Diabetes is an alarming disorder of the third world. The prevalence of diabetes is likely to increase by 35% by the year 2025 according to the World Health Organization (WHO) projections. Currently, India is the diabetic capital of the world. Diabetes mellitus, a group of metabolic diseases is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.

Diabetes mellitus is generally categorized as type 1 (insulin dependant diabetes or Juvenile-onset diabetes), type 2 (non-insulin dependent or adult onset diabetes) and gestational diabetes. Type 1 results due to autoimmunity and type 2 because of insulin resistance. There is yet another form of diabetes which phenotypically resembles type 2, but genotypically resembles type 1. This form of diabetes is known as type 1.5 diabetes or latent autoimmune diabetes (LADA). Type 1.5 diabetes is often misdiagnosed as type 2 diabetes and hence, treatment for type 1.5 diabetes is similar to type 2 diabetes. As the pathophysiology of different types of diabetes varies, interventions for treating them should also be specific to its type. This essentiates the need for correct categorization of diabetes.

The objectives of the study were to identify suitable methods and biochemical markers for the identification, confirmation and categorization of the different types of diabetes, to analyse the anthropometric and biochemical parameters to study the association of these types of diabetes with other metabolic disturbances and function of selected organs, to adopt a proper interventional strategies, to study the impact of interventions on microvascular and macrovascular complications and to identify a suitable drug for type 1.5 diabetes by an in silico approach. The study was conducted in three phases.
PHASE 1
Screening and differential diagnosis of the three types of diabetes

Patients who visited two hospitals and one diabetes care centre during two and a half years study period were screened for diabetes by analysing random blood glucose level. Participants found to be diabetic were further selected for the study based on selected inclusion criteria namely age above 20 and below 65 years, symptoms like polyuria, polydypsia and unintentional weight loss, baseline random blood glucose > 200mg/dl and glycosylated hemoglobin > 7.0%. The selected participants were subjected to differential diagnosis by measuring fasting C-peptide levels, a biochemical marker to distinguish type 1 diabetes from type 2 diabetes as this directly reflects endogenous insulin reserve. Patients with a fasting C-peptide level < 0.9 ng/ml were categorized as type 1 diabetics and those having a value > 0.9 ng/ml were categorized as type 2 diabetics.

In order to identify patients suspected to have latent autoimmune (type 1.5) diabetes among the type 2 diabetes categorized patients, the identified type 2 diabetes patients were further subjected to LADA risk score calculation based on five clinical features namely age at onset <50 years, acute symptoms of hyperglycemia, body mass index (BMI) <23 kg/m², personal history of autoimmune disease and family history of autoimmune disease. Patients with a LADA risk score > 2 who were suspected to have type 1.5 diabetes were confirmed by analyzing the autoimmune diabetes marker- glutamic acid decarboxylase antibody (GADA). Patients with a GADA titre value > 10 units / ml were confirmed to belong to type 1.5 diabetes.

PHASE 2
Profile of the diabetic patients who were categorized into type 1, type 1.5 and type 2 and impact of interventions among these patients

The study was carried out with 136 type 1, 86 type 1.5 and 164 type 2 diabetic patients. The general profile of these patients were recorded to
analyse the impact of age, sex, family history of diabetes, autoimmunity and life style pattern on the types of diabetes. Anthropometric measurements namely height, weight, BMI and blood pressure were recorded to study the relationship between blood pressure, obesity and diabetes in the three types of diabetes.

All the diabetic patients of the study were asked to strictly follow the diet plan according to their categorization as underweight, overweight or normal weight along with life style modification. Interventions were given to all the participants of the study with insulin for type 1 diabetics, with insulin+Voglibose/insulin+Metformin for type 1.5 diabetics and with Glimepiride/Metformin/Voglibose/Pioglitazone/Sitagliptin+Metformin for type 2 diabetics for a period of six months.

Voglibose, an alpha-glucosidase inhibitor impedes absorption of carbohydrate and therefore reduces glucose levels. Metformin, a biguanide acts primarily by lowering hepatic glucose production and may also improve insulin resistance. Glimepiride, a third generation sulfonylurea drug stimulates insulin release from pancreatic β-cells. Pioglitazone, a member of the thiazolidinedione drug family, improves glycemic control by improving insulin sensitivity. Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor improves glycemic control by reducing both fasting and postprandial glucose concentrations.

Biochemical analysis namely fasting blood glucose, postprandial blood glucose, glycated hemoglobin (HbA1c), fasting C-peptide, hemoglobin, lipid parameters (total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C, lipoprotein-A, apolipoprotein-A1 and apolipoprotein-B), total protein, albumin:globulin ratio, albumin:creatinine ratio, urine microalbumin, hs-CRP (high sensitive-C-reactive protein) and activities of selected liver enzymes namely serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) gamma glutamyltransferase (GGT) and alkaline
phosphatase (ALP) were carried out at diagnosis and after six months interventions to study the impact of the interventions along with patient awareness and strict diet practice on the different types of diabetes and their relationship with these parameters.

A substudy comprising of eighteen participants (six from each type—three men and three women in each type) was carried out to assess the impact of interventions on microvascular and macrovascular complications. Biothesiometer reading, fundoscopy examination, analysis of urine microalbumin and albumin: creatinine ratio, electrocardiogram, carotid doppler study and doppler study were carried out at diagnosis and after intervention.

PHASE 3

**In silico docking of drugs for type 1.5 diabetes**

In the autoimmune type 1.5 diabetes, β-cell destruction is caused by glutamic acid decarboxylase antibody (GADA) and the heat shock protein (Hsp) is found to be involved in selectively regulating Major Histocompatibility Complex (MHC) class II presentation of the diabetes autoantigen glutamic acid decarboxylase (GAD). Experimental studies have shown that inactivation of Hsp90 function inhibited MHC class II presentation of exogenous and endogenous GAD presentation. Hence, the heat shock protein (Hsp90 α) was used as the target protein and docking study was conducted with four ligands—geldanamycin, radicicol, radanamycin and mangiferin. Geldanamycin and radicicol are two natural inhibitors of Hsp90. Radanamycin is a chimeric product of geldanamycin and radicicol. Mangiferin is a plant product of *Mangifera indica* which has been reported to have multiple pharmacological potentials like antioxidant activity, immunomodulatory effect, anti-inflammatory effect as well as antidiabetic effect on both type 1 as well as type 2 diabetes.

Structure of the target protein was retrieved from protein databank (PDB) and the structures of the ligands were retrieved from PubChem and Drugbank. Glide module of Schrodinger software was used for docking. Drug
Salient findings of the study

**PHASE 1**

1,569 out of 4,250 patients who visited the two hospitals and one diabetes care centre during a period of two and a half years were found to be diabetic. 1,386 diabetic patients out of 1,569 patients were selected for the categorization of type 1 and type 2 diabetes as they met all the four inclusion criteria namely age above 20 and below 65 years, symptoms like polyuria, polydypsia and unintentional weight loss, baseline random blood glucose > 200mg/dl and glycosylated hemoglobin > 7.0%. Based on C-peptide levels and GADA positivity, 136 patients were categorized as type 1, 86 as type 1.5 and 164 as type 2 diabetic patients.

**PHASE 2**

Family history of diabetes was found to be closely associated with type 2 diabetes and family history of autoimmune disease was found to be associated with type 1 and type 1.5 diabetes.

Sedentary life style was found to be more pronounced in type 2 diabetics (70 %) compared to type 1 (38 %) and type 1.5 diabetics (27 %). The mean BMI of type 2 diabetic patients of all the three age groups and both sexes were found to be more compared to type 1 and type 1.5 diabetic patients.

In the present study, abdominal obesity measured in terms of waist to hip ratio > 0.85 among female diabetic patients and > 0.90 among male diabetic patients was noticed in female type 1.5 diabetics of the age group 20-35 years, in male type 2 diabetics belonging to the age groups 20-35 years and 50-65 years and in female type 2 diabetic patients in all the three age...
groups. Blood pressure was found to be increased in type 2 diabetics indicating a relationship between blood pressure, obesity and type 2 diabetes.

A significant positive correlation was observed in all the three types of diabetics between fasting blood glucose and HbA1c levels. A negative correlation (p<0.05) was observed between fasting blood glucose and C-peptide levels as well as between HbA1c levels and C-peptide levels in type 1 and type 1.5 diabetic patients. A significant decrease (p< 0.01) was observed in fasting and postprandial blood glucose levels among all the three types of diabetic patients after intervention compared to the levels at diagnosis. A significant decrease (p<0.05) in cholesterol and triglyceride levels were observed in all the three types of diabetic patients after intervention. A significant decrease (p<0.05) was observed in the LDL : HDL ratio in type 2 diabetic patients after intervention.

A significant positive correlation was observed between apolipoprotein-A1 and HDL-C as well as between apolipoprotein-B and LDL-C. The levels of hs-CRP were found to be increased in diabetic patients with heart disease. Incidence of heart disease among the diabetics in the present study was found to be associated (p<0.01) with each of the lipid parameters namely total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, VLDL cholesterol, apolipoprotein - A1 and apolipoprotein-B. An increase in hemoglobin level was observed in all the three types of diabetics after intervention.

There was no significant difference observed in urea, creatinine, protein, albumin and globulin levels before and after intervention. No significant difference was observed in the activities of liver enzymes namely serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, gamma glutamyl transferase and alkaline phosphatase after intervention. However they were within the normal levels at diagnosis and after intervention.
An improvement in the symptoms and findings of both microvascular and macrovascular diseases was observed in all the patients selected for the substudy. After six months intervention, severe neuropathy became moderate, moderate neuropathy became mild and mild became normal. Patients in whom nephropathy was found to be in incipient stage became normal and overt nephropathy became non-progressive. Incidence of non-proliferative as well as proliferative retinopathy were observed. After six months intervention, neither reversal of the condition nor absence of further deterioration was noticed.

Atherosclerotic changes with lumen narrowing in limb vessels in the patients with peripheral vascular disease remained static without any further deterioration after six months interventions. In some cases, atherosclerotic changes with lumen narrowing in limb vessels observed at diagnosis changed to non-progressive status after six months intervention period. Ischemic changes observed in cardiovascular diabetic patients at diagnosis reverted to normal and coronary heart disease became non-progressive after the intervention period. In patients presenting with cerebrovascular disease, atherosclerotic changes with lumen narrowing in limb vessels after intervention became non-progressive.

PHASE 3

Docking of the target protein Hsp90 α with the ligand molecule geldanamycin at the particular pose was found to involve five hydrogen bonds with a Glide score value of -7.13, with radicicol involving two hydrogen bonds with a score value of -5.93, radanamycin involving five hydrogen bonds with a score value of -5.44. The ligand molecule mangiferin was found to involve six hydrogen bonds with a score value of -8.49.

The following conclusions may be drawn from the present investigation:

Serum C-peptide levels can be used for categorizing type 1 and type 2 diabetes. LADA risk score can be used for identifying type 1.5 diabetics.
among type 2 diabetics and GADA test can be used for confirming type 1.5 diabetes.

Obesity and sedentary life style were found to be associated with type 2 diabetes. There was a significant positive correlation between fasting blood glucose, HbA1c and C-peptide. C-peptide status of type 1.5 diabetics was found to be between that in type 1 and type 2 diabetics. Abnormalities in lipid profile was found to be associated with all the three types of diabetes. Proper interventions proved to be effective in controlling all the three types of diabetes and the associated microvascular and macrovascular complications.

The ligands geldanamycin and mangiferin were found to be effective in docking with the target protein Hsp90 α - the glutamic acid decarboxylase antibody presenting protein.

To conclude, the diagnostic approaches and interventional strategies adopted in the present study proved to be effective in diagnosing and categorizing diabetes, in treating diabetes and its complications. Mangiferin may prove to be a suitable compound to treat type 1.5 diabetes.

Scope for future work

- A study may be conducted to understand the effect of long term interventional strategies in type 1.5 diabetes.

- The impact of interventions at genetic level can be attempted in suspected inherited cases of diabetes.

- The utility of diagnostic approaches and impact of interventions adopted in the present study can be attempted in other ethnic groups.

- Mangiferin - a compound present in the bark and leaves of the plant *Mangifera indica* can be subjected for further attempted clinical trials.