncontrolled diabetes with strict adherence to medical nutrition therapy and maximum dose of oral hypoglycemic drugs.
- Profound weight loss due to uncontrolled hyperglycemia

- Type 2 DM patients planning to pregnancy whose blood sugar is uncontrolled with oral agents.
- Severe hypertriglyceridemia
- Surgery in type 2 DM
- Florid sepsis with hyperglycemia where blood sugar has to be controlled instantly to save the life
- Acute Diabetes complication like diabetic ketoacidosis, hyper-osmolar non ketotic coma.

Prevention

Prevention should be the prime initiative at this moment to halt the alarming rise of diabetes in our population. Preventative care need not involve costly treatment or medication. Preventive measures can be categorized as primary prevention i.e. measures to reduce the new onset diabetes mellitus in the community and secondary prevention i.e., the measures to be taken to reduce the occurrence of diabetes complication.

From the analysis of CURE study it has clearly mentioned that only 22.2% of the whole population and 41.0% of the known diabetic subjects were aware that diabetes could be prevented. It also states that awareness and

knowledge regarding diabetes is still grossly inadequate in India. Massive diabetes education programmes are urgently needed both in urban and rural India.

Primary prevention of new onset diabetes

Type 1 DM

Primary prevention in case of type 1 DM can be targeted for the people with high risk like positive history of type 1DM in any of the family members. Incidence of Type 1 DM is very high in the first degree relatives of established type 1 DM patients. These high risk patients are to be screened for detection of auto antibodies than screening the entire population. Preventive measure adopted was giving prophylactic low dose insulin for these patients. But the results of these trials showed no efficacy in preventing or even delaying the onset of diabetes. Several approaches were carried out to prevent the onset of type 1 diabetes. No approach was beneficial and none of them have been shown to work. Preventive aspects of type 1 diabetes remain an objective for the future till date.

Type 2 DM

Preventive aspects hold very well in type 2 diabetes. Measures such as adopting simple lifestyle have shown to be effective. These activities are useful not only in preventing but also delaying the onset of type 2 diabetes.

1. Healthy balanced diet – avoiding high calorie foods rich in trans fatty acid is helpful.
2. Avoiding sedentary leisure activities with a goal to improve physical activity
3. Weight loss in order to maintain ideal body weight
4. Quit smoking habit.

Secondary Prevention of diabetes;

Type 1 DM:

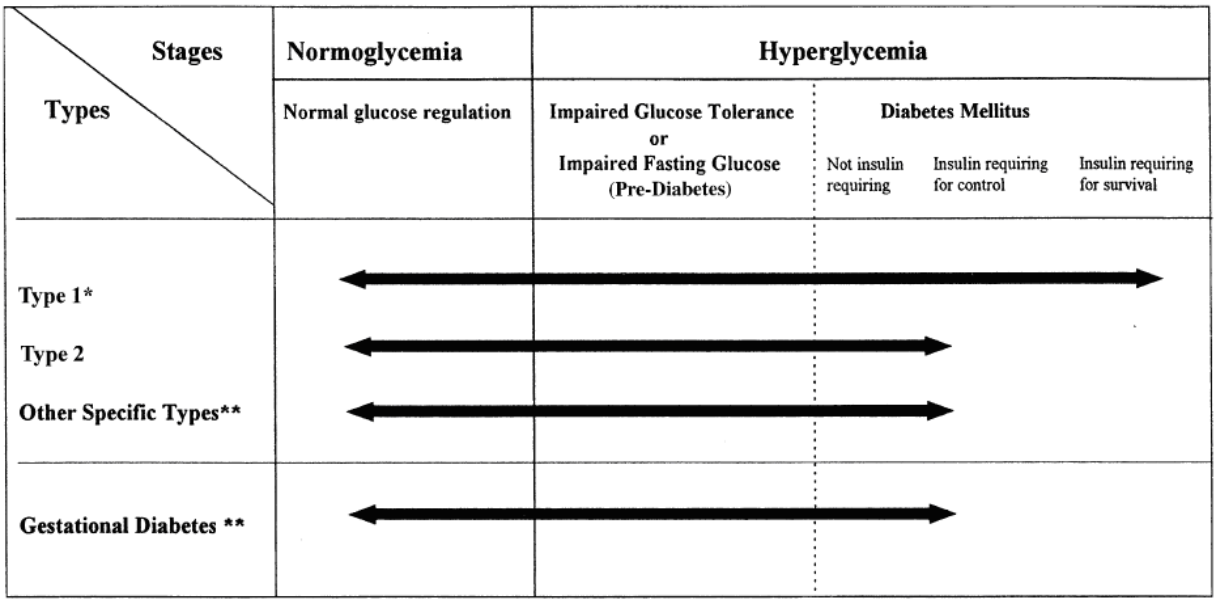
Secondary prevention in new onset type 1 DM was not fruitful to increase the rate and duration of type 1 DM remission. Immunosuppressive therapy with drugs like cyclosporine were tried but was not successful.

Regarding prevention of diabetic complication, DCCT – diabetes Control and Complication Trail has demonstrated that intensive insulin therapy can delay the onset and even slow the progression of diabetic complication

Type 2 DM:

Complications of diabetes can be prevented or delayed through effective management. Treatment of diabetes is not only concerned with lowering glucose, but also involves monitoring as well as reduction in the risk factors for diabetic complications such as control of blood pressure and blood lipids. People with type 2 DM can be managed with life style changes, oral drugs to control blood sugar and sometimes insulin to control blood sugar. People with type 1 diabetes require insulin for their survival.

Stages of Diabetes:



Type 1 DM which has high incidence rate in younger age especially before the age of 20 with a peak around puberty. Type 1 DM also occurs in middle aged people which are often not appreciated because of greater frequency of Type 2 DM in that age group. Hence it becomes dilemma to classify these patients as type 1 DM or Type 2 DM whose treatment is going to differ to achieve glycemic control. Detection of antibodies which are characteristic of type 1 DM in these patients can differentiate this type 1 DM from similar clinical phenotype of type 2 DM. This subset has been described as Latent autoimmune diabetes in adults, late onset type 1 DM or type 1.5 diabetes.

Latent Autoimmune Diabetes of Adult:

LADA are those patients with type 2 diabetes phenotype with presence of auto antibodies to islet or insulin and slowly progressive β –cell failure. Studies like UKPDS reveal that between 5%-30% of patients initially thought as type 2 DM are found to be

Type 1. Expression of autoantibodies to islets like GAD -65 in these patients becomes then paramount importance for diagnosis. In countries like Japan, majority of type 1 diabetes cases develop in adults.

Regarding HLA alleles, these patients resemble as that of type 1 diabetes with less frequent of DQ8/DQ2. Autoimmune β – cell destruction process in LADA proceeds more slowly in these middle aged people on comparison with young patients with classical type 1 DM. Obesity does not exclude the diagnosis of LADA. It's also knows slow onset type 1 DM or autoimmune diabetes of adult onset (ADA)

Clinically these patients present as weight loss, unstable blood glucose levels and extremely diminished C-peptide. Prospective observation of these patients disclosed the clinical finding which included a late onset, positive family history of type 2 DM, slow progression of β – cell failure over several years with incomplete β – cell loss.

Regarding treatment of these patients, oral hypoglycemic therapy will be ineffective and these patients are prone for ketoacidosis, Insulin becomes the choice of therapy. Immediate initiation of insulin in these patients is associated with preservation of C-peptide secretion. On contrary to type 1 Diabetes these patients can be treated with low doses of insulin.

From the above discussion it is very apparent that without determination of islet antibodies, it is not possible to separate type 1 diabetes from type 2 diabetes among obese young adults.

Autoantibodies

Regarding the recognization of target auto antigen there has been remarkable progress for the past 2 decades. Earlier islet autoantibodies was the cytoplasmic islet cell antibody (ICA) which was less specific in diagnosing type 1 DM. Autoantibodies reactive with antigens contained in pancreatic islet cells are common in type 1DM.

Auto immune process in Type 1 DM is most commonly characterized on the presence of auto antibodies such as ICA, IAA, GAD and IA-2. There is a remarkable inverse relationship between the age at which diabetes develops and the levels of insulin auto antibodies.

Epidemiological studies have clearly defined autoimmunity as the presence of auto antibodies in contrast to cellular markers as measurement of autoantibodies is reliable and standardized across laboratories. These auto antibodies are sensitive and predictive in diagnosing type 1 DM in general population.

The best current predictor for the development of type 1A diabetes is the expression of two or more islet auto antibodies like GAD-65, IA-2, IAA, ICA. 80% of individuals developing type 1A diabetes express two or more of these auto antibodies

ICA – (Islet cell antibody)

Islet cell antibody assay was the earliest test done to predict or diagnose type 1 DM. These auto antibodies are called restricted or selective and these are associated with relatively low and slow progression t diabetes. The detection of ICA in the absence of the other autoantibodies is associated with a very low risk of progression to diabetes. There is also difficulty in standardization of this test with poor reproducibility in laboratories. In final, detection of ICA in the absence of the other auto antibodies is associated with a very low risk of progression to diabetes.

Insulin auto antibodies (IAA)

It was the first islet auto antigen to be present especially in children diagnosed as type 1 DM before the age of 5 years. If diagnosed after the age of 15 years, insulin auto antibodies will be difficult to demonstrate in half of the people. Insulin auto antibodies can develop in patients treated with insulin including human insulin after months of therapy. Methods of detection of IAA are also cumbersome with ELISA or fluid phase radio assays which also require large volume of serum. There is a chance of trans placental transfer of insulin antibodies that usually disappear by 6 month of life but may persist up to 1 year. Thus, IAA provides no information relative to the diagnosis of Type 1 DM in middle aged person and persons treated with insulin.

Glutamic acid decarboxylase (GAD)

GAD is one of the most important assays used to diagnose type 1 DM. GAD exists in two major isoforms, GAD-65, GAD-67 where GAD-65 is the immunogen during β-cell destruction. Expression of GAD is relatively common for patients with type 1 diabetes. Although GAD distribution is found in neural tissue, antibodies to GAD-65 are predominantly associated with type 1 DM. GAD auto antibodies are also seen in rare neurologic

disorder like stiffman syndrome. GAD antibodies may provide useful data in detecting auto immune destruction particularly in population screening.

Insulinoma associated protein 2 auto antibodies (IA-2)

IA-2 auto antibodies were detected to about 70% in patients with new onset type 1DM. IA-2 have sequence homology to tyrosine phosphatase like molecules without any enzyme activity. This autoantibody is probably the most specific of the antibodies identified to date. When IA-2 auto antibodies are detected, individuals usually express GAD 65 or IAA. For few individuals, IA-2 auto antibodies may be the only autoantibody detected in the pre-diabetic phase of the disease.

Following are the study that clearly illustrates the importance of autoantibodies in the pathogenesis of type 1 diabetes mellitus.

Heike E.Naserke et al (1998), In this study they analyzed the epitope maturation of IA-2 autoantibodies from the birth. The results of this study gave a picture that antibodies to IA-2 were found to be a risk factor for type 1 diabetes mellitus. The study also highlighted the fact that these autoantibodies were high in prevalence in young individuals who are at risk of type 1 diabetes mellitus.

Anette-G.Ziegler et al (1999)- This study was conducted in the off-springs of type 1 diabetes mellitus patients for the appearance of autoantibodies and development of type 1 diabetes mellitus. Detection of autoantibodies such as GAD, IA-2 and IAA were performed in these patients at the time of birth followed by ninth month of life with subsequent analysis at second year and fifth year of age of these individuals. The results clearly showed that IAA was the prevalent autoantibody in children on comparison to other two autoantibodies. There was a strong co-relation with likelihood of development of type 1 diabetes. Earlier appearance of these autoantibodies showed the faster destruction of the beta cells of the pancreas resulting in earlier development of type 1 diabetes.

Borg.H et al (2001) – This study showed the high prevelance of antibodies to GAD and IA-2 that foretells the β-cell failure in patients with new onset of type 1 diabetes in adult age. They also demonstrated that presence of these autoantibodies is associated with low fasting plasma C-peptide levels. On conclusion it is clear that high level of autoantibodies to GAD-65 as well as IA-2 can predict the destruction of β-cell of pancreas and eventual development of type 1 diabetes especially in the adult population.

Katelijn Decochez et al (2000) – This is another study which clearly revealed the loss of β cell function in patients with autoantibodies like

GAD,IA-2 as well as ICA in the age group of people with diabetes less than 40 years of age.

Kimpimake.T et al (2000) – This study correlated presence of autoantibodies with genotyping in the individual of type 1 diabetes mellitus. Genotyping was done both in type1 diabetes and in their siblings using the PCR technique. Autoantibodies were assessed by radiobinding assay for IAA, GAD and IA-2. At the conclusion it clearly said that type 1 diabetes patients showed increased HLA susceptible gene and increased titres of various autoantibodies. Thus autoantibodies can be used as surrogate markers for the diagnosis of type 1 diabetes mellitus due to auto immune destruction.

Peter A.M.Weiss et al (2000) – This study investigated the impact of type 1 diabetes during pregnancy. The results demonstrated that offsprings of these type 1 diabetes mothers had an increased risk for diabetes later in life with high relative risk for type 1 diabetes with 71.6% on comparison with relative risk of 3.2% for type 2 diabetes mellitus.

Lindsay.R.S et al (2004) – This study was conducted on the cord blood from the off spring of mothers with type 1 diabetes mellitus for the analysis of autoantibodies such as IAA, IA-2 and GAD. They analyzed the influence of presence of these autoantibodies in utero with birth weight of the off-

springs. These autoantibodies does not influence on the birth weight of the offspring born to type 1 diabetic mothers.

Peter Achenbach et al (2004) – This is large cohort study conducted on relatives of patients who are positive for autoantibodies. In this study detailed examination was done that included determination of autoantibodies epitope specificity, titer and IgG subclass. Highest risk for type 1 diabetes mellitus was seen in the population showing high titer of IA-2 and IAA autoantibodies with IgG 2 and or IgG4 subclass of IA-2.

Charlotta Nilsson et al (2007) – This study was unique as the study groups involved were women with gestation diabetes mellitus. This study was done to assess the frequency of β-cell specific autoantibodies in the women with gestational diabetes mellitus. Follow up of these women was done to estimate the risk of development of type 1 diabetes later. The results of these study showed that 6% of the study population were found to be positive for at least one of the autoantibodies to GAD-65, IA-2, IAA. On follow up of this high risk group, 50% of the women who are positive for autoantibodies developed type 1 diabetes on comparison with GDM women with antibody negative group.

Charles F.Verge et al (1998) – In this study they analyzed the precision of combined assay for autoantibodies like ICA,IAA,IA-2 and GAD-65 to forecast the severity of the disease in type 1 diabetes patients and their siblings and this study involved various population and conducted at different centers. The results from the various centers highlighted that IA-2 was useful as a marker of type 1 diabetes mellitus and can be measured consistently without variations. Presence of multiple autoantibodies has achieved high sensitivity with low false positive rate.

James M.Lagasse et al (2002) - This is a cohort study where presence of multiple autoantibodes were evaluated in school children with the median age of approximately 14 years of Washington state and study was followed up for a period of 8 years. The study revealed that presence of single autoantibody was associated with slow progression of the disease whereaspresences of two or more autoantibodies are related with faster disease progression.

Jeffrey P. Krischer et al (2003) - In this study all the four autoantibodies such as ICA, IAA, IA-2 and GAD were studied in the relatives of type 1 diabetes mellitus. Study reported that presence of single autoantibody was more in subjects. Antibodies to GAD were found to be more sensitive than ICA. Increased sensitivity was found in the combination of IA-2 with GAD

with sensitivity of 97% in comparison to IA-2 plus ICA whose sensitivity is 93%.

In the DASP 2000 workshop, both GAD65 autoantibody and IA-2 autoantibody radio immunoassays were found to have high sensitivity (80 and 58%) and specificities (90 and 100%, respectively). The quest to identify one type of autoantibody as a better predictor than another has failed, because no clear order of appearance has been detected. Rather, several studies taken together suggest that the number of autoantibodies is predictive rather than the order of their appearance. Individuals with two autoantibodies had a 68% 5 year risk for developing type 1 diabetes where as those with three autoantibodies had an estimated risk of 100% in 5 years. TrialNet oversight committee has proposed an antibody screening paradigm in which GAD65 Antibody and IA-2 Antibody in younger subjects, are all measured on initial screening

Need of the study:

With the escalating epidemic of obesity in young adults, incidence of type 2 DM is increasing especially before the age of 40 years. On the other hand there is significant increase in the incidence of type 1 DM presenting as either overweight or obese. Whether it is type 1 or type 2, glycemic control is the prime target to avoid diabetes related complication. But the treatment part of type 1 diabetes differs from that of type 2 diabetes in achieving glycemic control.

Hence the clinical criteria to distinguish between type 1 or type 2 diabetes based on age at onset and obesity becomes difficult and this clinical misclassification leads to delay in achieving euglycemic level. Since in many population type 2 DM is either increasing in younger age or already the predominant form of diabetes in young is unanswered. For a clinician to differentiate between type 1 and type 2 diabetes, the most direct way is assess the presence or absence of autoantibodies to islet of pancreas.

Several studies in the past have clearly demonstrated the prevalence of auto antibodies against insulin or islets of pancreas in type 2 diabetes in overseas. Some studies correlated the antibodies level with prevalence of metabolic syndrome in diabetes. The information regarding the phenotypic and

metabolic characteristics of these groups of patients is limited in comparison to the majority patients with type 2 diabetes without autoantibodies.

In this study I am planning to assess the prevalence of auto antibodies – markers of beta cell dysfunction in newly diagnosed type 2 diabetes in young adults. This will help to identify the type of diabetes in young adults and will guide to initiate appropriate treatment to control hyperglycemia there by preventing chronic complications.

Materials and Methods

This is a cross sectional study and it was conducted at Institute of Internal Medicine and Institute of Diabetology, Madras Medical College and Rajiv Gandhi Government General Hospital for a period of 6 months from----------. The protocol for this study was approved by Institutional Ethical Committee (IEC), MadrasMedicalCollege - RajivGandhiGovernment GeneralHospital, Chennai - 600003.

Inclusion criteria for participation in the study

1. Type 2 diabetes mellitus –phenotypically diagnosed as per WHO guidelines
2. Age less than 40 years
3. No diabetes related complications at time of enrollment into the study
4. No other co-existing autoimmune disease
5. Diabetes patients on oral hypoglycemic agents with or without Insulin

Exclusion criteria

1. Not willing to participate in the study after clear explanation
2. History of diabetic ketoacidosis in the past
3. Micro or macro vascular diabetic complications
4. Pregnant diabetes patients
5. Patients known to have pancreatitis disease
6. Patients with autoimmune disease
7. Patients on drugs that may be the cause of diabetes

After meticulous assessment of inclusion and exclusion criteria, 82 patients were enrolled in this study. Verbal explanation about the study was clearly enlightened to the participants and their doubts in the study was addressed and clarified. From those are willing to enroll in the study an informed consent in the prescribed format submitted to ethical committee was obtained. Participants who can't understand English language, the consent form was obtained in their mother tongue (Tamil) so that the participants can have better understanding about the study details.

From the eligible subjects, history such as the duration of diabetes, onset of the disease, cause of detection and family history of diabetes with types were asked and noted in the prescribed format. Regarding treatment, details about diabetes management as well as other diseases were then recorded.

These subjects were subjected to general examination and anthropometric measurements were recorded. In anthropometry height, weight, calculation of BMI by the formula using height and weight, waist hip ratio were recorded. External markers for insulin resistance like acanthosis nigricans, hirsutism were made out by inspection.

Tape marked in centimeters was used to measure the height and it was measured to nearest centimeter. To measure the height participants were requested to stand upright without any foot wear with heels together. They are requested to look forward while measuring the height. Universally accepted and calibrated weighing machine was utilized to assess the weight of the participants. Weight was calibrated to the nearest rounded kg without decimals. Weigh machine was kept on a level flat surface while measuring. Wearing of thin cloths which will not interfere with weight was allowed. To assess the obesity or overweight body mass index (BMI) was calculated. BMI was calculated by dividing weight in kilogram by height in meter square. Reference for body mass index in our study was adopted from the

guidelines released by Health Ministry- Government of India, Indian Council of Medical Research (ICMR) and the National Institute of Nutrition (NIN).

Body mass index for Indian population:

Less than 18.4	Underweight
18.5 - 22.9	Normal
23 - 24.9	Overweight
More than 25	Obese

Measurement of blood pressure was done in a quiet room, patient in seated position for 5 minutes. Well calibrated and accepted blood pressure machine with appropriate cuff size working on mercury scale was used to measure the resting blood pressure. Blood pressure was recorded in the two arms and the highest of these two recording were mentioned. Patients who are on medication for blood pressure control were allowed the take the morning dose as scheduled and BP was recorded under the productive cover of antihypertensive only.

Waist circumference:

Waist circumference is used to assess the central obesity. Measurement of waist circumference was done at the mid-point between the iliac crest and sub costal margin with the help of non-stretching tape. Waist circumference of the participants where measured in standing position with feet together and measurement was rounded to the nearest whole number. Thin layer of cloth is allowed on measuring the circumference. For Indian men cut-off limit for waist circumstances is below 90 cm as opposed to 102 cm worldwide and in case for Indian women it is 80 cm as opposed to 88 cm at the worldwide.

Assays and calculations:

Under sterile precaution, sufficient blood samples were drawn both in fasted and post prandial state. Biochemical investigations were done at quality control laboratory at Institute of Diabetology, RajivGandhiGovernment GeneralHospital, Chennai. Fasting samples were utilized for assessing fasting plasma glucose, lipid profile, renal parameter, c-peptide, insulin auto antibodies like GAD 65 and IA-1. Postprandial blood sample was drawn 2 hours from the ingestion of breakfast with scheduled anti diabetic medication the patient is taking already.

Fasting plasma glucose and post prandial plasma glucose were tested by Glucose Oxidase – Peroxidase method using the validated semi-autoanalyser named Erba Mannheim cehm plus-7.

Urea was estimated by Urease-Glutamte dehydrogenase (GLDH) method and creatinine was assessed by modified Jaffe's method by means of the same semi-autoanalyser named Erba Mannheim cehm plus-7.

With the use of semi-autoanalyser named Erba Mannheim cehm plus-7. lipid profile was estimated. Total cholesterol was assessed by cholesterol oxidase-peroxidase (CHOD-PAP) method, triglycerdies by Glycerophosphate oxidase-peroxidase (GPO-PAP) method and high density lipoprotein was estimated by third generation direct homogeneous assay. Low density lipoprotein was calculated by using the Friedewald equation for the sample with triglycerides less than 400mgs/dl. Urine was test for the estimation of spot protein by 3% sulphosalicylic acid test and normal urine spot protein patients were included in the study.

Immunological assays such as estimation of C-peptide level and autoantibodies such as GAD-65 as well as IA-2 were done at central lab, department of Immunology, the TamilNaduDr.M.G.R.MedicalUniversity, Chennai using the ELISA technique in quality control Elisa reader made be

Biorad. GAD-65 estimation was done by using the Euroimmun Anti-GAD IgG Elisa kit and IA-2 was done by using Euroimmun Anit- IA2 IgG Elisa.

To determine the presence of metabolic syndrome in these individuals, NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III)

Criteria were used. Following are the criteria

1. Blood pressure ≥ 130/85 mmHg or ≤ 130/85 mmHg with antihypertensive medications.
2. Fasting plasma glucose ≥ 6.1 mmol/l (110 mgs/dl)
3. HDL cholesterol < 40 mg/dl for men or < 50mg/dl for women,
4. Triglycerides > 150 mgs/dl or patients on medication with fibrate drugs
5. Waist circumference (WC) cut-offs were taken as >90 cm for males and >80 cm for females to define overweight

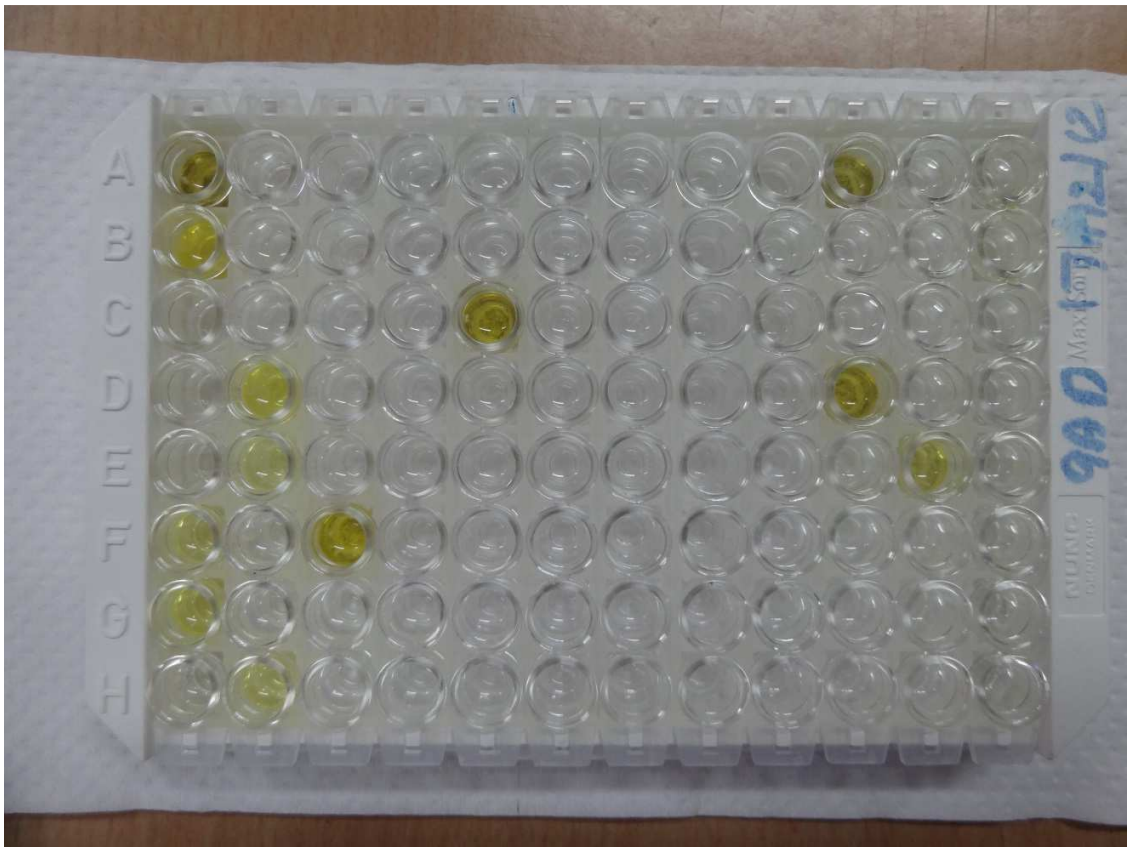
As Indians have higher body fat content than their western counterparts for the same BMI, lower cut-offs of waist circumference were used as suggested by Asia-Pacific guidelines in contrast the globally accepted waist circumference ≥ 102 cm for men or ≥ 88 cm for women.

The diagnosis of the metabolic syndrome required individuals to have at least three of these five criteria. Because all of the subjects in this report had diabetes, they only had to have two of the remaining four criteria to meet the definition.

Statistical method

Data reported in this study include descriptive statistics of the participants at the time of enrollment. Qualitative variables in this study such as sex, family history etc are analyzed by chi-square test. The quantitative data in this study were analyzed using independent sample t tes

ANTI—GAD ANTIBODIES POSITIVE PICTURE



ANTI-IA2 ANTIBODIES POSITIVE:

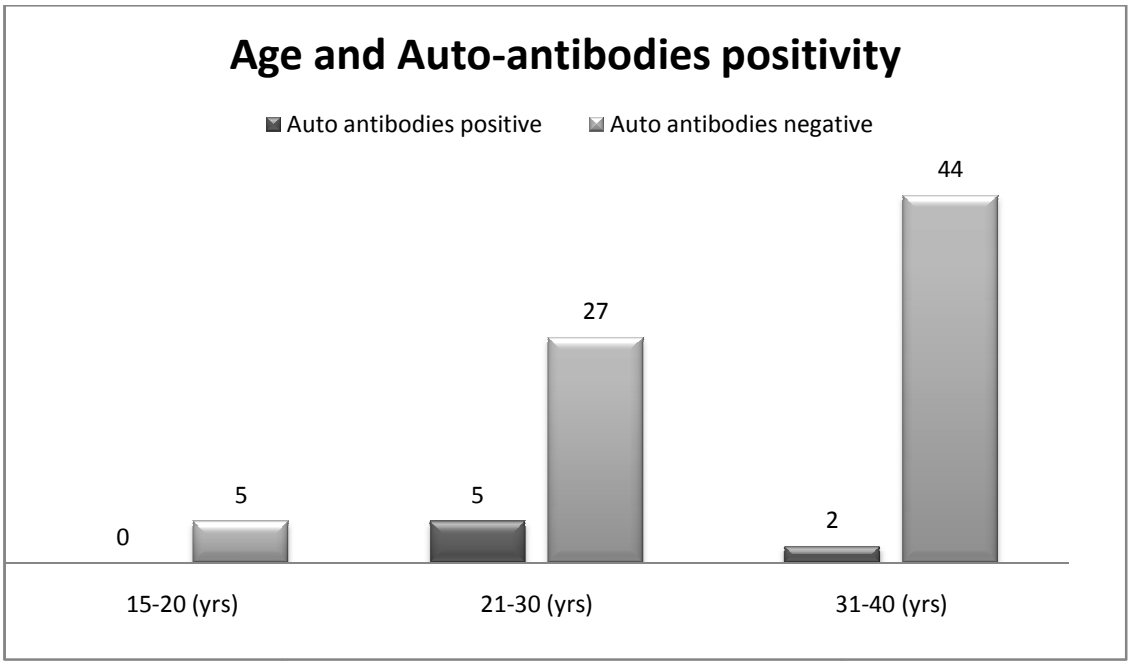
FASTINGT C-PEPTIDE TESTING:

OBSERVATION AND RESULTS

DEMOGRAPHIC PROFILE

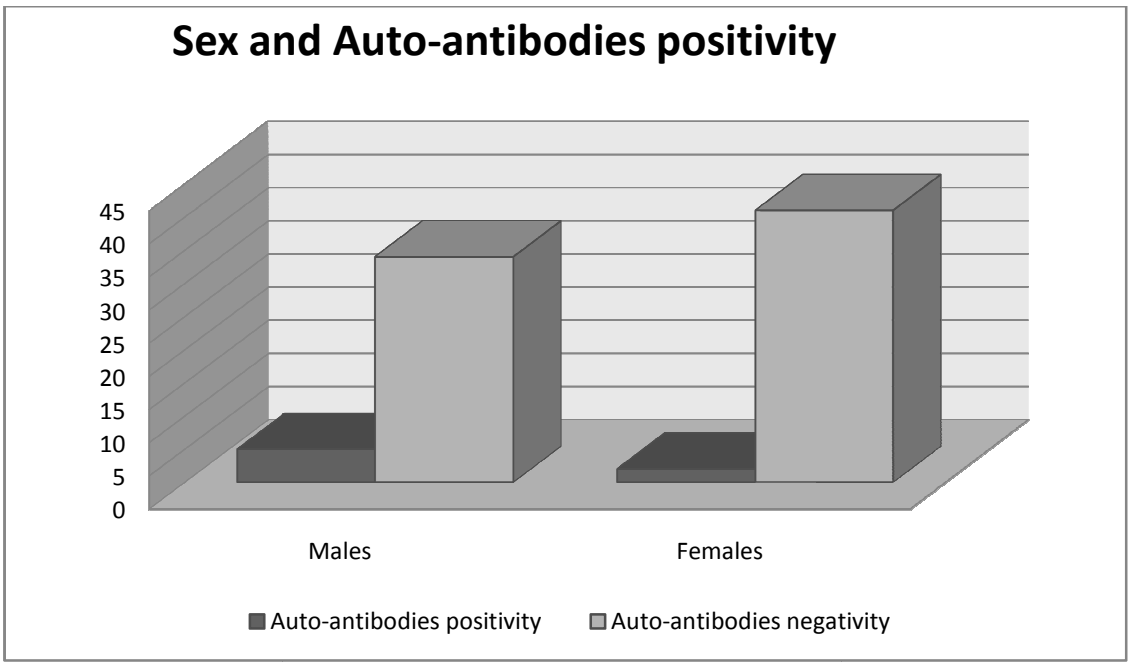
Age groups and Auto-antibodies positivity

Age group(in years)	Auto antibodies positive	Auto antibodies negative
15-20	0	5
21-30	5	27
31-40	2	44



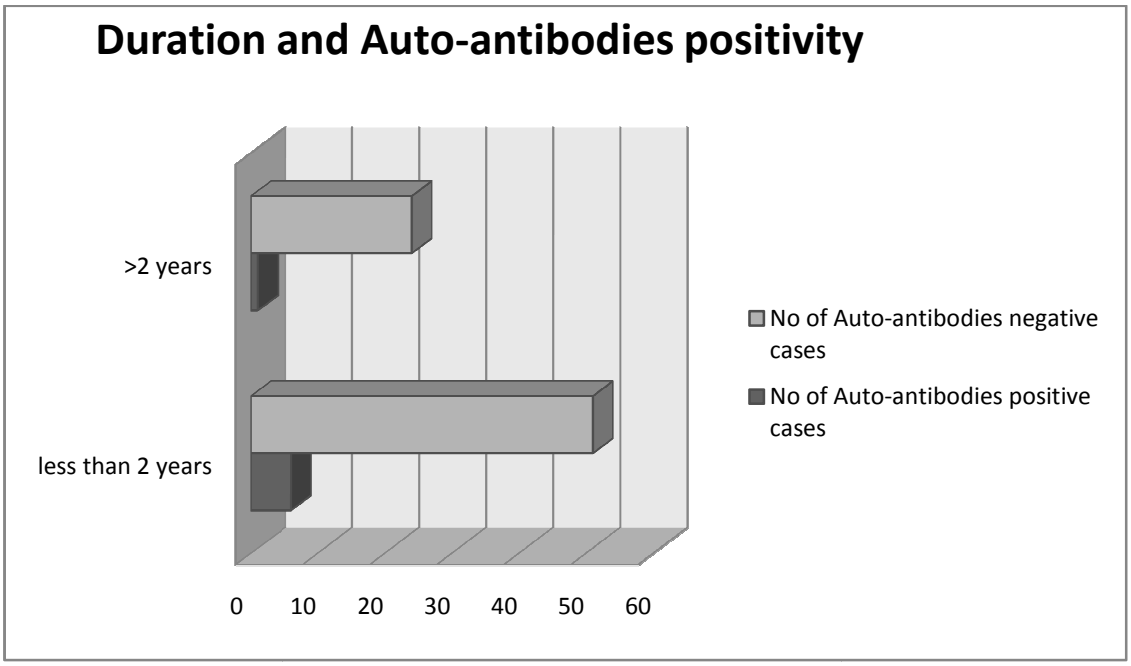
Sex and Auto antibody positivity:

Sex	Auto-antibodies positivity	Auto-antibodies negativity
Males	5	34
Females	2	41



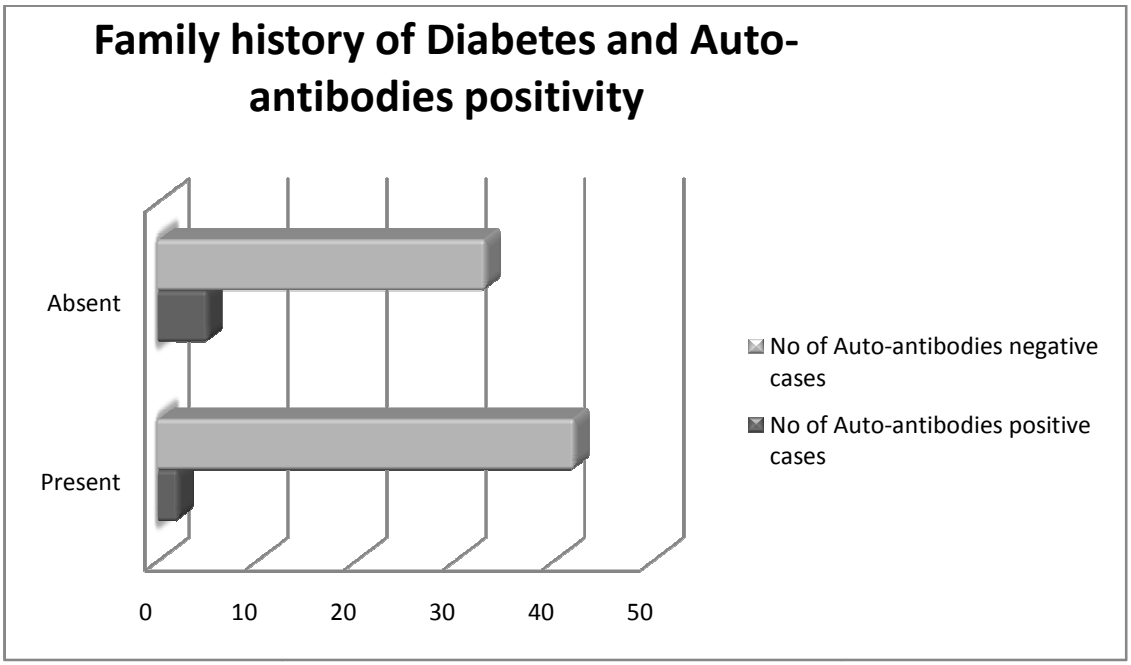
Duration and Auto-antibodies positivity:

Duration of Diabetes(in Years)	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
2	6	51
>2	1	24



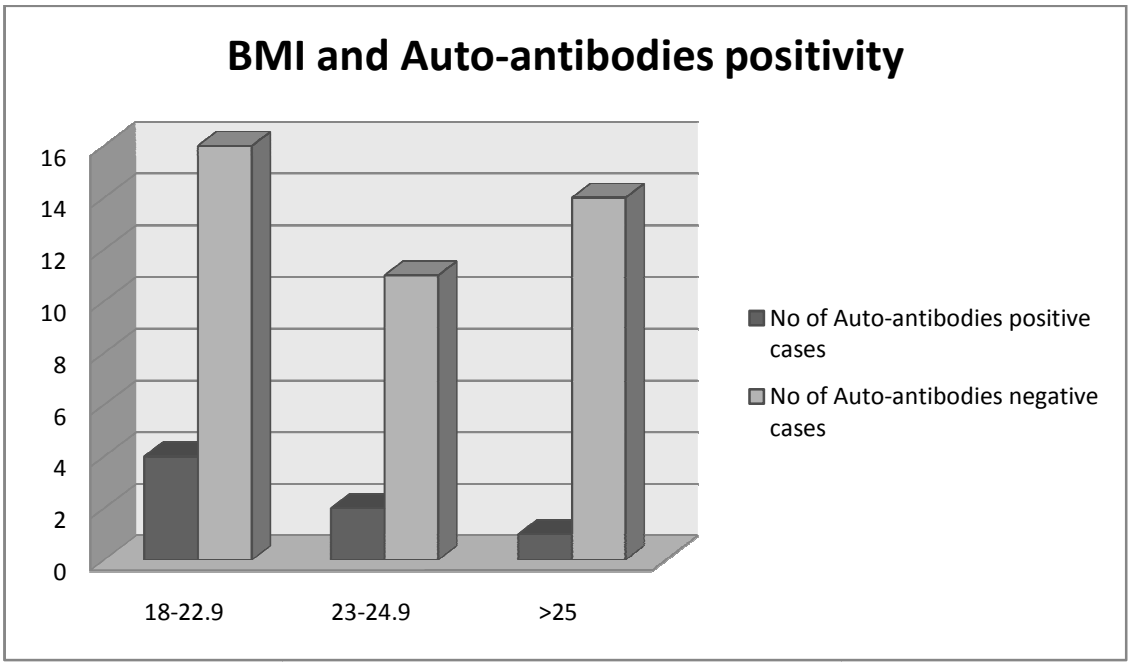
Family history of Diabetes and Auto-antibodies:

Family history of Diabetes	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
Present	2	42
Absent	5	33



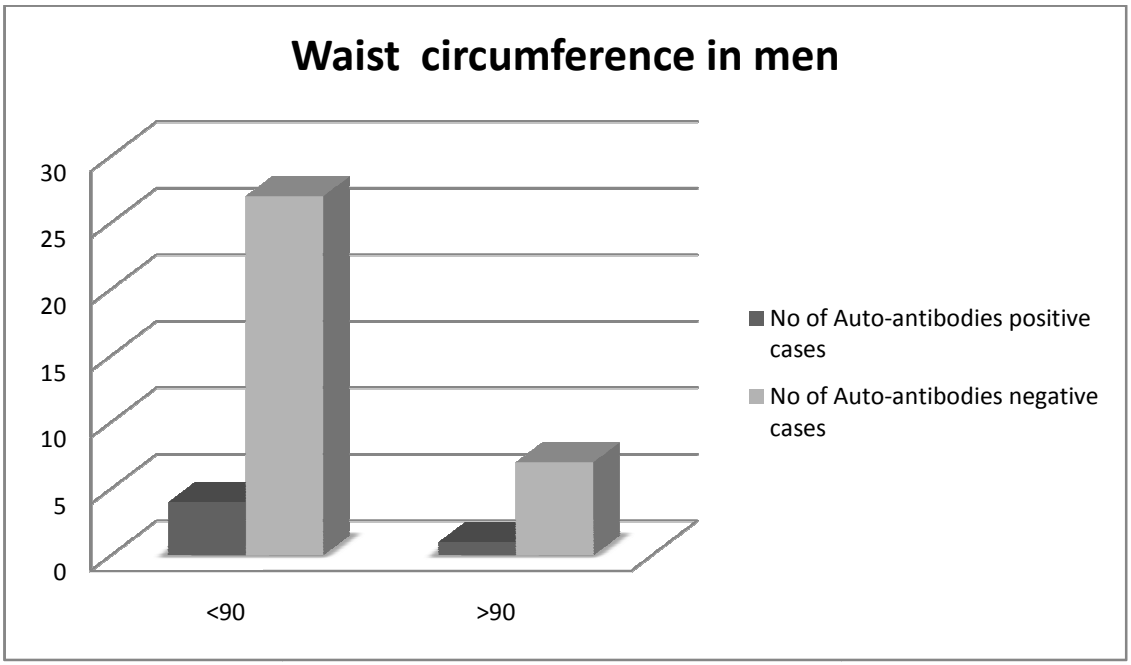
BMI and Auto-antibodies positivity:

BMI	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
18-22.9	4	16
23-24.9	2	11
>25	1	14



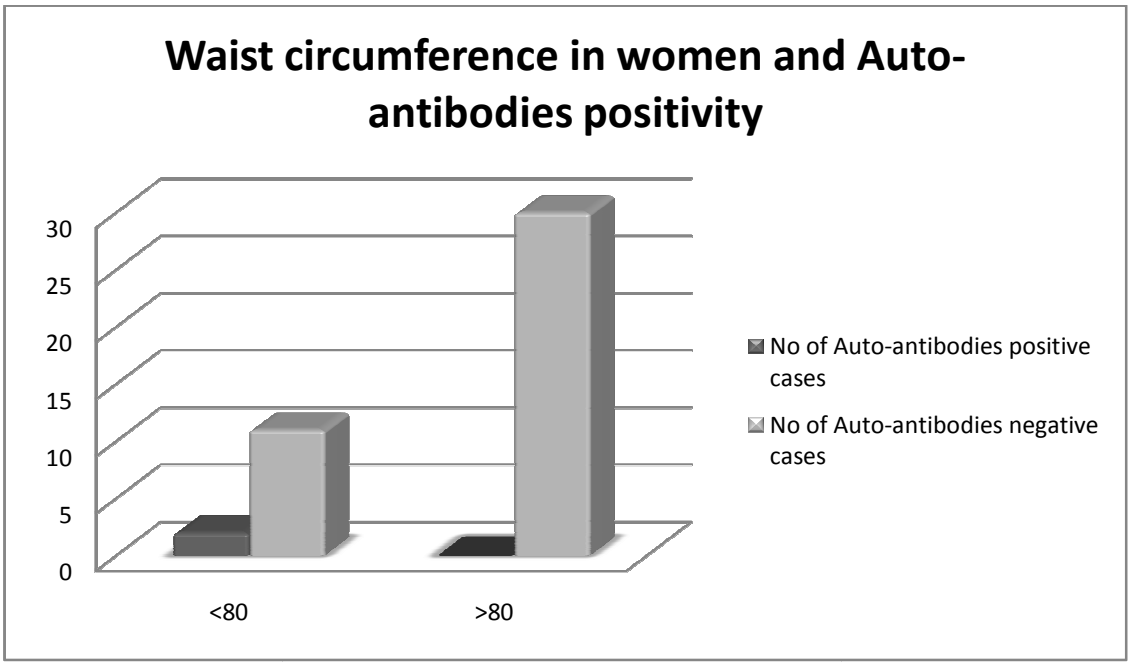
Waist circumference for men:

Waist circumference for men(in cm):	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
<90	4	27
>90	1	7



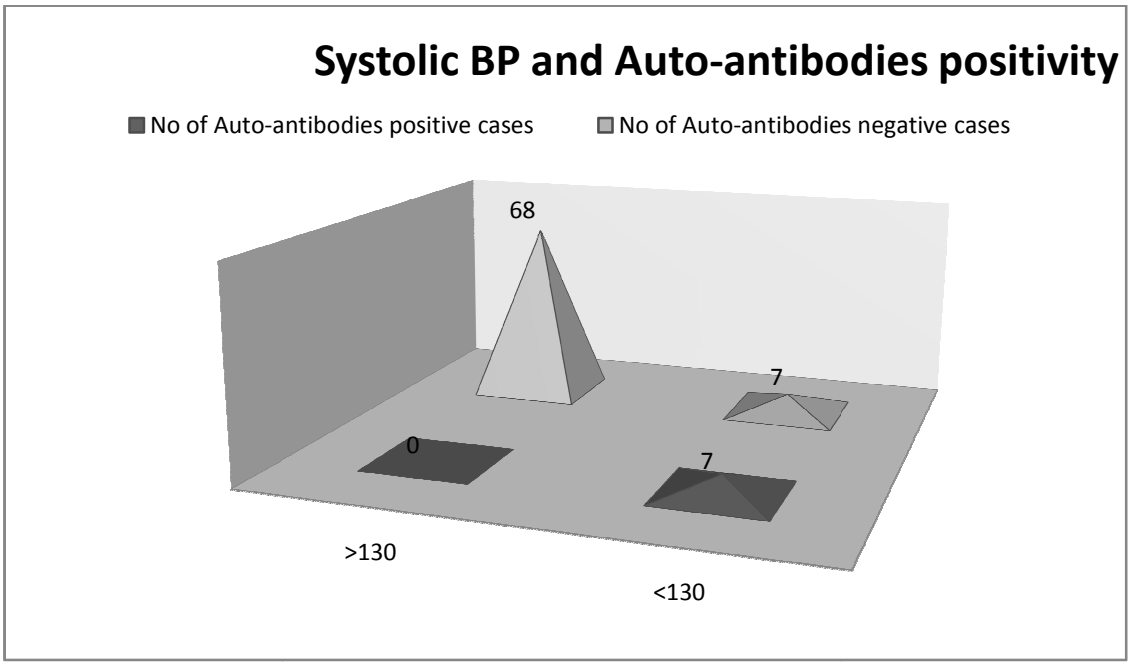
Waist circumference for women:

Waist circumference for women(in cm)	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
<80	2	11
>80	0	30



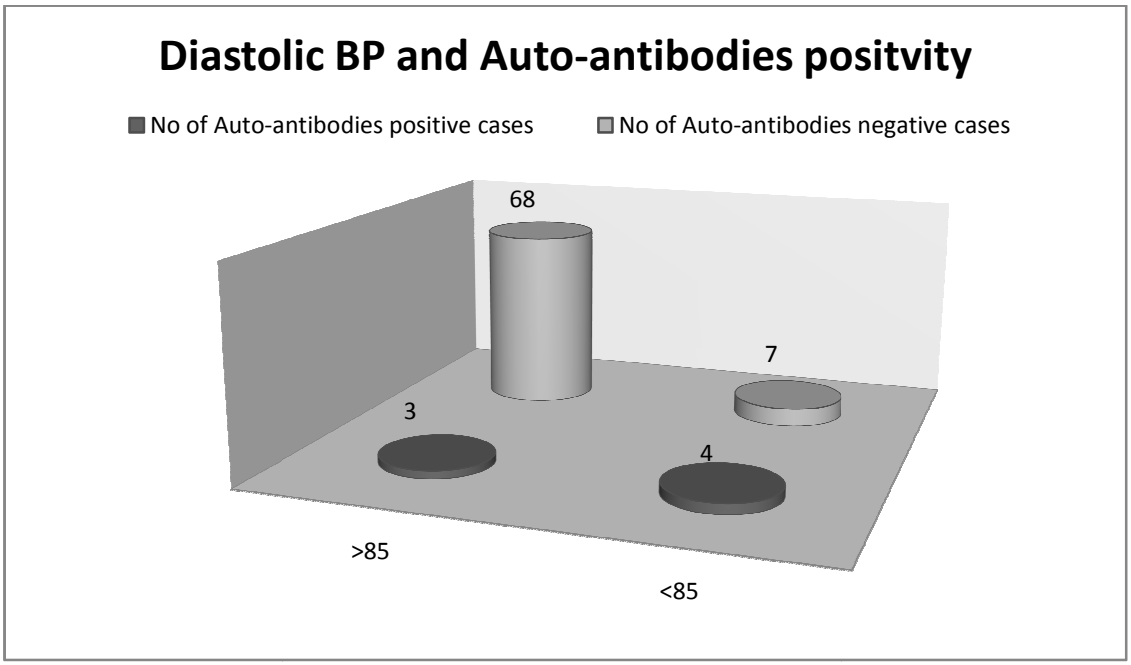
Systolic BP and Auto-antibodies:

Systolic BP(mmhg)	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
>130	0	68
<130	7	7



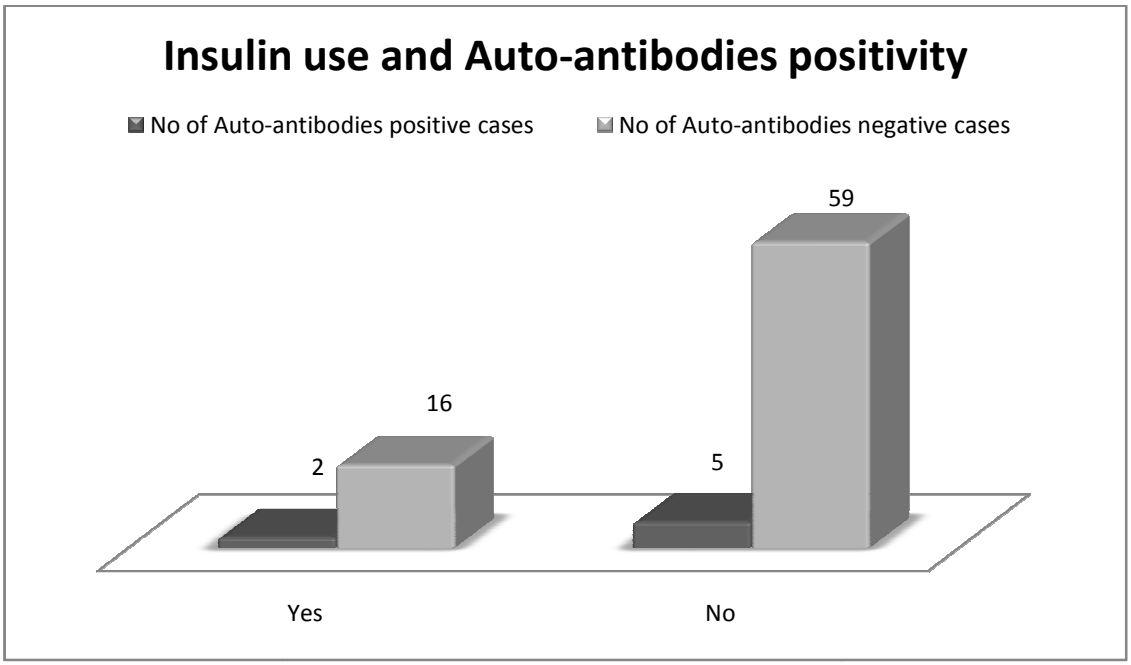
Diastolic BP and Auto-antibodies positivity:

Diastolic BP	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
>85	3	68
<85	4	7



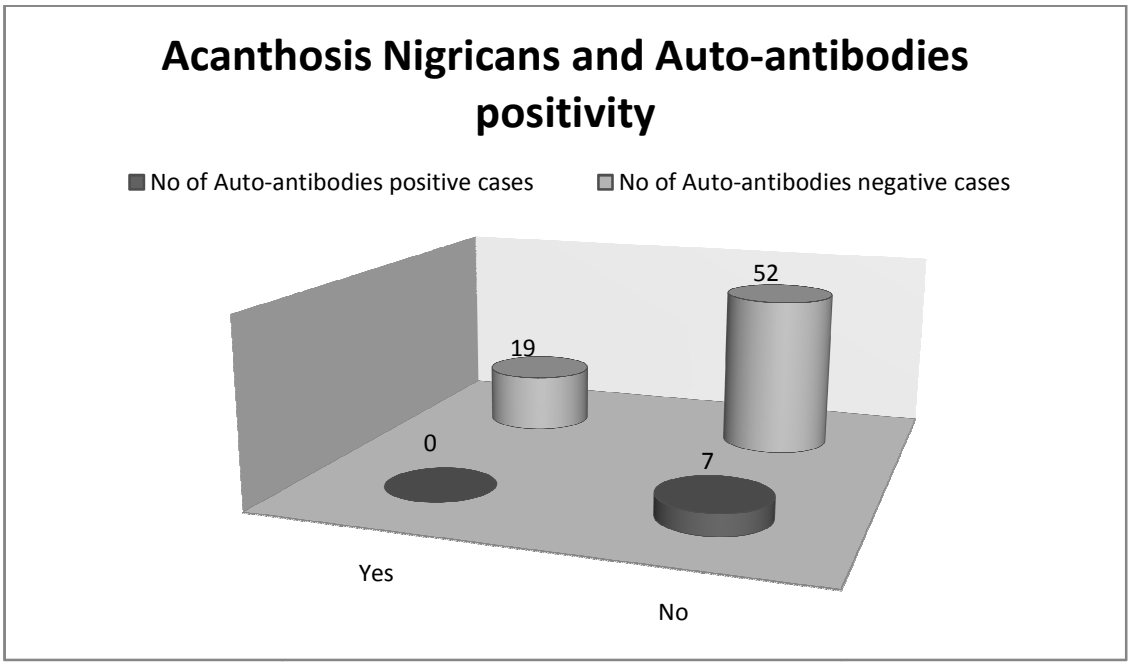
Insulin use and Auto-antibodies:

Insulin use	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
Yes	2	16
No	5	59



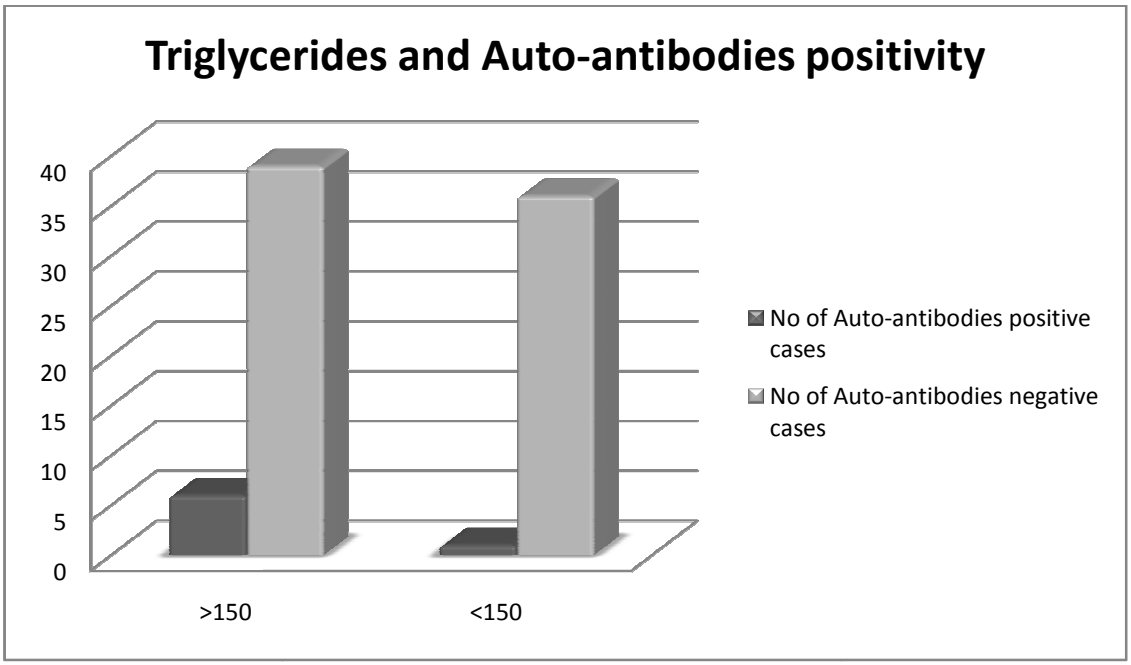
Acanthosis Nigricans and Auto-antibodies Positivity

Acanthosis Nigricans	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
Yes	0	19
No	7	52



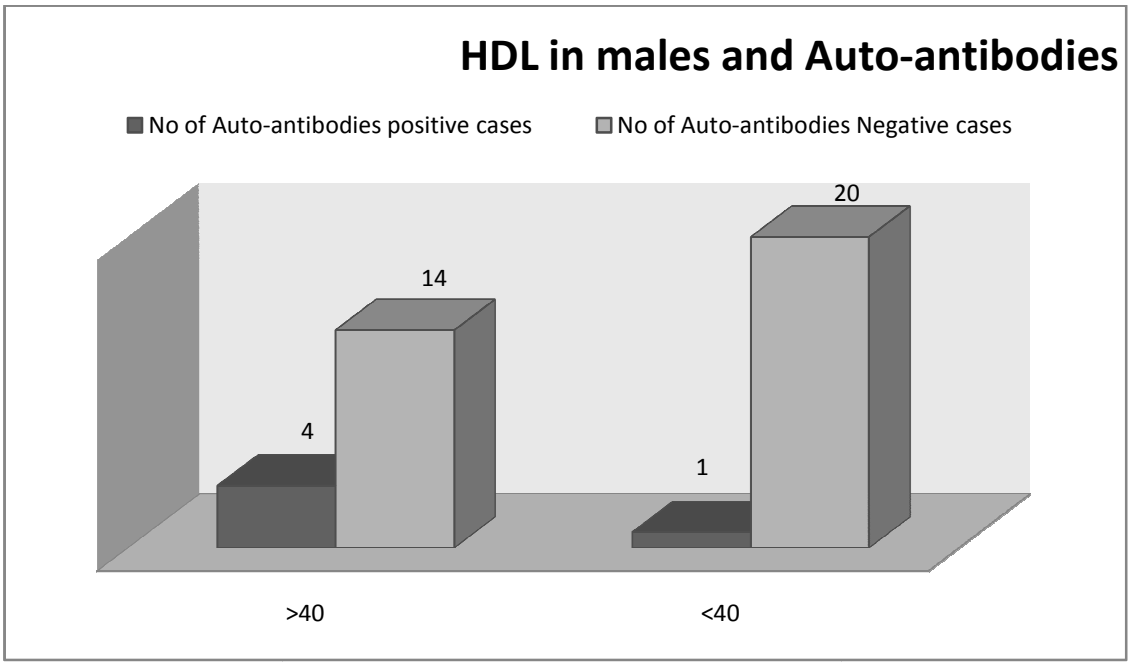
Triglycerides and Auto-antibodies positivity:

Triglycerides(in mg/dl)	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
>150	6	39
<150	1	36



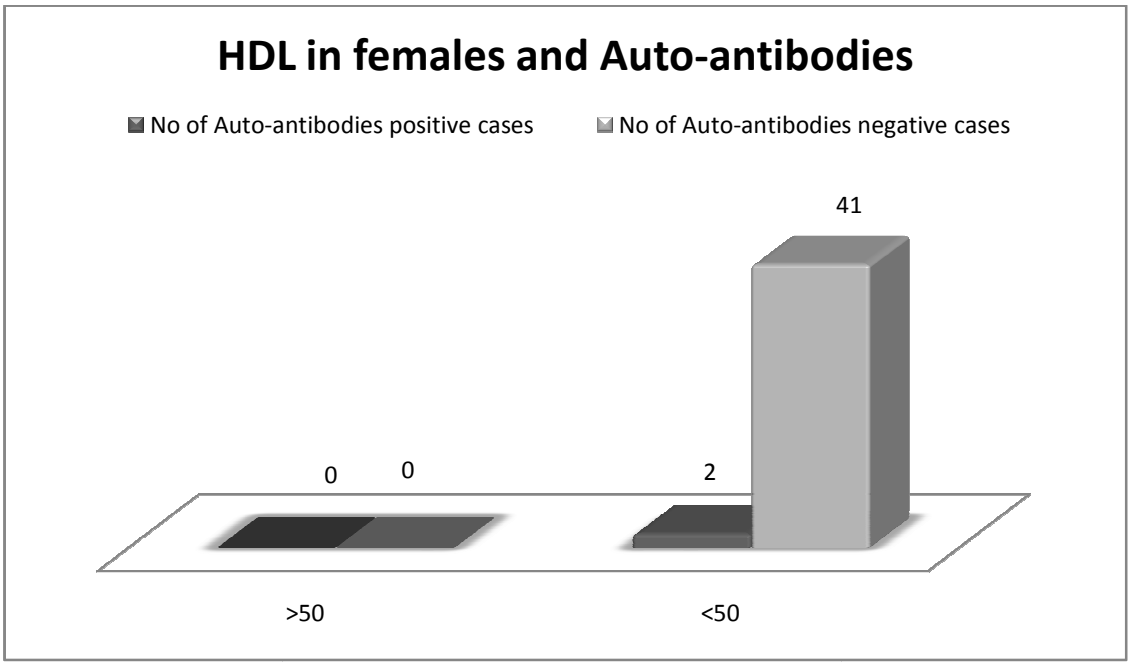
HDL in males and Auto-antibodies:

HDL in males	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
>40	4	14
<40	1	20



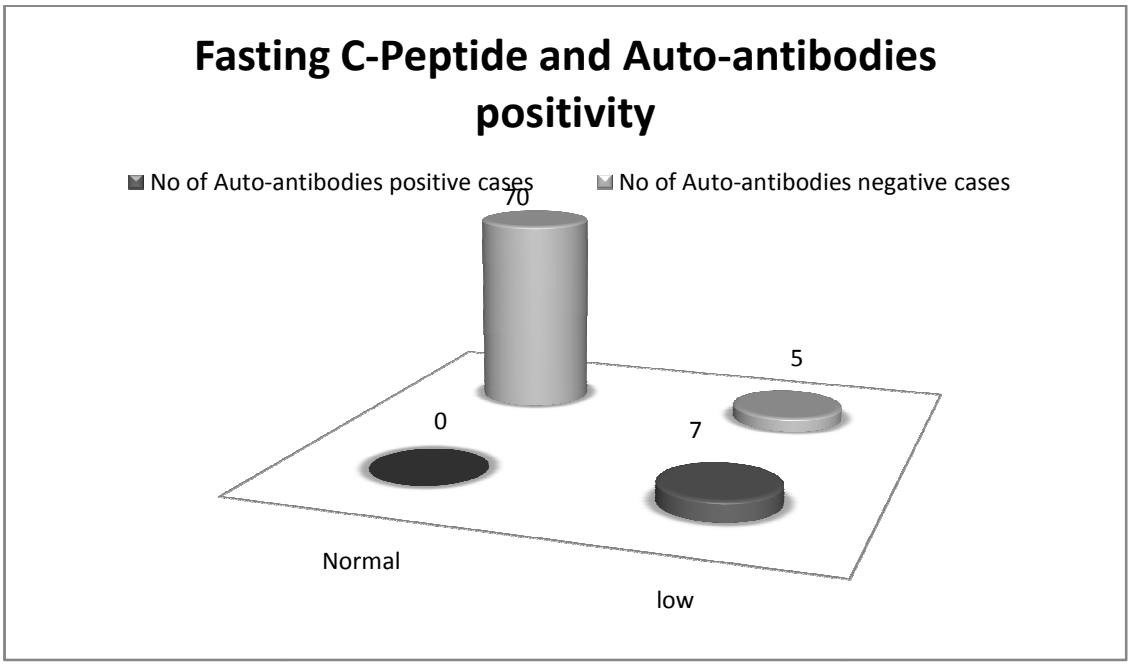
HDL in females and Auto-antibodies:

HDL in females	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
>50	0	0
<50	2	41



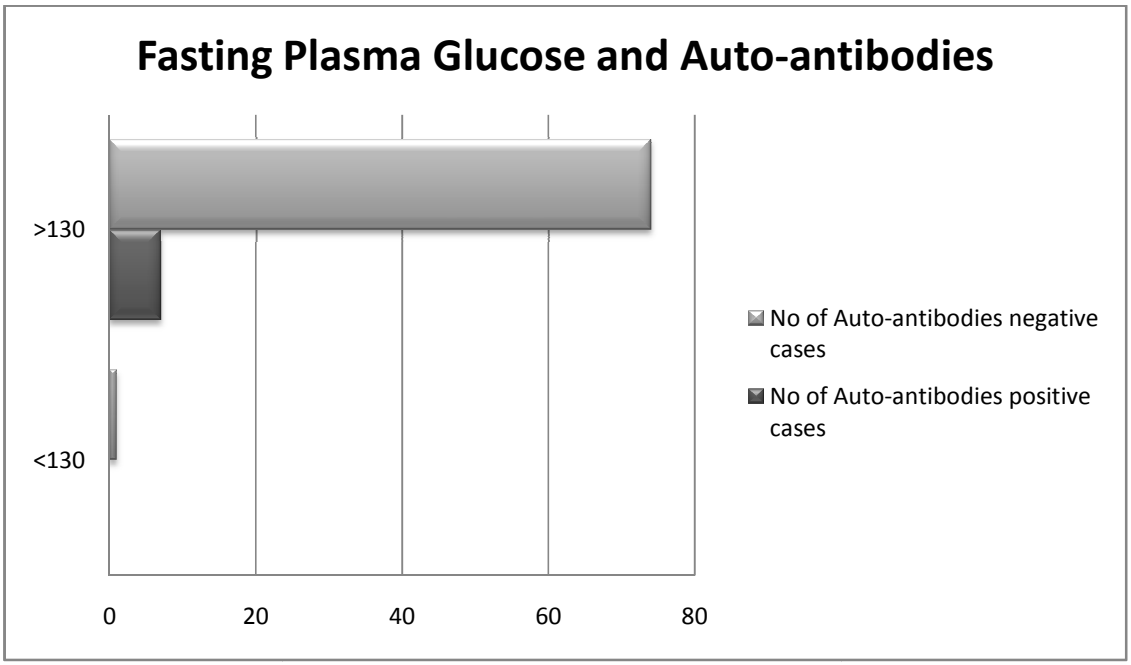
Fasting C-Peptide level and Auto-antibodies:

Fasting C-Peptide level	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
Normal	0	70
low	7	5



Fasting Plasma Glucose and Auto-antibodies positivity:

FPG	No of Auto-antibodies positive cases	No of Auto-antibodies negative cases
<130	0	1
>130	7	74



RESULTS

Results: Total number of participants in this study was 82 in number. In this study group 43(52.44%) were females and 39 (47.56%) were males. On analysis of 82 people for the presence of autoantibodies to GAD-65 and IA-2, 7 were positive which represents 8.53% in percentage. Of the two autoantibodies, antibodies to GAD was prevalent in adults and was seen in 6 peoples whereas IA-2 was seen in one patient and none of the patients in this study showed positivity for both autoantibodies.

Age and autoantibodies positivity:

Out of 7 positive for autoantibodies patients, 5 were in the age group between 21 to 30 years of age and 2 were in between the age group of 31-40 years of age. Although there was no statistical significance in the age distribution between autoantibodies positive group from the autoantibodies negative group, clustering of autoantibodies positive cases occurs earlier.

Sex and autoantibodies positivity:

In sex ratio within the autoantibody positive group, out of 7 autoantibodies positive 5 (71.4%) of them were male and 2(28.6%) of them were female.

On analyzing the sex ratio between antibody positive group vs antibody negative group, out of 39 males participated 5(12.8%) were positive for autoantibodies and in case of female out of 43 participants 2(4.7%) were positive for autoantibodies. There was no statistical significance between these two groups.

Family history of Diabetes and autoantibodies positivity

Both the autoantibodies positive group and negative group did not show any variations regarding the prevalence of family history in their group. In positive group 2 patients had a positive family history of diabetes out of total 7 patients. In negative group 42 cases out of 75 cases showed positive family history.

Duration of Diabetes:

Antibodies positive group did not vary from that of negative group in the aspect of duration of the study. Out of 7 autoantibodies positive patients, 6 patients presented with duration of diabetes less than 2 years where as out of 75 autoantibodies negative group 51 cases presented with less than 2 years duration.

The demographic parameters such as age, sex, duration of diabetes and family history did not differ between the two groups. So using the age or

family history as cut off to diagnose type 1 or type 2 diabetes clinically becomes blurred.

Clinical and Laboratory profile:

Body Mass Index: (BMI)

Reference for body mass index in our study was adopted from the guidelines released by Health Ministry- Government of India, Indian Council of Medical Research (ICMR) and the National Institute of Nutrition (NIN).

Body mass index between these two groups showed no statistical variations which is also required as screening eligibility for the study. On comparing the median BMI between these two groups autoantibodies positive group median BMI (22.47 ± 2.03) was lower than that of antibodies negative group (24.45±3.88).

Waist circumference and autoantibodies positivity

As per the ICMR recommendation; cut off limit for waist circumference is below 90cms for Indian men and 80 cms for Indian women.

In case of male population in our study, out of 5 autoantibodies positive patient one patient had central obesity whereas 27 patients of total 75 autoantibodies negative patients had central obesity of waist circumference

more than 90 cms. On statistical analysis these findings became not significant.

In case of female participants in our study none of the antibody positive patient had central obesity whereas 30 out of 43female participants had waist circumference more than 80 cms fitting in the criteria for central obesity. However autoantibodies positive female number is less this cannot be extrapolated to a huge population. On statistical analysis of these parameters comparing both the groups, waist circumference was not varying between the two groups.

Fasting C-peptide concentration and autoantibodies positivity:

Estimation of fasting C-peptide which is surrogate marker of endogenous insulin secretion was done in all the 82 participants and compared with the two groups.

Comparison revealed statistical significance between the two groups. All the 7 autoantibodies patients showed marked reduction in the plasma fasting C-peptide concentration. In the autoantibodies negative group out of 75 patients, only 5 patients showed significant low levels of plasma C-peptide concentration.

Metabolic syndrome and autoantibodies positivity

Estimation of systolic and diastolic blood pressure between the two groups was done to analyze the prevalence of metabolic syndrome. Mean systolic BP in antibody positive group is 117.14± 4.88 whereas in the antibody negative group is 117 ± 7.01 which was statistically insignificant. Mean diastolic BP in the antibody positive group is 82.29 ± 7.06 where mean diastolic BP in the antibody negative group is 78.83 ± 5.47 which is also statistically insignificant.

Triglycerides and autoantibody positivity

Mean triglycerides level in autoantibody positive group is 186.86 ± 34.84 whereas mean triglycerides level in autoantibody negative group is 164.47 ± 63.50. Statistical analysis revealed there is no significant difference between these two groups.

HDL and autoantibody positivity

HDL analysis revealed statistical significance between the two group. Mean HDL in autoantibody group is 41 ± 1.41 whereas in autoantibody negative group is 38.61 ± 4.62.

Insulin usage in the management of diabetes and autoantibodies positivity

Out of 7 autoantibodies positive patients 2 patients were on insulin before the enrollment into study and 16 patients out of 75 patients in autoantibodies negative group were on insulin before the start of the study. Statistically insulin usage between the two groups was not significant.

Fasting plasma glucose and autoantibodies positivity

Fasting plasma glucose between the autoantibodies positive and autoantibodies negative group did not reveal any statistical significance. Mean fasting glucose in autoantibodies positive group is 179 ± 14.99 whereas in the autoantibodies negative group is 173.99 ± 25.86.

Thus the BMI and Waist circumference which are the markers for assessing obesity is not fruitful to differentiate between autoimmune type 1 and type 2 diabetes mellitus.

Discussion

In our study , the prevalence of autoantibodies –GAD and IA-2 in phenotypically diagnosed type 2 diabetes mellitus is 8.53% whereas the prevalence in various studies are

1.Georgeanna J .Klingensmith et al– in Treatment Option for Type 2 Diabetes in Adolescent and Youth study (TODAY) the prevalence of autoantibodies in patients with clinically to have type 2 diabetes mellitus is 9.8%.The number of participants in the study was 1,206.

Bernard Zinman et al – A Diabetes Outcome Progression Trial (ADOPT) which was conducted to assess the autoantibody GAD in patients diagnosed as type 2 diabetes mellitus. The prevalence is this study is 4.2% for autoantibody to GAD. In this study 4134 type 2 diabetes mellitus participated.

United Kingdom Prospective Diabetes Study (UKPDS) – The major landmark study in diabetes showed the overall prevalence of GAD antibodies was 10%.

Tiinamaija Tuomi et al - Large scale study conducted involving 1122 type 2 diabetic subjects for the prevalence of GAD antibodies in western Finland. The prevalence of GAD antibodies was 9.3%Parameters

Parameters		TODAY Study	ADOPT Study	Present Study
Mean Age (in years)	Ab positive	13	56.5	29.71
	Ab negative	14	57	30.67
Duration of DM	Ab positive	2.5		1.37
	Ab negative	1.9		1.9
BMI	Ab positive	29.1	31.4	22.47
	Ab negative	34.9	32	24.45
SBP	Ab positive	110.5	131.2	117.14
	Ab negative	115	132.9	117.17
DBP	Ab positive	64.7	78.6	82.29
	Ab negative	68.3	79.7	78.83
Insulin use	Ab positive	54.2%		28.5%
	Ab negative	38.8%		21.33%
Low	Ab positive			100%

C-Peptide	Ab negative			6.6%
Triglycerides	Ab positive	77	147.9	186.86
	Ab negative	106.5	164.7	164.47
HDL	Ab positive	43	48.7	41
	Ab negative	39	46.79	38.6

The demographic profile and biochemical profile of our study is consistent with the findings of large study conducted at various parts of the world.

Thus antibody positive and negative groups did not differ with respect to age, sex, family history of diabetes, duration of diabetes, body mass index, waist circumference, fasting plasma glucose. These two groups also show similarity in relation to metabolic syndrome parameters such as blood pressure, triglycerides estimation. However there is statistical significance ($p < .005$) noted in HDL parameter where HDL is low in antibody negative groups

The fasting C-Peptide was low in all the patients belonging to antibody positive group with **P value < 0.001**. These results indicate that type 2 diabetes in young and type 1 diabetes have similar clinical and metabolic

parameters. Thus diagnosing diabetes on the basis of clinical criteria such as age, family history, obesity becomes blurred in young individuals.

Group Statistics

	Auto Antibody	n	Mean	Std. Deviation	Std. Error Mean	P value
Age	Yes	7	29.71	3.592	1.358	
	No	75	30.63	5.474	.632	0.668
Duration (in years)	Yes	7	1.371	.8845	.3343	
	No	74	1.950	1.1684	.1358	0.207
BMIKg/m2	Yes	7	22.4743	2.03219	.76810	
	No	75	24.4528	3.87623	.44759	0.188
Waist(Cms)	Yes	7	80.71	6.075	2.296	
	No	75	85.40	9.681	1.118	0.214
SBP	Yes	7	117.14	4.880	1.844	0.991

(mmhg)						
	No	75	117.17	7.007	.809	
DBP (mmhg)	Yes	7	82.29	7.064	2.670	0.123
	No	75	78.83	5.473	.632	
TGL (mgs/dl)	Yes	7	186.86	34.840	13.168	0.168
	No	75	164.47	63.504	7.333	
HDL(mgs/dl)	Yes	7	41.00	1.414	.535	0.005
	No	75	38.61	4.623	.534	
FPG(mg/dl)	Yes	7	179.00	14.989	5.665	0.616
	No	75	173.99	25.865	2.987	
PPG(mg/dl)	Yes	7	245.00	39.323	14.863	0.288
	No	75	262.97	42.190	4.872	

P value <0.005-significant >0.005 Not significant

The findings of autoantibodies positive patients who took part in our study were consistent with other studies. Diabetes diagnosed as type 2 in young individuals may have clinically in apparent islet cell autoantibodies which may culminate in metabolic derangement.

We conclude that young patients diagnosed as type 2 diabetes mellitus be screened for the presence of autoantibodies.

LIMITATIONS OF THE STUDY

One limitation of the study is that our study involved low number of participants in comparison to other studies. Hence these results need to be confirmed in large group of patients and longitudinal studies.

HbA1c was not done in our study to assess exact glycemic control.

CONCLUSION

Diagnosing type 2 diabetes in young individuals by using the classical clinical criteria such as age and weight has to be revised as these patients may have evidence for autoimmune destruction of islet of pancreas. These antibody positive individuals have similar demographic, clinical as well as biochemical profile as that of type 2 diabetes. Thus screening autoantibodies is useful to distinguish between type 1 diabetes and type 2 diabetes in young.

BIBILIOGRAPHY

1. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF consultation World Health Organization 2006

2. Diagnosis and Classification of Diabetes Mellitus- position statement, American Diabetes Association, *Diabetes care*; Vol -35: Supplement 1; Jan 2010, S64-67

3. International Diabetes Federation Atlas, 5th edition, 2012

4. Textbook of Joslin's Diabetes Mellitus – 14th edition, C.Ronald Kahn, George L.King, Alan C. Moses, Gordon C.Weir, Alan M. Jacobson, Robert J.Smith – Lippincott Williams & Wilkins

5. Textbook of Diabetes Mellitus by Ellenberg & Rifkin's – 6th edition; Daniel Porte, Robert S.Sherwin, Alain Baron; McGraw-Hill

6. Warnick GR, Knopp RH, Fitzpatrick V, Branson L (January 1990). "Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints". *Clinical Chemistry***36** (1): 15–9. PMID 2297909

7. WHO Expert Consultation- Appropriate body mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; *363* : 157-63.

8. Snehalatha C, Viswanathan V, Ramachandran A. Cutoff values for normal anthropometric variables in Asian Indian adults. *Diabetes Care* 2003; *26* : 1380-4

9. The Chennai Urban Population Study (CUPS) – Methodological details – paper no.1 C.S. Shanthi Rani, M. Rema, R. Deepa, G. Premalatha, R. Ravikumar, Anjana Mohan, N. G.Sastry, M. Ramu, R. Saroja, G. Kayalvizhi, V. Mohan; *Int. Journal* - Diabetes Developing countries ,VOL. 19, 149-155; 1999

10. The Chennai Urban Rural Epidemiology Study (CURES) - Study design and methodology (Urban Component) ,M Deepa, R Pradeepa, M Rema, Anjana Mohan, R Deepa, S Shanthirani, V Mohan, JAPI Vol 51 :863-867, September 2003

11. Prevalence and Risk Factors of Diabetic Nephropathy in an Urban South Indian Population. The Chennai Urban Rural Epidemiology Study (CURES 45), Ranjit Unnikrishnan, I, Mohan Rema, Rajendra

Pradeepa, Mohan Deepa,Coimbatore Subramaniam Shanthirani, Raj Deepa,Viswanathan Mohan: *Diabetes Care* 30:2019–2024, 2007

12. Prevalence and risk factors of Peripheral Vascular Disease in a Selected South Indian Population , The Chennai Urban Population Study; Gopal Premalatha, Subramaniam Shanthirani,Raj Deepa, Jerome Markovitz, Viswanathan Mohan :*Diabetes Care* 23:1295–1300, 2000

13. Global Prevalence and major risk factors of Diabetic Retinopathy- Joanne W.Y.Yau,Sophie L.Rogers, Ryo Kawasaki, Ecosse L Lamoureux, Jonathan W.Kowalski: Diabetes Care 35:556–564, 2012

14. Autoimmune Diabetes Not Requiring Insulin at Diagnosis (Latent Autoimmune. Diabetes of the Adult) Definition, characterization, and potential prevention - Paolo Pozzilli, MD,Umberto Di Mario, MD: *Diabetes Care* 24:1460–1467, 2001

15. Latent Autoimmune Diabetes in Adults -Definition, Prevalence, Cell Function and Treatment,Gunnar Stenstrom, Anders Gottsater, Ekaterine Bakhtadze, Bo Berger and Goran Sundkvist:*Diabetes* 54 (Suppl. 2):S68–S72, 2005

16. Natural History of Type 1 Diabetes,Peter Achenbach, Ezio Bonifacio, Kerstin Koczwara, and Anette-G. Ziegler: *Diabetes* 54 (Suppl.2):S25–S31, 2005

17. A 12-Year prospective study of the relationship between islet antibodies and cell function at and after the diagnosis in patients with adult-onset diabetes, Henrik Borg, Anders Gottsa ter, Per Fernlund and Goran Sundkvist: *Diabetes,* 51:1754–1762, 2002

18. Autoantibodies in Diabetes - Catherine Pihoker, Lisa K. Gilliam, Christiane S. Hampe, and Åke Lernmark: *Diabetes* 54 (Suppl. 2):S52–S61, 2005

19. Pinhas Hamiel O, Dolan LM, Daniel ST, et al: Increased incidence of non insulin dependent diabetes mellitus among adolescents, Jour Pediatr 1996;128:Poart 1:608

20. Increasing prevalence of type 2 diabetes mellitus in American Indian children, Dabelea D, Hanson RL, Bennett PH et al; Diabetologia - 1998;41:904

21. The epidemiology of insulin dependent diabetes with particular reference to the relationship of virus infection to its etiology, Gamble DR; Epidemiol Rev 1980;2:49

22. Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study.Georgeanna J,Klingensmith, Laura Pyle, Silva Arslanian, Kenneth C.Copeland, Leona Cuttler, Francine Kaufman: *Diabetes Care* 33:1970–1975, 2010

23. Phenotypic characteristics of GAD antibody positive recently diagnosed patients with Type 2 Diabetes in North America and Europe, Bernard Zinman, Steven E. Kahn, Steven M. Haffner, M. Colleen O'Neill, Mark A. Heise, and Martin I. Freed, for the ADOPT Study Group:*Diabetes* 53:3193–3200, 2004

24. Environmental Triggers and Determinants of Type 1 Diabetes, Mikael Knip, Riitta Veijola, Suvi M. Virtanen, Heikki Hyoty, Outi Vaarala and Hans K. Åkerblom: *Diabetes* 54 (Suppl. 2):S125–S136, 2005

25. Clinical and Genetic characteristics of Type 2 Diabetes with and without GAD Antibodies,Tiinamaija Tuomi, ÅsaLinda Carlsson, Haiyan Li, Bo Isomaa, Aaro Miettinen, Anita Nilsson, Michael Nissén, Björn-Olof Ehrnström, Björn Forsén, Börje Snickars, Kaj Lahti, Carol Forsblom, Carola Saloranta, Marja-Riitta Taskinen, and Leif C. Groop: *Diabetes*, Vol. 48, 150-157; Januray 1999

PROFORMA

THE PRESENCE OF GAD & IA-2 ANTIBODIES IN YOUNG ADULTS DIAGNOSED AS TYPE 2 DIABETES MELLITUS

Name :

Patient ID No:

Age/Sex :

IP No :

Patient characteristics		**Drugs**
Diabetes	Yes or No	❑Sulphonylurea
❑ Duration		❑ Metformin / Pioglitazone
❑ Mode of Onset: Insidious or sudden		❑ α – glucosidase inhibitors
❑ Symptoms : Polyuria / Polydipsia / Wt. loss		❑ Others :
❑ Family history:		❑ Treatment for other illness
❑ Any other illness:		❑ Ketosis prone
❑ H/O GDM/ BOH:		❑ Microvascular complication
❑ Childhood rashes with lymphadenopathy		❑Macrovascular complication

CLINICAL PARAMETERS			
Pulse	bpm	Blood Pressure	mmhg
❑ Height:		❑ Weight:	
❑ BMI:		❑ W/H :	
❑ Acanthosis nigricans:		❑ Xanthelesma/Xanthoma:	

Investigations:

Glycemic profile			**RFT**		
FPG		mg/dl	Bl.urea		mg/dl
PPG		mg/dl	Sr.Creatinine		mg/dl
C-peptide			Urine spot protein		Mg/day
LIPID PROFILE					
Total cholesterol		Mgs/dl	**INSULIN ANTIBODIES**		
TGL		Mgs/dl	GAD	Positive	Negative
HDL		Mgs/dl	IA-2	Positive	Negative
VLDL		Mgs/dl			
LDL		Mgs/dl			

KEY WORDS

Y	yes
N	no
M	male
F	female
BMI	Body mass index
GDM	Gestational diabetes mellitus
BOH	Bad obstetric history
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
FPG	Fasting plasma Glucose
PPG	Post prandial plasma Glucose
TGL	Triglycerides
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein

VLDL	Very Low Density Lipoprotein
GAD	Glutamic Acid Decarboxylase
IA-2	Insulinoma Associated protein 2

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E-mail	daffodils.amm@gmail.com
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DISSERTATION TITLED ”PRESENCE OF GAD AND IA-2 ANTI-BODIES IN YOUNG ADULTS DIAGNOSED AS TYPE 2 DIABETES MELLITUS Submitted in partial fulfilment of requirements for M.D.DEGREE EXAMINATION BRANCH-I GENERAL MEDICINE of THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY CHENNAI INSTITUTE OF INTERNAL MEDICINE MADRAS MEDICAL COLLEGE CHENNAI - 600003. APRIL 2013 CERTIFICATE This is to certify that the dissertation entitled “PRESENCE OF GAD AND IA-2 ANTI-BODIES IN YOUNG ADULTS DIAGNOSED AS TYPE 2 DIABETES MELLITUS ” is a bonafide work done by DR. V. MAHADEVAN , Post Graduate Student, Institute of Internal Medicine, Madras Medical College, Chennai-3, in partial fulfillment of the University Rules and...

Sl.No	Name	Sex	Age	Duration (in years)	Mode of onset (Insidious or Sudden)	Family History (Mother or Father)	H/o GDM / BOH	Sulphonylurea	Metformin / Pioglitazone	Insulin	Pulse (bpm)	SBP (mmhg)	DBP (mmhg)
1	Karthikeyan	M	34	4	I	YES	Nil	Y	Y	Nil	88	130	84
2	Juliet Shakina	F	31	2	I	YES	GDM +ve	Y	Y	Nil	84	120	78
3	Sharmila Begum	F	28	2	I	YES	Nil	Y	Y	Nil	78	110	80
4	Ananthi	F	27	2	I	NO	Nil	Y	Y	Nil	88	120	86
5	Gomathi	F	36	4	S	YES	GDM +ve	Y	Y	N	74	110	80
6	Diana	F	26	1.5	I	YES	Nil	Y	Y	N	78	120	80
7	Sulthana	F	39	4	I	NO	Nil	Y	Y	N	82	130	80
8	Samuthrakani	F	35	2	I	YES	Nil	Y	Y	Y	84	110	80
9	Sathya	F	30	3	I	NO	GDM +ve	Y	Y	N	76	130	80
10	Venugopal	M	33	1	I	YES	Nil	Y	Y	N	82	120	80
11	Selvakumar	M	37	3	I	NO	Nil	Y	Y	N	88	120	90

Sl.No	Name	Sex	Age	Duration (in years)	Mode of onset (Insidious or Sudden)	Family History (Mother or Father)	H/o GDM / BOH	Sulphonylurea	Metformin / Pioglitazone	Insulin	Pulse (bpm)	SBP (mmhg)	DBP (mmhg)
12	Ramesh	M	38	2	I	YES	Nil	Y	Y	N	82	110	80
13	Mythili	F	32	4	I	NO	Nil	Y	Y	N	78	120	80
14	Suresh Kumar	M	31	1	I	NO	Nil	Y	Y	Y	86	120	90
15	Nirupam	F	31	1	I	NO	Nil	Y	Y	Y	84	120	70
16	Dhanasekar	M	37	3	I	YES	Nil	Y	Y	N	82	130	80
17	Selvi	F	15	0.8	I	YES	Nil		Y	Y	78	120	80
18	Mahalingam	M	31	2	I	NO	Nil	Y	Y	N	82	110	80
19	Thasleema	F	16	0.6	I	YES	Nil	Y	Y	N	88	120	80
20	Thulasi	F	37	3		NO	Nil	Y	Y	N	76	120	70
21	Raja Manickkam	M	32	1	I	NO	Nil	Y	Y	N	78	120	80
22	Amalraj	M	29	1	I	YES	Nil	Y	Y	N	76	120	80
23	Das	M	33	1		NO	Nil	Y	Y	N	84	110	90

Sl.No	Name	Sex	Age	Duration (in years)	Mode of onset (Insidious or Sudden)	Family History (Mother or Father)	H/o GDM / BOH	Sulphonylurea	Metformin / Pioglitazone	Insulin	Pulse (bpm)	SBP (mmhg)	DBP (mmhg)
24	Pandidurai	M	32	1	I	YES	Nil	Y	Y	N	78	120	80
25	Geetha	F	30	3.5	I	YES	GDM +ve	Y	Y	Y	82	120	70
26	Dhanalakshmi	F	32	3.2	I	YES	GDM +ve	Y	Y	Y	72	110	80
27	Sarath Babu	M	30	1.5	I	NO	Nil	Y	Y	Y	84	120	80
28	Balamurugan	M	35	2	I	NO	Nil	Y	Y	N	86	110	80
29	Dhilip	M	24	1	I	YES	Nil	Y	Y	Y	78	120	80
30	Raju	M	28	0.6	I	NO	Nil	Y	Y	N	82	120	70
31	Selvam	M	35	2		NO	Nil	Y	Y	Y	78	110	90
32	Selvi	F	38	1.5	I	NO	Nil	Y	Y	N	80	120	90
33	Jeevan	M	34	1	I	NO	Nil	Y	Y	N	82	120	80
34	Udhayakumari	F	25	1	I	NO	Nil	Y	Y	N	82	120	80
35	Sumathi	F	30	0.6	I	NO	Nil	Y	Y	N	82	120	80
36	Pooshanam	F	21	2	I	NO	Nil	Y	Y	N	82	120	80

Sl.No	Name	Sex	Age	Duration (in years)	Mode of onset (Insidious or Sudden)	Family History (Mother or Father)	H/o GDM / BOH	Sulphonylurea	Metformin / Pioglitazone	Insulin	Pulse (bpm)	SBP (mmhg)	DBP (mmhg)
37	Chales	M	34	3.8	I	YES	Nil	Y	Y	Y	72	110	80
38	Venkatesan	M	30	1	I	NO	Nil	Y	Y	N	76	120	70
39	Mani	M	35	4	I	NO	Nil	Y	Y	Y	88	110	70
40	Subash	M	38	2.6	I	YES	Nil	Y	Y	Y	84	110	80
41	Annapoorani	F	24	2	I	YES	GDM +ve	Y	Y	Y	76	120	80
42	Thurab Ali	M	36	0.8	S	YES	Nil	Y	Y	Y	80	120	70
43	Rajaram	M	27	2.8	I	YES	Nil	Y	Y	N	78	120	80
44	Annalakshmi	F	31	1.5	I	YES	Nil	Y	Y	N	82	120	80
45	Jeya	F	29	1	I	YES	Nil	Y	y	N	72	110	80
46	Raman	M	35	1	I	NO	Nil	Y	Y	N	78	120	70
47	Thiyagarja	M	35	4	I	YES	Nil	Y	Y	N	84	110	80
48	Chitra	F	32	3.2	I	YES	GDM +ve	Y	Y	N	84	120	80
49	Martin	M	23	0.6	I	NO	Nil	Y	Y	N	84	110	80
50	Kavitha	F	30	4	I	NO	GDM +ve	Y	Y	N	82	120	80

Sl.No	Name	Sex	Age	Duration (in years)	Mode of onset (Insidious or Sudden)	Family History (Mother or Father)	H/o GDM / BOH	Sulphonylurea	Metformin / Pioglitazone	Insulin	Pulse (bpm)	SBP (mmhg)	DBP (mmhg)
51	Karpagam	F	35	0.6	I	YES	Nil	Y	Y	N	78	120	70
52	Kumar	M	30	2	I	YES	Nil	Y	Y	N	84	110	90
53	Jansi	F	28	0.6	I	YES	Nil	Y	Y	N	82	120	80
54	Yegavalli	F	36	4	I	YES	Nil	Y	Y	N	82	110	80
55	Lakshmanan	M	30	3	I	YES	Nil	Y	Y	N	82	120	80
56	Pavani	F	29	2	I	YES	Nil	Y	Y	N	84	120	70
57	Saraswathy	F	33	2	I	YES	Nil	Y	Y	N	80	120	80
58	Sasikala	F	34	2	I	NO	Nil	Y	Y	N	78	110	80
59	Thirumal	M	34	0.6	I	NO	Nil	Y	Y	N	76	130	80
60	Kalpana	F	23	1 Yr	I	YES	GDM +ve	Y	Y	N	74	120	80
61	Amudha	F	30	0.6	I	NO	GDM +ve	Y	Y	N	82	130	70
62	Dhanalakshmi	F	32	2	I	YES	GDM +ve	Y	Y	N	82	134	80
63	Sarala	F	27	2	I	NO	GDM +ve	Y	Y	N	82	110	80
64	Radha	F	27	2	I	YES	GDM +ve	Y	Y	N	80	120	80
65	Pushpa	F	27	0.5	I	YES	Nil	Y	Y	N	84	120	70
66	Sivakumar	M	35	4	I	NO	Nil	Y	Y	N	78	120	80

Sl.No	Name	Sex	Age	Duration (in years)	Mode of onset (Insidious or Sudden)	Family History (Mother or Father)	H/o GDM / BOH	Sulphonylurea	Metformin / Pioglitazone	Insulin	Pulse (bpm)	SBP (mmhg)	DBP (mmhg)
67	Yaseer	M	35	3	I	NO	Nil	Y	Y	N	79	120	70
68	Sivaraman	M	27	1	I	NO	Nil	Y	Y	N	80	110	80
69	Pushpa Malliga	F	34	2	I	YES	Nil	Y	Y	N	88	110	80
70	Wasim Akram	M	27	2.5	S	NO	Nil	Y	Y	Y	74	100	70
71	Jeevitha	F	20	0.9	S	NO	Nil	Y	Y	Y	71	110	80
72	Ramzan Beevi	F	27	3	I	YES	GDM +ve	Y	Y	N	78	120	80
73	Mohamed Rafiq	M	34	1	I	NO	Nil	Y	Y	N	78	120	80
74	Sentilkumar	M	32	1.5	I	YES	Nil	Y	Y	N	78	120	90
75	Sarhu Nisha	F	30	1	I	YES	Nil	Y	Y	N	82	110	70
76	Murugeswari	F	38	3	I	YES	Nil	Y	Y	N	80	120	90
77	Muneeswaran	M	29	0.6	I	YES	Nil	Y		N	88	110	80
78	Ramesh	M	36	1	I	NO	Nil	Y	Y	N	76	120	90
79	Jeya Bharathi	F	32	3	I	NO	Nil	Y	Y	N	78	120	80
80	Rajendra Prasath	M	31	0.2	S	NO	Nil	Y	Y	N	79	100	80
81	Meena	F	15	0.1	S	YES	Nil	Y	Y	Y	84	100	70
82	Raja	M	17	0.1	S	YES	Nil	Y	Y	Y	76	114	70
Total			**83**	**1.9**									

Height (Cms)	Weight (Kgs)	BMI Kg/m2	Waist (Cms)	Acanthosis nigricans	FPG (mg/dl)	PPG (mg/dl)	C-Peptine	Blood Urea (mg/dl)	Sr.Creatinine (mg/dl)	Total cholesterol (mgs/dl)	TGL (mgs/dl)	HDL(mgs/dl)	VLDL (mgs/dl)	LDL (mgs/dl)
180	106	32.71	104	YES	180	255	Normal	28	0.9	232	225	32	45	155
145	49	23.3	74	NO	163	221	Normal	29	0.8	185	171	39	34	112
142	52	25.8	81	YES	173	229	Normal	23	1.0	165	150	41	30	94
152	48	20.78	73	NO	202	331	**LOW**	28	1.0	183	195	41	39	103
154	77	32.4	102	YES	192	287	Normal	18	0.9	194	167	35	33	126
157	53	21.5	79	NO	158	248	Normal	22	0.7	209	163	36	33	130
158	87	34.85	105	YES	183	278	Normal	27	0.8	210	122	41	22	147
144	47	22.7	74	NO	165	217	Normal	21	0.7	174	112	37	22	114
154	84	35.41	110	YES	189	247	Normal	18	0.8	224	199	29	40	155
169	80	28.01	95	NO	180	317	Normal	28	1.0	199	180	36	36	127
165	60	22.03	81	NO	183	217	**LOW**	23	0.8	175	130	42	26	107

Height (Cms)	Weight (Kgs)	BMI Kg/m2	Waist (Cms)	Acanthosis nigricans	FPG (mg/dl)	PPG (mg/dl)	C-Peptine	Blood Urea (mg/dl)	Sr.Creatinine (mg/dl)	Total cholesterol (mgs/dl)	TGL (mgs/dl)	HDL(mgs/dl)	VLDL (mgs/dl)	LDL (mgs/dl)
150	46	20.4	73	NO	163	215	Normal	18	0.9	201	253	27	51	123
155	46	19.1	71	NO	159	209	Normal	21	0.7	178	108	36	22	120
165	70	23.8	81	NO	161	228	**LOW**	40	1.2	239	172	41	34	164
150	55	24.4	86	YES	180	233	Normal	20	0.9	179	163	40	33	106
170	72	24.91	85	NO	167	263	Normal	21	1.0	110	70	45	14	51
158	60	24.03	91	YES	210	325	**LOW**	33	0.7	150	187	46	37	67
163	62	23.33	103	YES	199	317	Normal	20	1.0	191	165	37	33	121
157	58	23.53	85	NO	151	265	Normal	19	0.7	180	132	42	26	112
164	74	27.5	92	NO	183	274	Normal	18	0.8	174	98	46	20	108
160	74	28.9	105	YES	180	245	Normal	28	1.1	163	122	42	25	96
167	81	29.1	87	YES	155	215	Normal	23	0.9	202	143	38	29	135
175	70	22.1	84	NO	168	261	Normal	18	0.8	145	111	47	22	76

Height (Cms)	Weight (Kgs)	BMI Kg/m2	Waist (Cms)	Acanthosis nigricans	FPG (mg/dl)	PPG (mg/dl)	C-Peptine	Blood Urea (mg/dl)	Sr.Creatinine (mg/dl)	Total cholesterol (mgs/dl)	TGL (mgs/dl)	HDL(mgs/dl)	VLDL (mgs/dl)	LDL (mgs/dl)
162	53	20.2	79	NO	210	334	**LOW**	23	0.9	183	134	39	27	117
153	64	27.3	93	NO	171	253	**LOW**	30	0.9	189	172	38	34	117
153	63	26.9	98	YES	155	222	Normal	31	1.0	211	108	41	22	148
166	71	25.7	91	NO	161	220	**LOW**	38	1.2	189	174	42	35	112
161	54	20.83	76	NO	151	209	Normal	26	0.8	130	112	42	22	66
171	63	21.24	87	NO	163	237	Normal	33	1.0	187	130	41	26	120
164	67	24.9	89	NO	189	255	Normal	22	0.7	188	145	39	29	120
170	72	24.91	81	NO	192	277	Normal	28	0.8	182	190	28	38	116
155	63	26.22	81	YES	170	231	Normal	30	1.0	174	123	36	25	113
161	60	23.1	83	NO	220	340	Normal	27	1.1	197	117	37	23	137
149	48	21.62	80	NO	201	322	Normal	16	0.7	215	356	40	71	104
160	67	26.17	85	NO	210	318	Normal	28	0.9	177	154	45	31	101
149	47	21	81	NO	151	227	Normal	23	0.8	160	137	41	27	92

Height (Cms)	Weight (Kgs)	BMI Kg/m2	Waist (Cms)	Acanthosis nigricans	FPG (mg/dl)	PPG (mg/dl)	C-Peptine	Blood Urea (mg/dl)	Sr.Creatinine (mg/dl)	Total cholesterol (mgs/dl)	TGL (mgs/dl)	HDL(mgs/dl)	VLDL (mgs/dl)	LDL (mgs/dl)
164	64	23.7	81	NO	132	224	Normal	21	0.9	171	102	43	20	108
173	59	19.71	75	NO	154	284	Normal	19	0.7	152	90	42	18	92
173	58	19.39	79	NO	211	373	Normal	22	0.9	252	153	42	50	160
170	55	19	73	NO	205	351	Normal	35	1.2	252	301	39	60	153
143	50	24.45	83	NO	183	294	Normal	27	0.9	191	173	41	35	115
166	61	22.1	81	NO	124	302	**LOW**	21	0.7	174	136	45	27	102
163	78	29.32	98	YES	145	253	Normal	18	0.9	213	194	38	43	132
147	55	25.4	86	YES	180	247	Normal	19	0.9	202	218	41	44	117
147	55	25.4	91	YES	173	234	Normal	18	0.7	185	152	40	30	115
165	67	24.6	84	NO	140	254	Normal	23	0.6	184	142	38	28	118
174	64	21.1	79	NO	165	324	Normal	20	0.7	152	89	43	18	91
144	65	31.3	106	YES	198	270	Normal	18	0.8	146	225	27	45	74
167	60	21.58	79	NO	201	256	Normal	24	1	131	109	38	22	71
144	41	19.5	81	NO	148	251	Normal	20	0.9	206	215	41	43	122

Height (Cms)	Weight (Kgs)	BMI Kg/m2	Waist (Cms)	Acanthosis nigricans	FPG (mg/dl)	PPG (mg/dl)	C-Peptine	Blood Urea (mg/dl)	Sr.Creatinine (mg/dl)	Total cholesterol (mgs/dl)	TGL (mgs/dl)	HDL(mgs/dl)	VLDL (mgs/dl)	LDL (mgs/dl)
153	74	31	105	YES	135	245	Normal	21	1	205	245	37	49	119
171	68	23.3	79	NO	162	264	Normal	36	1	186	214	35	43	108
160	62	24.2	84	NO	136	241	Normal	17	0.8	170	106	45	21	104
151	52	22.8	81	NO	230	340	Normal	23	0.8	234	256	41	51	142
180	78	24	84	NO	191	387	Normal	19	1.2	185	210	34	42	109
147	43	19.8	75	NO	165	239	Normal	17	0.5	153	74	42	15	96
153	57	24.34	81	NO	147	253	Normal	20	1.1	207	242	35	48	124
155	51	21.25	75	NO	141	245	Normal	17	0.8	185	284	41	57	87
161	67	25.8	86	NO	136	185	Normal	19	1	115	73	39	15	61
148	78	35.6	102	NO	145	256	Normal	21	0.9	194	200	34	40	120
145	53	25.2	84	YES	156	284	Normal	18	0.7	194	206	29	41	124
147	50	23.15	89	NO	141	263	Normal	19	0.8	162	120	41	24	97
153	54	23.0	79	NO	180	233	Normal	21	0.8	201	152	36	30	135
163	60	25.3	85	NO	201	311	Normal	18	0.8	141	163	37	33	71
150	40	19.7	74	NO	190	233	**LOW**	24	0.8	209	191	42	38	129
167	61	21.86	74	NO	196	273	Normal	19	1.0	163	125	40	25	98

Height (Cms)	Weight (Kgs)	BMI Kg/m2	Waist (Cms)	Acanthosis nigricans	FPG (mg/dl)	PPG (mg/dl)	C-Peptine	Blood Urea (mg/dl)	Sr.Creatinine (mg/dl)	Total cholesterol (mgs/dl)	TGL (mgs/dl)	HDL(mgs/dl)	VLDL (mgs/dl)	LDL (mgs/dl)
170	66	22.84	79	NO	135	201	Normal	24	0.8	172	98	42	20	110
168	50	21.71	81	NO	182	247	**LOW**	27	0.7	214	245	41	49	124
154	55	22.3	85	NO	173	220	Normal	19	0.9	163	134	42	27	94
187	79	22.57	83	NO	144	201	Normal	18	1.0	185	91	39	18	128
154	52	21.9	75	NO	190	281	Normal	21	0.9	162	98	44	20	98
160	65	25.39	86	NO	191	238	Normal	21	1.0	177	199	40	40	97
166	67	24.3	84	NO	200	260	Normal	22	0.8	182	104	39	21	122
168	59	21	76	NO	153	217	Normal	22	1.1	203	241	41	48	114
145	60	28.53	98	YES	160	220	Normal	18	0.8	163	187	37	37	89
160	61	23.82	79	NO	180	250	Normal	23	0.9	199	252	34	50	115
168	51	23.6	84	NO	174	239	**LOW**	28	1.1	184	201	38	40	106
164	74	27.6	96	NO	166	219	Normal	24	0.9	214	301	41	60	113
140	50	26.3	94	NO	193	270	Normal	19	1.1	186	324	25	65	96
169	68	23.8	91	NO	190	276	Normal	23	0.9	187	225	32	45	110
137	35	18.62	69	NO	245	302	**LOW**	22	0.7	148	118	39	24	85
138	37	19.47	72	NO	227	286	Normal	20	0.8	126	75	38	15	73

[redacted]

GAD	IA-2
NO	NO
NO	NO
NO	NO
YES	NO
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NO	**YES**

GAD	IA-2
NO	NO
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GAD	IA-2
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GAD	IA-2
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GAD	IA-2
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GAD	IA-2
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YES	NO
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NO	NO