



## **STUDY OF BIOCHEMICAL TEST IN DIABETIC DISEASE**

**BIOCHEMISTRY SEMESTER - IV**

**SUBMITTED TO**

**DEPARTMENT OF LIFE SCIENCES AND BIOTECHNOLOGY  
FACULTY OF SCIENCE**



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**Submitted To**

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**Research Centre**

**HEALTH SOLUTIONS PATHOLOGY PVT.LTD**

## **CERTIFICATE**

## **DECLARATION**

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I would like to acknowledge that this project was completed entirely by me and not by someone else.

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### LIST OF ABBREVIATIONS

CVD	Cardiovascular Disease
IDDM	Insulin Dependent Diabetes Mellitus
NIDDM	Non- Insulin Dependent Diabetes Mellitus
MRDM	Malnutrition -related Diabetes Mellitus
GDM	Gestational Diabetes Mellitus
IND	International Nomenclature of Diseases
ICAs	Islet Cells to the Antibodies
GAD65	Glutamic Acid Decarboxylase
IAAs	Insulin-auto Antibodies
LADA	Latent Autoimmune Diabetes in Adults
ASCVD	Atherosclerotic Cardio-vascular Disease
IADPSG	International Association of Diabetes and Pregnancy Study Groups
GLT	Glucose Load Test
MODY	Maturity Onset Diabetes of the Young
DKA	Diabetes related Ketoacidosis
PCOS	Polycystic Ovary Syndrome
UTIs	Urinary Tract Infections
PPBS	Post Prandial Blood Sugar
FBS	Fasting Blood Sugar
IFG	Impaired Fasting Glucose
HbA1c	Hemoglobin A1c
SGL	Sodium- Glucose co-transporter
GLP	Glucagon-like peptide
ICD	International Classification of Diabetes



## **ABSTRACT**

Due to increasing number of cases of diabetes among people. It has been categorized as a common disease caused simply due to increase of sugar level in bloodstreams causing “Diabetes”. Unknown to the other consequences forthcoming, people take it as a normal cause.

Therefore, it is necessary to examine, diagnose and spread awareness about the diseases and its diagnostic parameters depending on which it can be tested examined and can be treated well utmost. Awareness of the metabolic, physio-biochemical, and structural changes that occurs in an individual body when he\she gets affected, knowingly or unknowingly leads to a life -threatening diseases.

Hence, the main objective to create awareness and make feasible availability of the diagnostic tests and criteria depending on which examination of the diseases can be taken place. Biochemical changes in the body may lead to various other dead causing illness, likewise myocardial infarction, vascular complications, oxidative stress, and many other causes.

Thus, for the above, certain tests are being performed by various medical health centers to overcome and can be diagnosed before time, so that it must not bring changes to our biochemical reactions taking place in an individual’s bodies.

For this purpose, biochemical changes must be studied in diabetic patient to check the biochemical reactions in an individual body without the diabetes, with the diabetes and after the diagnostic tests changes that took place after the tests are performed.

The goal of diabetes management is to keep blood glucose levels as close to normal as safely possible. As diabetes may greatly increase risk for heart disease and peripheral artery disease, measures to control blood pressure and cholesterol levels are an essential part of diabetes treatment as well.

As the well said proverb is exactly applicable here., i.e.,

“Prevention is better than cure”

## 1. INTRODUCTION

Math problems are weird; “I had 10 chocolate bars and ate 9. What do I have now?” Oh, I don’t know, DIABETES may be?”

Let’s go through one of the funniest moments related to this “sugar sickness” diseases.

You peed on tape and compared the color it turned to on a chart. Anyway, my mother was always smelling my breath to make sure it didn’t smell “fruity” indicating my sugar was too high. People would be horrified when they heard her say “are you high? Let me smell your breath! “I didn’t get why people would stop and stare.” – *Jill Bryant Weaver*

“Diabetes” a very common disease nowadays among people. It has various nicknames people introduce it when they get caused of, likewise,” I have sugar”, “ sugar trouble”, “sugar problem”, “ sugar disease”, “sugar sickness”, “ sugar”, “ the sugar”, “ have the sugars”, “sweet blood”, etc. The very common source of cause said by people is, “the over-consumption of sugar in diet”. Eventually, normal or table sugar is not responsible for the cause of diabetes, the sugars present in the balanced diet in the various forms such as carbohydrates, lipids, starch, and many other variants, when they are not being synthesized properly by our body due to the ill-regulation of hormone or chemical releasing body organ, thus causes the problem of being diabetic. Leading to this, many biochemical reactions in the body gets altered causing other health related issues.

Scientifically, if we explain the diabetes, it can be said as a group of common endocrine diseases characterized by sustained high blood sugar levels.

Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. If it left untreated, leads to many health complications. Untreated or poorly treated diabetes accounts for approximately 1.5 million deaths per year.

## **1. OBJECTIVE OF RESEARCH**

- (A) To find out the biochemical changes that takes place in a diabetic patient before and after the cause of the disease.
- (B) To assist biochemical test profile of FBS/PPBS, HbA1c, Insulin and C-Peptide with respect to predicted outcomes and treatment and precaution of being diabetic and cautionary measures in day to day lifestyle.

## **2. NEED OF STUDY**

Diabetes contributes to the development of heart failure through various metabolic, structural and biochemical changes. The presence of diabetes increases the risk for the development of cardiovascular disease (CVD), and since the introduction of cardiovascular outcome trials to test diabetic drugs, the importance of improving our understanding of the mechanisms by which diabetes increases the risk for heart failure has come under the spotlight.

In addition to the coronary vasculature changes that predispose individuals with diabetes to coronary artery disease, diabetes can also lead to cardiac dysfunction independent of ischemic heart disease.

The hyperlipidemic, hyperglycaemic and insulin resistant state of diabetes contributes to a perturbed energy metabolic milieu, whereby the heart increases its reliance on fatty acids and decreases glucose oxidative rates. In addition to changes in cardiac energy metabolism, extracellular matrix remodeling contributes to the development of cardiac fibrosis, and impairments in calcium handling result in cardiac contractile dysfunction.

Lipotoxicity and glucotoxicity also contribute to impairments in vascular function, cardiac contractility, calcium signaling, oxidative stress, cardiac efficiency and lipoapoptosis.

Lastly, changes in protein acetylation, protein methylation and DNA methylation contribute to a myriad of gene expression and protein activity changes. Altogether, these changes lead to decreased cardiac efficiency, increased vulnerability to an ischemic insult

and increased risk for the development of heart failure. This review explores the above mechanisms and the way in which they contribute to cardiac dysfunction in diabetes.

Better diabetic education and knowledge to control and treat diabetes at right time can reduce the risk factors and minimize the chances to develop complications of diabetes and thus reduce morbidity and mortality in diabetics.

### **Diabetes: Where We Are Today**

Today, insulin is still the primary therapy used to treat type 1 diabetes; Other medications have since been developed to help control blood glucose levels. Diabetic patients can now test their blood sugar levels at home, and use dietary changes, regular exercise, insulin, and other medications to precisely control their blood glucose levels, thereby reducing their risk of heart attack.



**Fig 1: Introduction to Diabetes**

## II. LITERATURE REVIEW

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and, in modified form, in 1985. The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM (Insulin dependent diabetes mellitus) or Type 1, and NIDDM (Non-Insulin dependent diabetes mellitus) Type 2. In the 1985 Study Group Report the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition-related Diabetes Mellitus (MRDM) was introduced.

In both the 1980 and 1985 reports other classes of diabetes included, Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). These were reflected in the subsequent International Nomenclature of Diseases (IND) IN 1991, and the tenth revision of the International Classification of Diseases (ICD-10) in 1992. The 1985 classification was widely accepted and is used internationally. It represented a compromise between clinical and etiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or etiology was unknown. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complementary etiological classification.

Diabetes is a heterogeneous, complex metabolic disorder characterized by elevated blood glucose concentrations secondary to either resistance to the action of insulin, insufficient insulin secretion, or both. The most common classifications include:

- Type 1 diabetes mellitus
- Type 2 diabetes mellitus
- Type 3 diabetes mellitus (Alzheimer's disease) and,
- Gestational diabetes

## 1. CLASSIFICATION OF DIABETES MELLITUS

The major clinical manifestation of the diabetic state is hyperglycemia. However, insulin deficiency and/or insulin resistance also are associated with abnormalities in lipid and protein metabolism, and with mineral and electrolyte disturbances. The vast majority of diabetic patients are classified into two broad categories: type 1 diabetes mellitus, which is caused by an absolute or near absolute deficiency of insulin, or type 2 diabetes mellitus, which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. In addition, women who develop diabetes during their pregnancy are classified as having gestational diabetes. Finally, there are a variety of uncommon and diverse types of diabetes, which are caused by infections, drugs, endocrinopathies, pancreatic destruction, and genetic defects. These unrelated forms of diabetes are included in the “Other Specific Types” and classified separately.

### **Type 1 Diabetes Mellitus**

Type 1 diabetes results from autoimmune destruction of the pancreatic beta-cells. Markers of immune destruction of the beta-cell are present at the time of diagnosis in 90% of individuals and include antibodies to the islet cell (ICAs). To glutamic acid decarboxylase (GAD65), tyrosine phosphatases IA-2 and IA-2b, ZnT8, and insulin auto-antibodies (IAAs). Individuals may convert to negative if only one marker is positive, but individual risk of developing type 1 DM increases with the number of positive markers. Two positive antibodies are associated with a 75% chance of developing diabetes in the next 10 years. Diagnostic staging is now available for individuals with autoimmunity, even prior to diagnosis of type1 DM. While this form of diabetes usually occurs in children and adolescents, it can occur at any age. Younger individuals typically have a rapid rate of beta cell destruction and present with ketoacidosis, while adults often maintain sufficient insulin. But more indolent adult-onset variety has been referred to as latent autoimmune diabetes in adults (LADA). There is still controversy whether adult type 1 DM and LADA are the same clinical entity, but LADA patients are antibody positive and often require insulin therapy within years of diagnosis. Idiopathic forms of

type 1 DM often are of African or Asian descent. An intermittent risk of diabetic ketoacidosis, based on their varying insulinopenia, is present. Eventually, all type 1 diabetic patients will require insulin therapy to maintain normoglycemia.

### **Type 2 Diabetes Mellitus**

Type 2 diabetes is characterized by insulin resistance and, at least initially, a relative deficiency of insulin secretion. In absolute terms, the plasma insulin concentration (both fasting and meat-stimulated) usually is increased, although “relative” to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis. With time, however, there is progressive beta cell failure and worsening insulin deficiency ensues. Recently, more sophisticated analyses of the beta-cell response and regulation revealed that most subjects at risk for developing type 2 diabetes, i.e. those with combined impaired fasting glucose and impaired glucose tolerance already have a significant loss, close to 80% of the total insulin secretory capacity of the pancreas. In a minority of type 2 diabetic individuals, severe insulinopenia is present at the time of diagnosis and insulin sensitivity is normal or near normal. Most individuals with type2 diabetes exhibit intra-abdominal (visceral) obesity, which is part of the “ectopic fat” deposition pattern closely related to the presence of insulin resistance. In addition, hypertension, dyslipidemia (high triglyceride and low HDL-cholesterol levels; postprandial hyperlipemia), vascular endothelial dysfunction and elevated PAI-1 levels often are present in these individuals. This clustering of abnormalities is referred to as the “insulin resistance syndrome” or the “metabolic syndrome”. Because of these abnormalities, patients with type 2 diabetes are at increased risk of developing atherosclerotic cardiovascular disease (ASCVD) with macrovascular complications (myocardial infarction and stroke).

### **Type 3 Diabetes Mellitus**

Type 3 diabetes is a term used by some researchers to describe the theory that insulin resistance and insulin-like growth factor dysfunction in the brain may cause Alzheimer’s disease. More research needs to be carried out to understand the link between diabetes and Alzheimer’s disease.

Some research studies have suggested that Alzheimer's disease should also be classified as a type of diabetes, called type 3 diabetes. However, type 3 diabetes is not currently an official medical term. It is not recognized by National Health Organizations or the American Diabetes Association. This "type 3 diabetes" is a term proposed to describe the hypothesis that Alzheimer's disease is caused by a type of insulin resistance and insulin-like growth factor dysfunction that occurs specifically in the brain.

This condition also has been used by some to describe people who have T2D and also receive a diagnosis of Alzheimer's disease. The classification of type 3 diabetes is highly controversial, and it's not widely accepted by the medical community as a clinical diagnosis. Another classification of Diabetes includes type 3c diabetes mellitus (also called T3cDM, pancreatogenic diabetes, and type 3c diabetes). This type of diabetes develops due to conditions that affect the pancreas. Despite having a similar name, this is a separate condition.

T3cDM occurs when the exocrine pancreas glands become damaged and cause damage to the endocrine pancreas glands. Beta-islets cells in endocrine pancreas tissue produce and secrete insulin.

### **Gestational Diabetes Mellitus (GDM)**

Gestational diabetes mellitus (GDM) is defined as glucose intolerance which is first recognized during pregnancy. In most women who develop GDM, the disorder has its onset in the third trimester of pregnancy. At least 6 weeks after the pregnancy ends, the woman should receive an oral glucose tolerance test and be reclassified as having diabetes, normal glucose tolerance, impaired glucose tolerance, or impaired fasting glucose. Gestational diabetes complicates about 8-9% of all pregnancies, though the rates may double in populations at high-risk for type 2 diabetes. Clinical detection is important, since therapy will reduce perinatal morbidity and mortality. Two groups, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and the National Institutes of Health (NIH) Consensus Group recommend different testing methods for the diagnosis of GDM. A large-scale (~25,000 pregnant women) multinational epidemiologic study demonstrated that risk of adverse maternal and



neonatal outcomes continuously increased as a function of maternal glycemia at 24-28 weeks, even within ranges previously considered normal for pregnancy. These observations led to a revision in the diagnostic criteria recommended by IADPSG for GDM using a “one-step” 75-gram OGTT. All women not known to have diabetes should undergo glucose test screening between weeks 24 and 28 using the “one-step” 75 grams of glucose load in the morning after an overnight fasting period of at least 8 hours or the “two-step” method which starts with a non-fasting 50-gram glucose load test (GLT). A fasting 100-gram glucose tolerance test is only performed if the screening 50-gram GLT 1-hour plasma glucose value is  $>140\text{mg/dl}$  ( $7.8\text{ mmol/L}$ ).

### **Specific Types of Diabetes Mellitus**

**Genetic Defects:** Maturity Onset Diabetes of the Young (MODY) is characterized by impaired insulin secretion with minimal or no insulin resistance. MODY can be subtyped into neonatal and MODY-like. Neonatal diabetes usually has an onset in the first 6 months of life and can be transient or permanent. MODY may affect genes important for beta-cell glucose sensing, development, function, and regulation. Genetic inability to convert proinsulin to insulin results in mild hyperglycemia. Similarly, the production of mutant insulin molecules has been identified in a few families and results in mild glucose intolerance. MODY5 is most often associated with renal cysts and was not listed on the most recent ADA classification of diabetes, but can rarely cause diabetes. The natural history of MODY is highly dependent on the underlying genetic defect and most typically exhibit mild hyperglycemia at an early age. The disease is inherited in an autosomal dominant pattern.

Several genetic mutations have been described in the insulin receptor and are associated with insulin resistance. Type A insulin resistance refers to the clinical syndrome of acanthosis nigricans, virilization in women, polycystic ovaries, and hyperinsulinemia.

A variety of genetic syndromes have been described in which diabetes mellitus occurs with increased frequency. The etiology of the disturbance in glucose homeostasis in these diverse and seemingly unrelated syndromes remains undefined.

**Diseases of the Exocrine Pancreas:** Damage of the pancreas must be extensive for diabetes to occur. The most common causes are pancreatitis, trauma, and carcinoma. Chronic pancreatitis can cause general inflammatory/fibrotic changes in the pancreas which can cause diabetes. Cystic fibrosis causes a well-recognized pancreatic exocrine function insufficiency, but the same thick, viscous secretions cause inflammation, obstruction, and destruction of small ducts in the pancreas, which can lead to insulin deficiency. Hemochromatosis has also been associated with impaired insulin secretion and diabetes.

**Endocrinopathies:** Since growth hormone, cortisol, glucagon, and epinephrine increase hepatic glucose production and induce insulin resistance in peripheral (muscle) tissues, excess production of these hormones can cause or exacerbate underlying diabetes. Although the primary mechanism of action of these counter regulatory hormones is the induction of insulin resistance in muscle and liver, overt diabetes mellitus does not develop in the absence of beta cell failure.

**Infections:** A variety of infections have been etiologically related to the development of diabetes mellitus. Of these, the most clearly established is congenital rubella. Approximately 20% of infants who are infected with the rubella virus at birth develop autoimmune type 1 diabetes later in life. These individuals have the typical type 1 susceptibility genotype, DR3/DR4.

**Drugs:** A large number of commonly used drugs have been shown to induce insulin resistance and/or impair beta cell function and can lead to the development of diabetes mellitus in susceptible individuals. An extensive review of these drugs and their mechanism of action has been published. Drug classes which have been extensively associated with elevating glucose levels include: beta-blockers, thiazide diuretics, fluoroquinolones, atypical or second-generation anti-psychotics, calcineurin inhibitors, protease inhibitors, nicotinic acid, and corticosteroids. In addition, HMG-CoA reductase inhibitors (statins) have been shown to cause a small increase in the risk of diabetes, though the exact mechanisms of how it may increase the risk of diabetes are not completely understood.

## 2. CAUSES AND COMPLICATIONS

Long-term exposure to DM shows many other complications of diabetes. Diabetic nephropathy, diabetic retinopathy is caused due to prolonged high blood sugar level over time. When the level of blood glucose is high for a long period, it can increase myo-inositol oxygenase (MIOX) enzyme activity and enhances myo-inositol catabolism. The enzymatic degradation of myo-inositol alters the activity of  $\text{Na}^+/\text{K}^+$  ATPase and phosphatidylinositol synthases, the very important molecules in the secondary signaling pathway. Thus, high blood glucose levels due to DM results in diabetic nephropathy, retinopathy, neuropathy, and diabetic cataracts. In uncontrolled diabetes, the high blood glucose level in the delicate vessels of the retina increases osmotic pressure, and the vessels get leaked or rupture in some instances resulting in an impaired supply of blood to the retina. To compensate for the ruptured retinoid vessels, collateral blood vessels grow out of the retina and cause scar tissue to form resulting in impaired vision. Uncontrolled diabetes can affect kidneys, damaging the basement of glomerular capillaries, disrupting protein crosslinking, and allowing proteins in the urine to leak through, a process known as diabetic nephropathy. Ketoacidosis is common in diabetic patients due to the continuous production of ketone bodies. Diabetic ketoacidosis (DKA) is a feature of insulin insufficiency rather than resistance, which characterizes T2DM. The phenomenon is also known as Fournier's gangrene is a very rare condition whereas diabetes makes it more common. T1DM patients may develop obstructive pancreatitis because of inflammation in the pancreas, hyperplasia of the pancreatic duct gland resulting in obstructive pancreatitis. Diabetic patients are also at risk of free radical associated damage which is higher in diabetic patients than that of normal leading to atherosclerosis, cardiovascular disease, and hypertension. Prevalence of coronary artery disease (CAD), heart disease, and sudden cardiac death are elevated in diabetic patients. The high blood glucose level in diabetic patients stimulates superoxide production by the Maillard reaction. Several studies indicated that cognitive dysfunction occurs in T2DM affecting intelligence, attention, memory, learning, and perception. Diabetes is also diagnosed as a potent risk factor for cancer because both share some common risk factors including age, sex, obesity, diet, smoking, and alcohol. The contributing factor of cancer

due to DM is that in T2DM the hepatic production of IGF binding protein becomes low and circulating IGF-1 becomes high. In T2DM, mTOR is overactivated which phosphorylates IRS-1 and attenuates the metabolic pathway of insulin signaling. In T2DM, IRS-2 is upregulated and this activates the mitogen-activated protein kinase (MAPK) pathway and enhances cell proliferation. Other studies also indicated that different forms of cancer including liver cancer, pancreatic cancer, and non-Hodgkin's lymphoma are predominant in diabetic patients. Diabetes and chronic hepatitis C (CHC) alter the immune system concurrently. Diabetic CHC patients have an augmented risk of progression of cirrhosis and hepatocellular carcinoma. High blood glucose level decreases tubular reabsorption of magnesium resulting in lower blood Mg. Decreased Mg level in blood shows symptoms of many diseases caused by magnesium deficiency.

According to researches, type 3 diabetes mellitus had been found to have varied complications. In 2022 review of research, people who have T2D may be up to 45% to 90% more likely to develop Alzheimer's disease or another type of dementia, such as vascular dementia.

- A family history of diabetes
- High blood pressure (hypertension)
- Having overweight or obesity
- Certain chronic health conditions, such as depression and polycystic ovary syndrome (PCOS)
- Insulin resistance
- Elevated Cholesterol
- Hyperglycemia caused by oxidative stress
- Lipid peroxidation

### 3. PATHOPHYSIOLOGY

In normal pathophysiology of human body, the pancreas secretes digestive enzymes and the hormones insulin and glucagon into the bloodstream to control the amount of glucose in the body. The release of insulin into the blood lowers the level of blood glucose by allowing glucose to enter the body cells, where it is metabolized. If blood glucose levels get too low, the pancreas secretes glucagon to stimulate the release of glucose from the liver. Right after meal, glucose and amino acids are absorbed directly into the bloodstream, and blood glucose levels rise sharply. The rise in blood glucose levels signals important cells in the pancreas, called beta cells, to secrete insulin, which pours into the bloodstream.

#### Pathophysiology of type 1 diabetes

In this condition the immune system attacks and destroys the insulin producing beta cells of the pancreas. There is beta cell deficiency leading to complete insulin deficiency. Thus, is it termed an autoimmune disease where there are anti insulin or anti-islet cell antibodies present in blood. These cause lymphocytic infiltration and destruction of the pancreas islets. The destruction may take time but the onset of the disease is rapid and may occur over a few days to weeks. There may be other autoimmune conditions associated with type 1 diabetes including vitiligo and hypothyroidism. Type 1 diabetes always requires insulin-therapy, and will not respond to insulin-stimulating oral drugs.

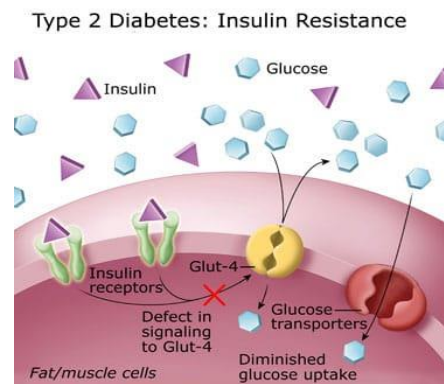


**Fig 2: Pathophysiology of Diabetes Mellitus type 1**

## Pathophysiology of type 2 diabetes

This condition is caused by a relative deficiency of insulin and not an absolute deficiency. This means that the body is unable to produce adequate insulin to meet the needs. There is beta cell deficiency coupled with peripheral insulin resistance.

Peripheral insulin resistance means that although blood levels of insulin are high there is no hypoglycemia or low blood sugar. This may be due to changes in the insulin receptors that bring about the actions of the insulin.



**Fig 3: Pathophysiology of Diabetes Mellitus type 2**

## Pathophysiology of Type 3 diabetes

In type 3 diabetes, the neurons lack glucose, a key element needed for the neurons to function effectively in body however more specifically the hippocampus and the cerebral cortex. This deficiency can lead to a decrease in memory, judgement and the ability to reason, of which are key symptoms of Alzheimer's disease. It involves plasma concentrations of glucose signaling the central nervous system to mobilize energy reserves. It is based on cerebral blood flow and tissue integrity, arterial plasma glucose, the speed that plasma glucose concentrations fall, and other available metabolic fuels.

## **Pathophysiology of Gestational diabetes**

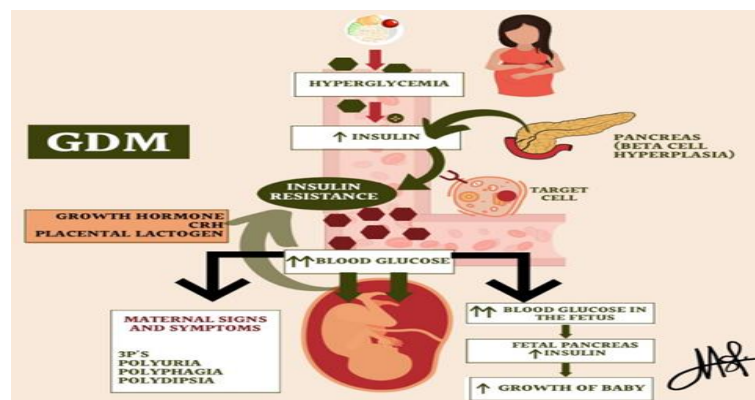
Gestational diabetes is caused when there are excessive counter-insulin hormones of pregnancy. This leads to a state of insulin resistance and high blood sugar in the mother. There may be defective insulin receptors.

Pathophysiology behind symptoms and complications of diabetes:

- Polydipsia or increased thirst is due to high blood glucose that raises the osmolarity of blood and makes it more concentrated.
- Polyuria or increased frequency of urination is due to excess fluid intake and glucose-induced urination.
- Weight loss occurs due to loss of calories in urine.
- Polyphagia or increased hunger due to loss or excess glucose in urine that leads the body to crave for more glucose.
- Poor wound healing, gum and other infections due to increased blood glucose providing a good source of nutrition to microbes and due to a diminished immunity.
- Heart disease-this occurs due to changes in the large blood vessels leading to coronary, cerebral, and peripheral artery diseases, atherosclerosis, dyslipidemia etc.
- Eye disease-this is termed diabetic retinopathy and occurs due to damage of the fine blood vessels of the retina in the eye due to long term exposure to high blood sugar.
- Kidney damage-similar damage to small and large blood vessels of the kidneys. Initially there is proteinuria or increased outflow of protein and may lead to end stage renal disease.
- Nerve damage-this can affect the arms and legs and is called stocking-glove numbness/tingling. It can also affect autonomic functions leading to impotence, erectile dysfunction, difficulty in digestion or gastroparesis etc.
- Diabetic foot-this occurs due to peripheral nerve damage as well as blood vessel affliction due to long term diabetes. Little trauma, sores and blisters go

unnoticed due to lack of sensation and peripheral vascular disease impairs healing and allows infection.

- Diabetic Ketoacidosis is caused in type 1 diabetes where there is complete lack of insulin and reliance on fatty acids for energy. This uncontrolled lipid breakdown leads to formation of ketones and causes acidosis and ketonemia.
- Non-Ketonic Hyperosmolarity-this is caused due to extreme rise of blood sugar. This is seen in type 2 diabetes. There is just enough insulin to suppress ketone synthesis. The high blood sugar leads to excessive concentration or osmolarity of blood which in turn leads to diuresis and collapse of the blood vessels and Cardiovascular shock.



**Fig 4: Pathophysiology of Gestational Diabetes Mellitus**

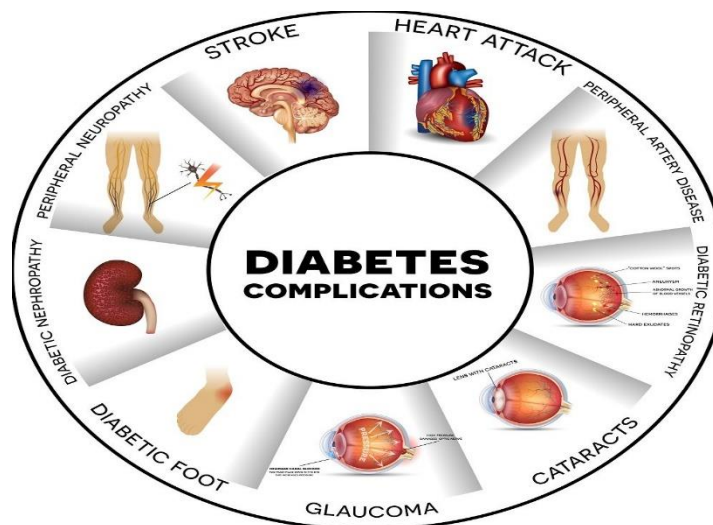


#### 4. SIGNS AND SYMPTOMS

Common symptoms include the following:

- Excessive thirst
- Unexplained weight loss
- Extreme hunger
- Sudden vision changes
- Tingling or numbness in the hands or feet
- Feeling very tired much of the time
- Very dry skin
- Sores that are slow to heal
- More infections than usual

Some people may experience only a few symptoms that are listed above. About 50 percent of people with type 2 diabetes don't experience any symptoms and don't know they have the disease.



**Fig 5: Complications of Diabetes**

### III. METHODOLOGY

FBS is a test that has to be performed by drawing a blood sample from a vein, after twelve hours of fasting. It measures the fasting blood sugar levels. After giving a blood sample to determine FBS, the patient consumes a heavy meal. Approximately between 1.5 to 2 hours after eating, a second blood sample is drawn from the vein for testing. This is known as the Post Prandial Blood Sugar test, or PPBS test. A third test is the Glucose Tolerance Test or GTT which is performed after consuming a concentrated amount of glucose dissolved in water.

#### **Why is this test consider an important diagnostic tool for diabetes?**

As untreated DM can lead to serious complications, early diagnosis of diabetes may prevent serious consequences due to the illness. Primary symptoms of diabetes include high blood glucose levels over a prolonged period, frequent urination, increased thirst, and elevated hunger. Some biochemical tests are routinely carried out to make a diagnosis of prediabetes or diabetes. Glycosylated hemoglobin (HbA1c) and oral glucose tolerance tests (OGTT) are commonly demonstrated for screening diabetes. OGTT test measures how well body cells can absorb glucose after consuming a specific amount of sugar. Usually, the suspected individual is treated with 75 g glucose orally and the plasma glucose level is measured 2 h after ingestion. If the plasma glucose level is found  $\geq 11.1$  mmol/L, then the individual is diagnosed as diabetic. Fasting plasma glucose test is another reliable routine method for the diagnosis of diabetes. Diabetes patients usually have a fasting glucose level of  $\geq 7.0$  mmol/L. If a person has a plasma glucose level  $\geq 7.8$  mmol/L after 2 h of ingesting 75 g glucose, then it is said that the person has impaired glucose tolerance. HbA1c is also widely used as a diagnostic test for diabetes.

Patients with T2DM have a glycosylated hemoglobin level of  $\geq 48$  mmol/mol ( $\geq 6.5$  DCCT%). Random blood sugar monitoring is yet another prognostic marker for determining diabetes.

## Biochemical Test Profile

The research was conducted in a well-established laboratory, and the data was collected on 100 patients per month on a regular basis for dissertation periods for currents analysis of diabetic patients with different age groups. During this period, different types of biochemical tests were performed, including test for FBS/PPBS, test for HbA1c, test for Insulin and test for C-Peptide. The total number of cases is listed in the table below. The data collected during this period is compared to annual year with the following graphical presentation.

### 1. Test for FBS/PPBS

**Method:** Ortho-toluidine method (Mono step).

**Normal Values:**

Serum/plasma: (fasting):70-110mg/dl

Serum/plasma: Post Prandial (PP) (2hrs after lunch):70-130mg/dl

Specimen collection: Patient should fast for 12-16hrs

**Sample material:** Plasma

**Instrument:** Spectrophotometer/ Colorimeter



**Fig 6 : Spectrophotometer**



**Fig 7 : Colorimeter**

## 2.TEST FOR HbA1c

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse antihuman HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance.

### Normal Values:

Normal range: 4% and 5.6%

Prediabetics: 5.7% and 4.6%

Diabetics: 6.5% or more

**Sample material:** Blood

**Instrument:** HPLC (High- performance Liquid Chromatography)



**Fig 8: HPLC (High- performance Liquid Chromatography)**

### 3.TEST FOR INSULIN

The ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with anti-insulin antibodies bound to the micro-titration well and with peroxidase-conjugated anti-insulin antibodies. A washing step removes unbound enzyme labeled antibody. The bound conjugate was determined by reaction with TMB (3,3',5,5'-tetramethylbenzidine) a chromogen. The reaction is stopped by adding acid to give a colorimetric endpoint that is read Spectro-photometrically at a wavelength of 450nm using a microplate reader.

**Sample material:** Serum/Plasma

**Normal Range:**

Fasting: 2.6 – 37.6 mIU/L

Post Prandial: 16 – 166 mIU/L

**Instrument:** ELISA/ CLIA



**Fig 9 : ELISA**



**Fig 10 : CLIA**

#### 4.TEST FOR C-PEPTIDE

In-vitro determination of insulin and C-Peptide levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and C-Peptide are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic  $\beta$ -cells and is split into a 31 amino acid connecting peptide and insulin (MW 6000). C-Peptide is devoid of any biological activity but appears to be necessary to maintain the structural integrity of insulin. Although insulin and C-Peptide are secreted into portal circulation in equimolar concentrations, fasting levels of C-Peptide are 5-10folds higher than those of insulin owing to the longer half-life of C-Peptide.

**Sample:** Blood

**Normal values:**

Fasting: 0.9 – 1.8 ng/ml

Post prandial: 0.5 – 2.0 ng/ml

**Instrument:** ELISA/ CLIA



**Fig 11 : ELISA**

## IV. RESULT

### A) Analysis of different Biochemical tests for Diagnosis of type of diabetes

Sr. No	Patients	Age	Gender	FBS/PPBS	HbA1c	Insulin	C- Peptide	Diagnosis
1	Patient 1	19	female	92	6.5	120	60	Type 2 diabetes
2	Patient 2	23	male	100	7.0	150	70	Type 2 diabetes
3	Patient 3	30	female	88	8.0	90	75	Type 1 diabetes
4	Patient 4	33	male	76	5.5	2	14	Prediabetics
5	Patient 5	39	female	117	5.0	149	65	Gestational diabetes
6	Patient 6	41	female	97	4.5	198	62	Type 2 diabetes
7	Patient 7	50	male	106	6.0	7	79	Type 2 diabetes
8	Patient 8	63	male	63	7.5	180	64	Type 2 diabetes
9	Patient 9	78	male	76	9.0	40	89	Type 1 diabetes
10	Patient10	80	female	120	10.0	99	54	Type 1 diabetes

Table: 1 Showing number of patients obtained during biochemical tests performed and average resultant

As we can evaluate, the diagnosis of Diabetes is based on the following criteria:

- FBS level of  $>126$  mg/Dl
- HbA1c level of  $>6.5$  %
- Insulin level of  $<3$  mU/mL
- C- Peptide level of  $<0.2$  nmol/L

Based on these criteria, the following patients are diagnosed with diabetes:

- ✓ Patient 1
- ✓ Patient 2
- ✓ Patient 3
- ✓ Patient 6
- ✓ Patient 9
- ✓ Patient 10

The other four patients are either prediabetic or have gestational diabetes.

Hence, the type 3 diabetes is yet ongoing researches, as the cases had been found in other countries, on research basis.

It is important to note that this is just a small sample size, and further research is needed to confirm these findings. However, this study provides some valuable insights into the diagnosis of diabetes.

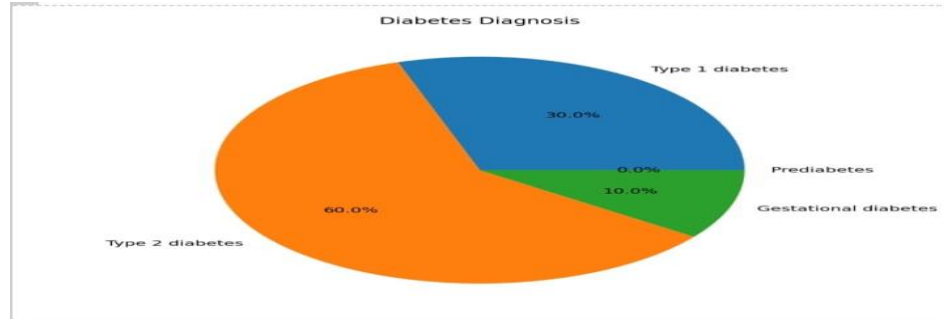
## **B) Interpretation**

The FBS (fasting blood sugar), HbA1c (glycated hemoglobin), insulin, and C-Peptide tests are all used to diagnose and manage diabetes as examined in above table.

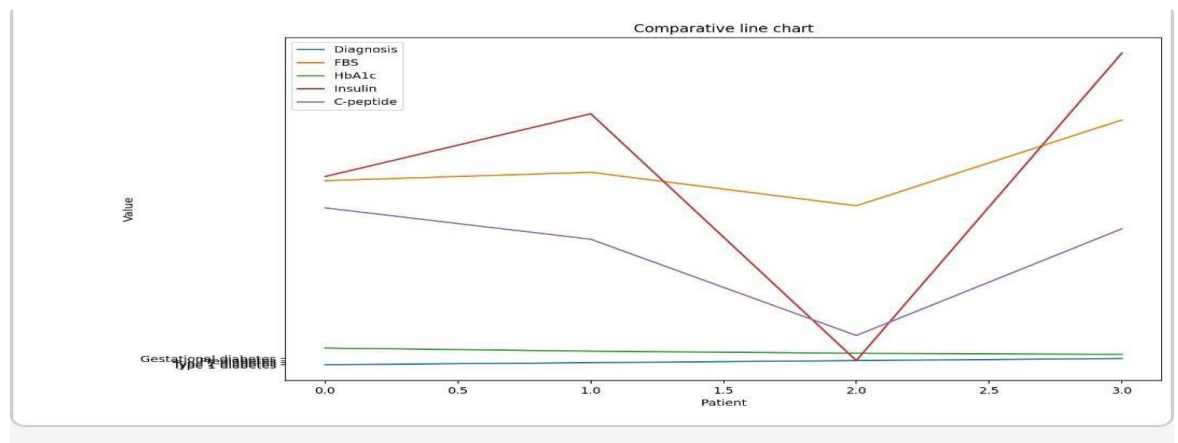
- FBS measures the amount of glucose in the blood after an overnight fast. A normal FBS level is less than 100 mg/dL. A level of 100 to 125 mg/dl is considered prediabetics, and a level of 126 mg/dl or higher is considered diabetes.



- HbA1c is a measure of the average blood sugar level over the past 3 months. A normal HbA1c level is less than 5.7%. A level of 5.7 to 6.4% is considered prediabetics, and a level of 6.5% or higher is considered diabetes.
- Insulin is a hormone that helps our body use glucose for energy. A normal insulin level is 2 to 25 mIU/L. A level of 26 to 100 mIU/L is considered elevated, and a level of 100 mIU/L or higher is considered high.
- C- Peptide is a protein that is released along with insulin. A normal C-Peptide level is 0.2 to 0.8 nmol/L. A level of 0.2 nmol/L or lower is considered low, and a level of 0 nmol/L is considered undetectable.
- A high FBS level and HbA1c level, along with a low C-Peptide level, suggest that a person have type 1 diabetes.
- A high FBS level and HbA1c level, along with a normal or elevated C-Peptide level, interprets that a person has type 2 diabetes.
- A normal FBS level and HbA1c level, along with a high insulin level, interprets that a person may have insulin resistance.



Graph 1



Graph2

## **V. DISCUSSION**

The analysis and determination of biochemical test profile of various diabetic patients of different age groups during this dissertation conclude that number of cases obtained in this vary period of months. As diabetes being common among the crowd, but we need to pay attention to control the roots of spreading diabetes because it contains mortality rate and complicated cases in worldwide. Number of cases in type 2 diabetes are found higher than other type 2, type 3 gestational or prediabetes. With increased in cases of diabetes, it has become difficult for other individuals to be self-aware about their own health.

Now-a-days every individual think diabetes as a common disease, thus unknowingly they become the prey of it. Proper healthcare measures and steps must be taken to prevent. Also, regular tests and check-ups are assisted by healthcare centers to get updated about one own self. The best way to prevent diabetes disease is to adhere to the proper guidelines related to physical, stress, diet and doctor recommended activities. Regular check-ups are crucial in the day to day busy life to prevent illness and other complications related to same.

According to WHO recommendations, it is advisable to help prevent type 2 diabetes and its complications, people should: reach and keep a health body weight. stay physically active with at least 30 minutes of moderate exercise each day. eat a healthy diet and avoid sugar and saturated fat.

## **VI. CONCLUSION**

Recent study of Biochemical tests of diabetic patients during dissertation periods includes some cases of type 1 diabetes, type 2 diabetes , prediabetics and gestational diabetes, which are seriously needed to pay attention, as compared to globally examined cases, thus they contain complications in some serious cases and insulin resistance is a major issue globally in type 2 diabetes. Proper guidelines and preventive measures must be implemented by oneself and regular checkup are mandatory.

Awareness measures and charts must be displayed in every hospital comprising endocrinologist and pathologists, who can make people aware of the common disease according to people and must provide a healthy interactive session to spread awareness about the same and upcoming complications when carelessness takes place

The endocrinologists and other health center members must consider this common disease as a fatal one so that before getting delayed an individual can seek with proper checkup, treatment, medications, tests and much more.

## VI. REFERENCES

1. WHO Expert Committee on Diabetes Mellitus. Second Report. Geneva: WHO, 1980. Technical Report Series 646.
2. World Health Organization. Diabetes Mellitus: Report of a WHO Study Group. Geneva: WHO, 1985. Technical Report Series 727.
3. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 26:5-20; 2003. [\[PubMed\]](#)
4. Introduction to Diabetes Mellitus. Diabetes by K Kaul · 2013 · Cited by 276 —citation · Published: 30 December 2012 · Publisher Name: Springer, New York, NY ·
5. Centers for Disease Control and Prevention. Diabetes Public Health Resource: Incidence and Age at Diagnosis. 2013. [2015 January 27]. [http://www.cdc.gov/diabetes/statistics/incidence\\_national.htm](http://www.cdc.gov/diabetes/statistics/incidence_national.htm) .
6. Anon. Economic costs of diabetes in the U.S. In 2007. *Diabetes Care*. 2008 Mar;31(3):596–615. [\[PubMed\]](#)
7. Anon. Standards of medical care in diabetes-2014. *Diabetes Care*. 2014;37(SUPPL.1): S14–S80. [\[PubMed\]](#)
8. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes: A patient-centered approach: Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Spectrum*. 2012;25(3):154–71. [PMC free article]
9. Anon. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998 Sep 12;352(9131):854–65.
10. Anon. Intensive blood-glucose control with insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998 Sep 12;352(9131):837–53. [\[PubMed\]](#)

- 11.** Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *The New England journal of medicine*. 2009 Jan 8;360(2):129–39. [\[PubMed\]](#)
- 12.** Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. *The New England journal of medicine*. 2008 Jun 12;358(24):2545–59. [PMC free article] [PubMed]
- 13.** Papademetriou V, Lovato L, Doumas M, et al. Chronic kidney disease and intensive glycemic control increase cardiovascular risk in patients with type 2 diabetes. *Kidney Int*. 2014 Sep 17;
- 14.** Saremi A, Schwenke DC, Bahn G, et al. The effect of intensive glucose lowering therapy among major racial/ethnic groups in the Veterans Affairs Diabetes Trial. *Metabolism*. 2015 Feb;64(2):218–25. [PMC free article]
- 15.** Bolen S, Wilson L, Vassy J, et al. Comparative Effectiveness and Safety of Oral Diabetes Medications for Adults with Type 2 Diabetes. Rockville, MD: Agency for Healthcare Research and Quality; 2007. (Comparative Effectiveness Review No 8). (Prepared by the Johns Hopkins Evidence-based Practice Center under Contract No. 290-02-0018) [PubMed]
- 16.** Bennett WL, Wilson LM, Bolen S, et al. Oral Diabetes Medications for Adults with Type 2 Diabetes: An Update. Rockville, MD: Agency for Healthcare Research and Quality; 2011. (Comparative Effectiveness Review No. 27). (Prepared by Johns Hopkins University Evidence-based Practice Center under Contract No. 290-02-0018.) AHRQ Publication No. 11-EHC038-EF. [PubMed]
- 17.** Bolen S, Feldman L, Vassy J, et al. Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. *Ann Intern Med*. 2007 Sep 18;147(6):386–99.
- 18.** Bennett WL, Maruthur NM, Singh S, et al. Comparative effectiveness and safety of medications for type 2 diabetes: an update including new drugs and 2-drug combinations. *Ann Intern Med*. 2011 May 3;154(9):602–13. [PMC free article] [PubMed]

19. Garber A, Abrahamson M, Barzilai J, et al. American association of clinical endocrinologists' comprehensive diabetes management algorithm 2013 consensus statement. *Endocrine Practice*. 2013;19(SUPPL. 2):1–48. [PMC free article] [PubMed]
20. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of Hyperglycemia in Type 2 Diabetes, 2015: A Patient-Centered Approach: Update to a position statement of the American diabetes association and the European association for the study of diabetes. *Diabetes Care*. 2015;38(1):140–9.
21. Type 2 Diabetes: National Clinical Guideline for Management in Primary and Secondary Care (Update). London: Royal College of Physicians of London; 2008. [PubMed]
22. Colhoun HM, Livingstone SJ, Looker HC, et al. Hospitalized hip fracture risk with rosiglitazone and pioglitazone use compared with other glucose-lowering drugs. *Diabetologia*. 2012 Nov;55(11):2929–37. [PMC free article] [PubMed]
23. Lu CJ, Sun Y, Muo CH, et al. Risk of stroke with thiazolidinediones: a ten-year nationwide population-based cohort study. *Cerebrovasc Dis*. 2013;36(2):145–51. [PubMed]
24. Mahaffey KW, Hafley G, Dickerson S, et al. Results of a reevaluation of cardiovascular outcomes in the RECORD trial. *Am Heart J*. 2013 Aug;166(2):240–9 e1. [PubMed]
25. Mamtani R, Haynes K, Bilker WB, et al. Association between longer therapy with thiazolidinediones and risk of bladder cancer: a cohort study. *J Natl Cancer Inst*. 2012 Sep 19;104(18):1411–21. [PMC free article] [PubMed]
26. Anon. (7) Approaches to glycemic treatment. *Diabetes Care*. 2015 Jan;38 Suppl: S41–8. [PubMed]
27. Turner LW, Nartey D, Stafford RS, et al. Ambulatory treatment of type 2 diabetes in the U.S., 1997-2012. *Diabetes Care*. 2014 Apr;37(4):985–92. [PMC free article] [PubMed]
28. Raebel MA, Xu S, Goodrich GK, et al. Initial antihyperglycemic drug therapy among 241 327 adults with newly identified diabetes from 2005 through 2010: a surveillance, prevention, and management of diabetes mellitus (SUPREME-DM) study. *Ann Pharmacother*. 2013 Oct;47(10):1280–91. [PubMed]

- 29.** Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2014 Jun 24;129(25 Suppl 2): S1–S45. [PubMed]
- 30.** Anon. (8) Cardiovascular disease and risk management. *Diabetes Care*. 2015 Jan;38 Suppl: S49–57. [PubMed]
- 31.** Tsertsvadze A, Maglione M, Chou R, et al. Updating comparative effectiveness reviews: current efforts in AHRQ's Effective Health Care Program. *J Clin Epidemiol*. 2011 Nov;64(11):1208–15. [PubMed]
- 32.** Higgins JPT, S. G. Cochrane handbook for systemic reviews of interventions Version 5.1.0. 2011. <http://handbook.cochrane.org/> Accessed Oxford, England.
- 33.** Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials*. 1996 Feb;17(1):1–12. [PubMed]
- 34.** Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomized and non-randomized studies of health care interventions. *J Epidemiol Community Health*. 1998 Jun;52(6):377–84. [PMC free article] [PubMed]
- 35.** Institute of Medicine. Finding What Works in Health Care: Standards for Systematic Reviews. Washington, DC: The National Academies Press; 2011. [2014 July 9]. books.nap.edu/openbook.php?record\_id=13059&page=81 [PubMed]