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STUDY OF EFFECTS OF DDP4 INHIBITORS AND METFORMIN ON INTERLEUKIN-1ß AND METABOLIC PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES IN IRAQI

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ATHMAR JAWAD AHMED ABUSIBA

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By Athmar Jawad Ahmed ABUSIBA

June 2022

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ABSTRACT

STUDY OF EFFECTS OF DDP4 INHIBITORS AND METFORMIN ON INTERLEUKIN-1ß AND METABOLIC PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES IN IRAQI

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Doctor of Philosophy in Chemistry

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June 2022

Hyperglycemia is caused by aberrant insulin synthesis, action, or both in many metabolic disorders. DM is a group of illnesses. This study evaluated DPP-4 and metformin's impact on metabolic indicators in type 2 diabetes and newly diagnosed individuals. This study included 30 to 83-year-olds. In this research, type 2 diabetics were divided into three groups based on their medication. Control group healthy patients numbered 49. DDP4 and IL-1ß levels were measured by ELISA, and biochemical features varied from healthy groups. Except for the metformin-only group, the DDP4 inhibitor drug is relevant in all biochemical markers. Newly diagnosed type 2 diabetics showed higher IL-1 levels than those taking DDP4 inhibitors and metformin. Higher IL-1 levels increased IL-1 activation. A systemic inflammatory process may indicate beta-cell death in early type 2 diabetes. DDP4 and DDP4 may assist more people, according to these data. Current recommendations recommend DDP inhibitors for type 2 diabetic management. This study focuses on diabetic cytokines like IL-1ß.

2022, 98 pages

Keywords: Diabetes mellitus, DDP4 inhibitors, Metformin, IL-1ß

ÖZET

IRAK'TA TİP 2 DİYABET HASTALARINDA DDP4 İNHİBİTÖRLERİ VE METFORMİNİN INTERLEUKIN-1ß VE METABOLİK PARAMETRELER ÜZERİNE ETKİLERİNİN İNCELENMESİ

Athmar Jawad Ahmed ABUSIBA

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Haziran 2022

Hiperglisemi, birçok metabolik bozuklukta anormal insülin sentezi, etkisi veya her ikisinden kaynaklanır. DM bir hastalık grubudur. Bu çalışma, DPP-4 ve metforminin tip 2 diyabet ve yeni tanı konmuş bireylerde metabolik göstergeler üzerindeki etkisini değerlendirdi. Bu çalışma 30 ila 83 yaşındakileri içeriyordu. Bu araştırmada tip 2 diyabet hastaları kullandıkları ilaçlara göre üç gruba ayrıldı. Kontrol grubu sağlıklı hastalar 49'du. DDP4 ve IL-1ß seviyeleri ELISA ile ölçüldü ve biyokimyasal özellikler sağlıklı gruplardan farklıydı. Yalnızca metformin grubu dışında, DDP4 inhibitör ilacı tüm biyokimyasal belirteçlerle ilgilidir. Yeni teşhis edilen tip 2 diyabet hastaları, DDP4 inhibitörleri ve metformin alanlara göre daha yüksek IL-1 seviyeleri gösterdi. Daha yüksek IL-1 seviyeleri, IL-1 aktivasyonunu arttırdı. Sistemik bir inflamatuar süreç, erken tip 2 diyabette beta hücre ölümünü gösterebilir. Bu verilere göre DDP4 ve DDP4 daha fazla kişiye yardımcı olabilir. Mevcut öneriler, tip 2 diyabet tedavisi için DDP inhibitörlerini önermektedir. Bu çalışma, IL-1ß gibi diyabetik sitokinlere odaklanmaktadır.

2022, 98 sayfa

Anahtar Kelimeler: Diyabetes mellitus, DDP4 inhibitörleri, Metformin, IL-1ß

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Assoc. Prof. Dr. Dr. Şevki ADEM

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LIST OF SYMBOLS

g	Gram
Kg	Kilogram
m^2	Square meters
mg	Milligram
mL	Milliliter
ng	Nanogram
nm	Nanometer
TG	Triglycerides
µg	Microgram
µL	Microliter

LIST OF ABBREVIATIONS

BMI	Body mass index± Plus minus
DDP4	Dipeptidyl peptidase 4
ELISA	Enzyme-linked immunosorbent assay
FFA	Free fatty acid
FSG	Fasting serum glucose
GDM	Gestational diabetes mellitus
GLUT4	Glucose transporter four
HbA1C	Glycated hemoglobin
HDL-C	High density lipoprotein cholesterol
IDF	International Diabetes Federation
IL-1β	Interleukin 1 beta
LDL-C	Low density lipoprotein cholesterol
SPSS	Statistical Package for the Social Sciences
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholestero

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1. INTRODUCTION

Diabetes is the 21st-century pandemic. One of the leading causes of death in America is diabetes mellitus (DM) (Berger and Moller 2002), Diabetes is projected to impact 246 million people worldwide. Diabetes will affect 380 million individuals worldwide by the year 2025. The fact is that diabetes is responsible for the deaths of 3.8 million individuals every year (Yin *et al.* 2008). After a fast or during an oral glucose tolerance test, a diabetic's plasma glucose levels are excessively high. This is known as hyperglycemia. Diabetes may be caused by insulin insufficiency, insulin resistance, or a combination of the two (Modak *et al.* 2007, Haque *et al.* 2011). Two forms of diabetes are recognized by the World Health Organization: type 1 (T1DM) and type 2 (T2DM) (T2DM). Type 2 Diabetes Mellitus (T2DM) is to blame for 90 to 95 percent of all cases of diabetic complications (Prabhakar and Doble 2008). Type 1 diabetes is linked to a number of environmental and genetic variables, as well as to maternal age at birth and exposure to particular viral infections. Type 2 Diabetes Mellitus has been linked to a rise in obesity and insulin resistance, as well as changes in diet and activity (T2DM) (Bnouham *et al.* 2006). More individuals are being diagnosed with diabetes due to rapid urbanization and changes in the diet and physical activity patterns. Both T1DM and T2DM need for close observation and supervision. Many organs and tissues might be permanently damaged if blood sugar levels are not appropriately controlled. People with diabetes are more likely to die from cardiovascular disease than the general population (Havale and Pal 2009) Nephropathy, neuropathy, and retinopathy are all possible complications of diabetes.

People with type 2 diabetes need to be identified and treated since there is no cure for any kind. Since the invention of insulin in 1921, all types of diabetes have been effectively treated. One of the most important goals in the treatment of type 2 diabetes is to boost insulin production in the pancreatic islet cells. Medications known as hypoglycemics, or anti-diabetic drugs, aim to lower blood glucose levels (i.e., the amount of sugar in the blood). There are a variety of anti-diabetic medications to select from, depending on your type of diabetes and other variables. Consequently, diabetes

medications work by increasing insulin levels, enhancing the body's insulin sensitivity, or decreasing intestinal glucose absorption (Brubaker and Drucker 2004). Most of these treatments are deemed useless because of their severe side effects in terms of preventing problems and preserving one's quality of life. -Glucosidase inhibitors like acarbose and miglitol, which limit the function of glucosidases at the brush border of the small intestine, seldom cause gas, diarrhea, or stomach bloating. Hypoglycemia is a side effect of traditional insulin secretagogues such sulfonylureas and meglitinides. Although metformin has been shown to prevent macrovascular events in T2DM, persons with impaired renal or hepatic function should not be given the drug. Metformin should be the first-line treatment for overweight or obese patients with T2DM who have normal renal function. On the other hand, side symptoms such as hypoglycemia and weight gain are not uncommon. Type 2 diabetes is a disease that requires new treatment methods. Identifying drugs that increase glucose (nutrient)-dependent insulin production may help accomplish this goal (Augeri *et al.* 2005). GLP-1 agonists and DPP-4 inhibitors, as well as thiazolidinediones (TZDs) as an alternate treatment, are advised for individuals with metabolic syndrome in addition to metformin. There has been an increase in the number of women in leadership positions in the public sector since

DPP-IV inhibition prolongs GLP-1 and GIP activity, improving glucose homeostasis with a low risk of hypoglycemia and the ability to change disease, according to this study, which examined a wide range of T2DM therapy medications, including metformin and DPP4 inhibitors. so that these medications' effects on a biochemical marker might be better understood and novel anti-diabetic therapeutic targets identified Type 2 diabetes must be controlled in part by addressing these treatment objectives.

T2DM patients have an overtaxed immune system, resulting in chronic inflammation and infection (Re 2017). According to a number of research, elevated inflammation and oxidative stress are caused by an increase in glucose. Chronic inflammation, which has only lately been identified as a risk factor for type 2 diabetes, has been related to the disease. Because chronic inflammation releases cytokines that alter insulin signaling, adipose, muscle, and liver tissue all suffer from insulin resistance. Systemic insulin 18 resistance is the primary cause of IR81, since it reduces insulin sensitivity throughout

the whole body. According to current research, DM2's pathophysiology includes inflammation. People who have greater baseline inflammatory markers are more likely to acquire diabetes82. Since DM2 has long been connected to inflammation, we don't know if this metabolic abnormality causes or causes inflammation.

One of the most essential factors in the treatment of type 2 diabetes is the role of IL-1ß, which is connected to insulin resistance and cell dysfunction. Many studies suggest that IL-1 interacts with insulin signaling in adipose tissue and the liver, reducing adiponectin release 44-46. It has been shown that modest concentrations of IL-1ß stimulate islet 12 insulin output and cell proliferation, and minimize cell death, but large doses of IL-1ß produced by increased glucose and/or free fatty acids have the opposite effect. IL-1 is often blocked by a naturally occurring antagonist41 because it stimulates the production and release of IL-1RA.

1.1 Research Questions

- 1. Do medications antihyperglycemic have an effect on biochemical parameters in patients with type 2 diabetes?
- Does age, gender and Body mass index BMI have an effect on newly diagnosed type 2 diabetes patients and patients who take antidiabetic medication.
- Is high cytokines, especially Interleukin-1beta, an inflammatory marker of diabetes?

1.2 The Primary Objectives

- An Iraqi population with type 2 diabetes and biochemical indicators for antidiabetic medications is the focus of this thesis.
- Type 2 diabetic Iraqi patients' responses to the inflammatory marker IL-1ß and the function it plays.

1.3 The Secondary Objectives

We'll do the following through a study of a representative sample of Iraqi type 2 diabetics:

- This study compares for Age, BMI, and gender levels of newly diagnosis individuals with type 2 diabetes and those on antihyperglycemic medication and non-diabetic healthy groups.
- Examine the differences in CRP between diabetic and non-diabetic groups at various glucose and HbA1c levels in patients and healthy groups.

2. LITERATURE REVIEW

2.1 Diabetes Mellitus

Defining characteristics of diabetes mellitus include abnormalities in the body's capacity to make and use insulin. Many people with diabetes may not have any symptoms at first, but they might have long-term repercussions such as cardiovascular comorbidities, kidney illness, limb amputations and even blindness because of an increasing blood glucose level (Dahlström and Sandholm 2017). There are four primary molecular instruments linked with blood vessel damage, and diabetes is a key cause of many of these illnesses. Figure 2.1 shows an increased excretion of hyperglycemia due to an increase in superoxide generation via the electron transport chain (Conget 2002).

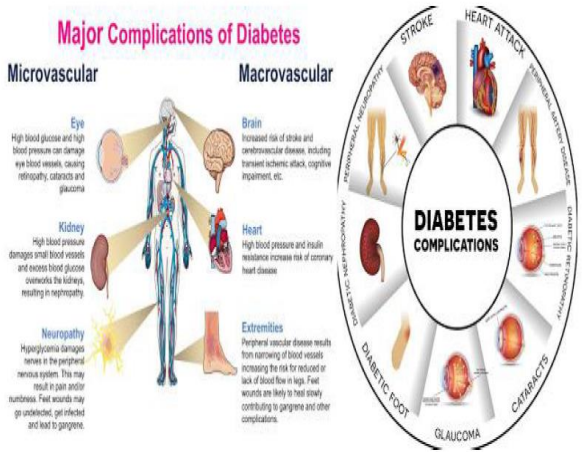

Figure 2.1 Major microvascular and macrovascular difficulties related with diabetesmellites (Ahmed *et al.* 2020)

The development of diabetes is linked to a number of different pathogenetic processes. Aside from those that induce insulin insufficiency by killing off the pancreas' beta cells, there are others that cause insulin resistance.

Insulin's poor impact on target tissues is a consequence of insulin sensitivity or insulin deficiency, which causes metabolic abnormalities in carbohydrates, fats, and proteins.

Diabetic symptoms, on the other hand, include thirst, polyuria, impaired vision, and weight loss. Often, the signs and symptoms are minimal or nonexistent.

2.1.1 History

Diabetes has been recognized as a dangerous and frequently deadly condition during the last two thousand years. A Greek physician named the condition "diabetes" after "siphon" because of its destructive tendency in the first century AD (www.diabeteshealth.com). Aretaeus and other ancient physicians were able to identify the symptoms of diabetes, but they were unable to treat it successfully. They should've known better than to put themselves in this situation.

Thomas Willis, a London physician of the 17th century, used urine collection to diagnose diabetes in his patients. Diagnosis would be made if it had a sweet taste, or "honey" diabetes, if it was present. For the most of the twentieth century, this method of checking blood sugar levels remained virtually unchanged.

Prior to the discovery of insulin, there was nothing that could be done for diabetics. They lived longer on low-calorie diets, but they also became weaker and more ravenous. Diabetic patients who were on the verge of death were treated with insulin in Canada in 1921, and their blood sugar levels were restored. Health-care developments since that time have allowed people with impairments to enjoy longer and better quality of life.

Diabetic type I and type II were initially found in the 1950s, respectively (type II). Diabetes was referred to as "the weird illness" by Aretaeus more than 2,000 years ago.

Since then, diabetes has been called "the weird illness" by ancient Greek physician Aretaeus. The search for a treatment has been a long and arduous one, requiring the combined knowledge of many generations of physicians and scientists. Due to a wealth

of information, a small Canadian laboratory found insulin. Since then, medical improvements have been made.

The search for a cure for diabetes continues today, in the twenty-first century. A significant breakthrough like insulin may be on the way, but researchers may have to settle with the slow grind of progress for now since the future is unknown (Lakhtakia 2013).

2.1.2 Pathophysiology

An in-depth knowledge of glucose metabolism and insulin action is required to fully grasp diabetes pathogenesis. Carbohydrates are converted to glucose molecules in the stomach after absorption. When glucose enters the body, it raises blood glucose levels. insulin is released by beta cells in the pancreas when blood sugar levels increase. Glucose enters most cells via insulin. Insulin interacts to certain receptors on cells in order to enhance the entrance of glucose into cells.

Higher quantities of pancreatic insulin are produced, which is then used up by cells in the body to reduce blood sugar levels Insulin production decreases as blood glucose levels fall.

It's possible that insulin release and synthesis will modify blood glucose dynamics in unwell persons. When insulin synthesis is low, glucose cannot enter cells, resulting in hyperglycemia. The same thing happens if the pancreas produces insulin but it is not properly used by target cells. Glucose enters tissue cells in large numbers and is expelled from the circulation when insulin levels are increased, resulting in dangerously low levels of blood glucose (hypoglycemia).

Hormones might have an impact on blood sugar levels. Insulin is the only other hormone capable of lowering blood glucose levels. Glucagon and catecholamines, as

well as growth hormone, thyroid hormone, and glucocorticoids, may boost blood glucose levels (Sima and Glogauer 2013).

2.1.3 Complication

In the United States, diabetes complications are the leading cause of disability, poor quality of life, and death.. Many different biological systems might be affected by diabetic complications, and each person's symptoms will differ. It is more common for people with diabetes to acquire a broad variety of major health issues. Men might suffer from erectile dysfunction, low testosterone levels, mental disorders such as melancholy and worry, and stress as a result. When it comes to diabetes, women have it the worst. A woman's chance of developing gestational diabetes rises if she already has diabetes when she becomes pregnant. The following is a list of diabetes complications *(Hahl et al.* 2002).

- Acute complication

Complicating factors include, but are not limited to:

Ketoacidosis: A glucose deficit inside fat cells makes them hungry, and if food intake is inadequate or insulin doses are excessive, fat cells begin to use fat as a source of energy, even while blood glucose levels are high. Unsaturated fats are converted into ketone groups by the liver cells. When glucose levels are low, high concentrations of ketones may make the pee acidic and produce a fruity odor in the urine; this scenario can proceed to a coma.

Hyperosmolar: Nonketotic Syndrome of Hyperglycemia and Hyperglycemia Hyperglycemic hyperosmolar nonketotic syndrome (HHNS) is a medical term for this condition. A person may become severely dehydrated as a consequence of increased urine induced by high blood glucose levels. Blood sugar levels that are too high might lead to mental disorientation.

- Chronic complication

Chronic complications, which include the following, are among the most well-known side effects of long-term diabetes:

In the latter stages of the illness, those with impaired blood glucose levels of at least one of these consequences require foot care as well. Individuals with poor blood glucose levels in the latter stages of the illness are more likely to have microvascular problems, which may lead to retinopathy, nephropathy, and nerve cell damage (Nathan 2014).

Venous complication; glycation, inflammation, fat buildup and other variables are the causes of venous difficulties in the veins. Significant vascular damage may lead to a variety of side effects, including myocardial necrosis, rheumatoid arthritis, stroke, osteoporosis, and degenerative joint disease (Singh *et al.* 2014).

The most concerning of these complications is cardiac dead tissue; nonetheless, it seems that blood glucose management does not totally eradicate or postpone the development of vascular issues. As seen in (Figure 2.2), extra clinical administration is required.

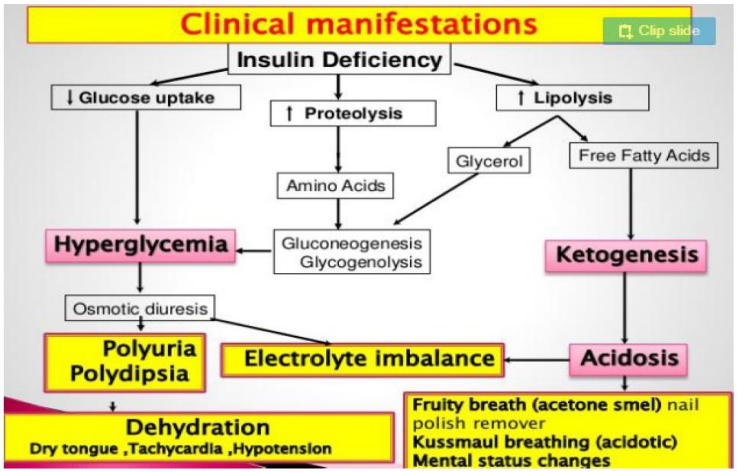

Figure 2.2 Mechanism of complication of diabetes (Ahmed *et al.* 2020)

As a consequence of diabetes, vascular disease occurs in various tissues, including the retina, peripheral limbs, and other parts of the body. Diabetic neuropathy, blindness, and renal failure are some of the most common complications of diabetes. An further consequence of diabetes is an increase in the risk of atherosclerosis, which may reduce the amount of blood flowing to the heart, brain, and legs (Lebowitz *et al.* 2017).

Myocardial infarction, amputation, and stroke are just a few of the extra problems that diabetics face as a consequence of their condition, and clinical testing show that diabetes and vascular disease are strongly linked (Singh *et al.* 2014).

Several vascular diseases have comparable physiological properties as a result of diabetes. In the early stages of diabetes, cell hyperglycemia leads to blood stream dysfunction and increased vascular porousness. (Serreze *et al.* 2000) The following are some of the repercussions of this:

- For example, the activity of nitric oxide decreases when the diameter of the blood vessels expands.
- The enhanced constriction action of endothelin-1 and angiotensin II, for example.
- An increasing amount of research is focused on developing new transport factors, such as vascular endothelial growth factor (VEGF).
- There is an increase in vascular porousness due to departures from the norm in the extracellular framework in terms of both quantitative and qualitative metrics.
- Cell death in microbes is caused by apoptosis.
- Each extracellular matrix causes progressive hair blockage as a consequence of growth factors like TGF development and the deposition of other plasma proteins.

Other complications include peripheral nerve damage, hypertrophic glomerular mesothelioma, glomerular nephritis, and retinal hypoxia, which are all caused by these changes.

Glucose intolerance and insulin resistance in diabetic arteries cause endothelial dysfunction, which results in decreased endothelial nitric oxide production for arteriosclerosis, an increase in smooth vascular cell proliferation, and the activation of RAZ RMAP via the protein kinase pathway by Plasmogen-1 (PAI-1). The production of PAI-1 and nitric oxide in blood vessel endothelial cells is reduced as blood sugar levels rise. This is based on the findings of Koya and King Hyperglycemia and insulin resistance both contribute to increased levels of cholesterol-containing B-cholesterol particles in the blood by lowering the outflow of heparan sulfate proteoglycan perlecan from hepatocytes. Atherosclerosis and the risk factors for atherosclerosis were shown to be associated with glucose tolerance throughout a greater range than is normal for diabetes, according to affiliations. Arteriosclerosis may be better predicted by postpartum hypoglycemia rather than plasma fasting glucose or hemoglobin A1 (Temelkova-Kurktschiev *et al.* 2000).

Diabetes Mellitus classification

- Type-1 diabetes mellitus (T1DM)

Even while Type I diabetes accounts for just 5 to 10 percent of all cases, its worldwide prevalence is growing at an alarming pace and has long-term consequences. Ketoacidosis and coma may develop if the pancreas' beta cells begin to deteriorate, and insulin is "essential for life" to prevent this from happening in diabetics with type I diabetes. In 2017, (Brison 2017). This kind of diabetes is marked by acute symptoms such as dry mouth and recurrent urinary tract infections as well as weight loss and delayed wound healing.

A person with type 1 diabetes must constantly check their blood sugar levels, administer insulin, plan meals, and test for diabetes-related complications. An integrated team of health care professionals is the best way to deal with this The bulk of Type I diabetes-related morbidity and mortality is caused by microvascular and macrovascular disorders (Rosenblat *et al.* 2014). Diabetes is brought on by antigen-presenting cells presenting -cell peptides (APCs). Activated T cells in the pancreatic lymph node make

autoantibodies against -cell proteins, which may be identified in the circulation and utilized as a diagnostic for type 1 diabetes (Figure 2.3).

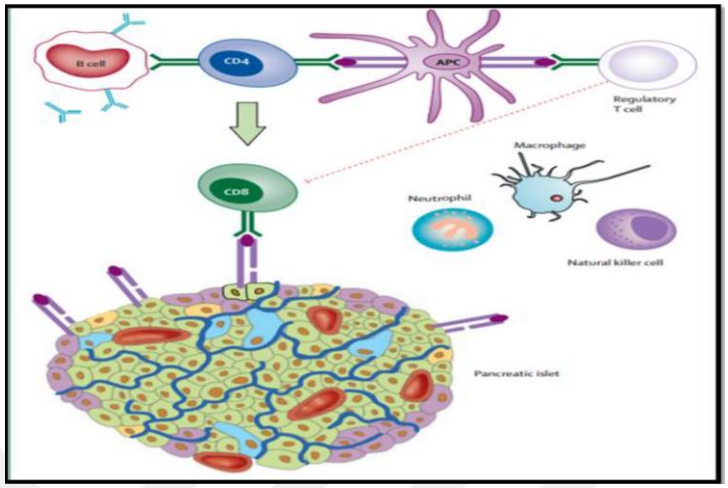

Figure 2.3 The pancreatic -cell interacts with the innate and adaptive immune systems (Dreyer 2019)

- Type- II diabetes mellitus

Type II diabetes is the most common kind. Millions of individuals throughout the globe have been diagnosed with type II diabetes, yet there are still many more who are not. Diabetics are more prone to suffer from heart attacks and strokes if they are not recognized or treated properly. As a result of nerve and blood vessel damage and renal failure, they are more likely to become blind, lose their limbs, or need dialysis or a kidney transplant (Pasinetti *et al.* 2011).

Those with "prediabetes" have blood glucose levels that are higher than normal but not high enough to be diagnosed with Type II diabetes prior to onset. According to recent research, pre-diabetes may already be causing long-term harm to the heart and circulatory system. The peptides are encased in cells.

Diabetics with type II diabetes are by far the most prevalent. Type II diabetes has been identified in millions of people throughout the world, and that figure is only going to

climb in the coming years. Without adequate treatment, diabetics are more likely to have heart attacks and strokes. Blindness is more common among diabetics because they either don't have enough insulin or their cells don't react to it. The body uses insulin to help transform glucose into energy when blood sugar levels are too high. Cells use glucose as their primary source of energy from all the sugars and carbohydrates they absorb. Insulin, a hormone responsible for controlling blood sugar levels, transports glucose into cells. Diabetic problems may result from a build-up in the blood sugar rather than being absorbed by the cells. If you have type 2 diabetes, you're more likely to have insulin resistance (IR). A rise in blood glucose levels is accompanied by an increase in insulin production as a result of insulin's inability to function properly in type 2 diabetics with insulin resistance (IR). Anti-hyperglycemic medications are used for treatment (Michael *et al.* 2019).

- Pregnancy and diabetes (gestational diabetes)

During a woman's pregnancy, she may be diagnosed with gestational diabetes for the first time. Overweight women, those with a history of gestational diabetes, or those with a substantial family history of the condition are more likely to develop gestational diabetes. Estrogen diabetes in pregnant women may have major effects for the unborn child if left untreated. For the remainder of their lives, both the mother and the infant are at a higher risk of acquiring Type II diabetes (Pathirana *et al.* 2021).

- Other kinds of diabetes

Diabetes may be caused by a variety of factors, including genetics, exocrine pancreas illness, endocrine disorders, and chemical or drug usage. Among these are newborn diabetes, maturity-onset diabetes of the young (MODY), pancreatitis, cystic fibrosis, and glucocorticoid use (Care and Suppl 2020).

2.1.4 Type 2 diabetes

When it comes to Type 2 Diabetes, it has long been known as NIDDM or adult-onset diabetes with insulin resistance that may evolve to full-blown insulin resistance. However, it has recently been highlighted as a serious problem in T2DM because of the decline in cell function (Saisho 2014). Diabetes mellitus type 2 (T2DM) and its complications, such as obesity and insulin resistance, are common in children (Ghosh *et al.* 2021)

- Diagnosis of type 2 diabetes

When a patient exhibits the classic symptoms of hyperglycemia and has a random blood glucose level of 200 mg/dL or greater, it is straightforward to diagnose diabetes mellitus. The following are examples of diagnostic procedures:

A fasting plasma glucose (FPG) test is performed on people who haven't eaten for at least eight hours. This test may identify people with diabetes or a pre-diabetic state. Glucose-containing beverages are consumed two hours after fasting to monitor blood glucose levels, which is known as an oral glucose tolerance test (OGTT). You may take this test to find out whether you have diabetes or if you are at risk for it. In order to identify diabetes, the FPG test is the go-to method because to its ease of use and inexpensive cost. However, it is likely that the OGTT may overlook certain cases of diabetes or prediabetes. First thing in the morning is the best time to take the FPG test. It's easy to use and maintain. This kind of blood glucose test does not consider when the person being tested last ate and is also known as a casual plasma glucose test. Diagnosis of diabetes can only be made with this test and a review of symptoms, not prediabetes. Regardless of the first results, a second test should be performed to confirm a diagnosis of diabetes (Merz *et al.* 2002) A fasting plasma glucose of 7.0mmol/l (126 mg/dl) or 2-hour plasma glucose of 11.1mmol/l (200 mg/dl) should be maintained as the current WHO diagnostic criteria for diabetes (Brison 2017). As shown in Table 2.1.

Table 2.1 Guidelines of diagnosing diabetes mellitus by ADA

STATE	CRITERIA	VALUE
Normal State	Oral glucose tolerance	<7.81 mmol/L (<140) mg/dl
	Fasting blood glucose	<5.61 mmol/L (<100) mg/dl
Diabetes Mellitus	Random blood glucose	≥11.1 mmol/L (≥ 200) mg/dl
	Oral glucose tolerance	≥11.1 mmol/L (≥ 200) mg/dl
	Fasting blood glucose	≥11.1 mmol/L (≥ 126) mg/dl
	Hb A1C	≥6.5%

- Epidemiology of T2DM

In all regions and populations, including rural areas in middle- and low-income nations, diabetes is an epidemic. Diabetes is on the rise across the world as a result of a changing diet and increasing obesity (Carrillo-Larco *et al.* 2019). According to the 9th edition of the IDF Diabetes Atlas, issued in 2019, there are 463 million individuals with diabetes in the United States. Children and adolescents under the age of 20 now have Type 1 Diabetes, an increase of 1.1 million since 2010. (T1DM) (Sinclair *et al.* 2020)

According to WHO (World Health Organization) forecasts, diabetes will be the seventh greatest cause of death in the world by 2030. Up to 438 million individuals throughout the world are expected to be affected by diabetes by 2025, based on current trends. As estimated in 2010, 438 million people would have diabetes by 2025; nevertheless, this figure has already exceeded 25 million. In 2010, this was predicted. International Diabetes Federation estimates that by 2030, 578 million people will have diabetes, and 700 million by 2045. (IDF).

Despite greater attention to T2DM risk factors and evidence for effective preventative strategies, the disease's prevalence and incidence continue to rise across the globe. Detection via screening programs and the availability of safe and effective medications reduces mortality and morbidity, as well as delays and avoids effects (Chatterjee *et al.* 2017).

- T2DM epidemiology in Iraq

More than 40.2 million people will live in Iraq in 2020; it's a multi-ethnic nation. There are 19,914,400 million individuals in the United States, of whom 1,505,000 million have diabetes. 7.6 percent of individuals develop diabetes as a consequence (Abusaib *et al.* 2020). (Figure 2.4) illustrates this.

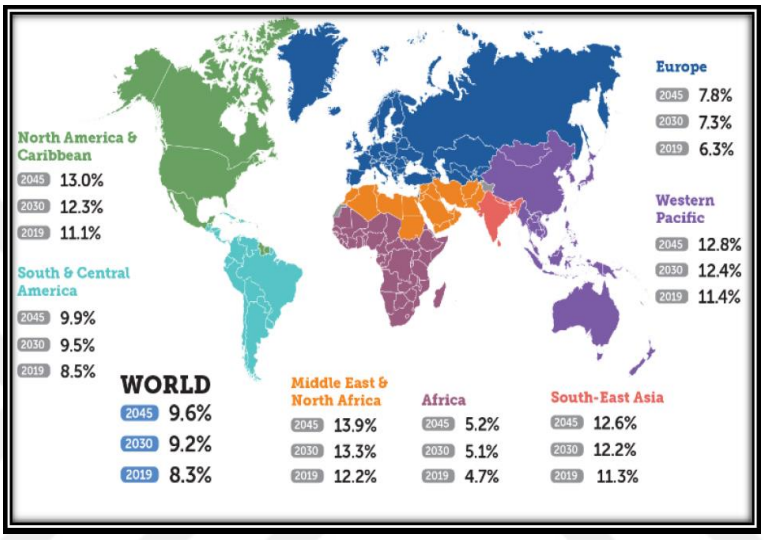

Figure 2.4 Incidence of diabetes in adults aged 20 to 79 in International Diabetes Federation IDF regions in 2019, 2030, and 2045 (Atlas 1955) International Diabetes Federation

- Risk factors

Obesity and inactivity are two preventable risk factors for diabetes. Race and heredity are two more important risk factors that cannot be changed.

The most major risk factor for acquiring type I diabetes is a genetic predisposition. People who have diabetes in their family are more likely to get their hands on the condition. Diabetic first-degree relatives should be tested for the condition, according to the American Diabetes Association (Care 2019). When it comes to diagnosing type I diabetes, all it takes is a simple blood test.

Type I diabetes may also be caused by injury or sickness to the pancreas, which is unable to generate insulin. Type I diabetes may be caused by a variety of unusual infections and disorders. Some risk factors for Type II diabetes include being overweight or obese, not eating healthily and exercising seldom, being older, having a family history of diabetes or specific genes, and being of a particular race or ethnicity. Gene 14 polymorphisms and a higher incidence of type 2 diabetes have been linked. Genes related with insulin sensitivity, insulin synthesis, and obesity have been linked to these genes (Diabetes 2013).

Race and ethnicity all have a role in the increased diabetes rates among various ethnic groups, including African Americans, Mexican-Americans, Native Americans, and Asian-Americans. Diabetes and heart disease are more prevalent among the demographics indicated above. Among these demographics, hypertension, obesity, and diabetes are more prevalent. African Americans are more likely to develop type II diabetes than any other ethnic group (Clifford *et al.* 2005).

In addition to a family history of diabetes and ethnicity, there are many more risk factors. Rapid technological advancements and urbanization have led to an increase in the prevalence of diabetes in many individuals. Those who have a history of substance abuse are more prone to acquire Type II diabetes at a younger age.

Those who have a history of drug misuse are more likely to develop diabetes at a younger age. It's also been connected to drug usage in studies done in the US, according to those findings (Lee *et al.* 2002). Cannabis and cocaine are the most often utilized illicit narcotics among diabetic primary care patients, but they aren't the only substances they use (amphetamines, heroin, hallucinogens, and nonmedical inhalants).

- Prevention and delay of type II diabetes

Those with "prediabetes" have blood glucose levels that are higher than normal but not high enough to be diagnosed with Type II diabetes prior to onset. Prediabetes is both

dangerous and treatable as a medical condition. By altering their diet and increasing their level of physical activity, persons with pre-diabetes may avoid acquiring Type II diabetes. Their blood glucose levels may be able to return to normal or even lower than previously.

On the other hand, lifestyle changes are essential. Maintaining a healthy weight and increasing your physical activity will help you live longer and reduce your chance of developing diabetes. In the Diabetes Prevention Program's results, weight loss might be delayed or prevented by mild dietary modifications and physical exercise (DPP). As a major clinical research trial, the Diabetes Prevention Program attempted to explore whether moderate weight reduction and increased physical activity, as well as therapy with the oral diabetes medicine metformin (Glucophage), might prevent or postpone the development of type 2 diabetes in study participants. Glucophage (Haus *et al.* 2010)**.**

2.1.5 Mechanism for regulation of blood glucose

2 percent of pancreatic islets contain Langerhans cells. Glucagon and insulin are secreted by cells in this organ. Insulin is released by cells when blood glucose levels are high. Cells, on the other hand, create glucagon when blood glucose levels are low (Da Silva Xavier 2018). For regulating blood glucose levels, insulin and glucagon are essential hormones. For example, (Van Den Berghe 2004) Insulin is released by the pancreas' islet cells as a result of a spike in blood sugar levels after a meal, which breaks down carbs into glucose. There are several reasons why a diabetic patient's blood sugar levels are not maintained at a proper level, and these causes vary from one kind of diabetes to the next. Due to a lack of insulin synthesis, type 1 diabetes (T1D) is caused by the death of cells. Whereas Type 2 Diabetes (T2D) may be caused by either a failure of the pancreas to generate or react to insulin, or by a combination of both. For example, see (Figure 2.5) (Ye *et al.* 2020).

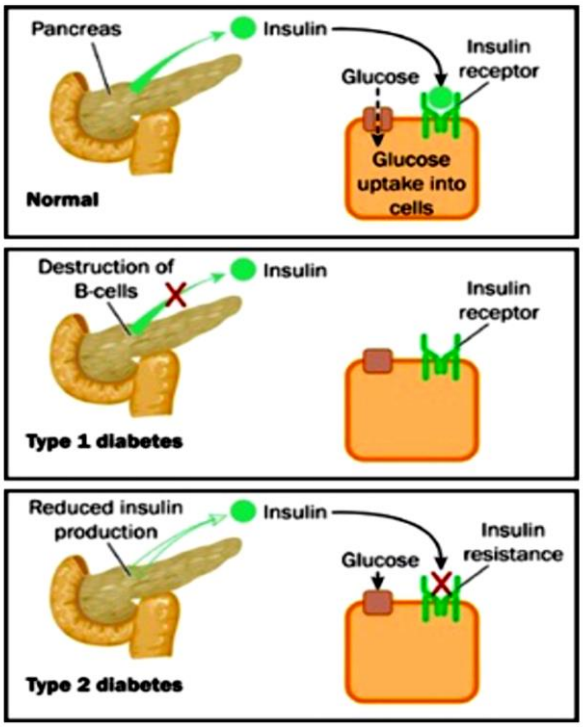

Figure 2.5 An overview of T1D and T2D by author (Ye *et al.* 2020)

Insulin is released by the pancreas in a physiologically normal condition (Normal) and instructs cells to take up glucose from the circulation. The pancreas produces very little or no insulin in people with type 1 diabetes (T1D). T2D occurs when the pancreas fails to generate enough insulin to maintain a normal glucose level, or when the cells reject the insulin's actions.

Adipocyte size expansion rather than an increase in the number of adipocytes is the source of obesity-related increases in adipose tissue mass, according to many hypotheses on how adipose tissue develops insulin resistance. Insulin's anti-lipolytic function is less effective in bigger adipocytes (Arafat *et al.* 2020).

Higher amounts of circulating NEFAs help in the accumulation of NEFAs in muscle and hepatocyte cells. Diarylglycerol (DAG) and ceramide (ceramide) buildup in the cell's cytoplasm (Skyler *et al.* 2017) may activate the serine kinase cascade, causing insulin signaling problems as well as damage to the glucose transporters GLUT (Figure 2.6) (Ieronymaki *et al.* 2019).

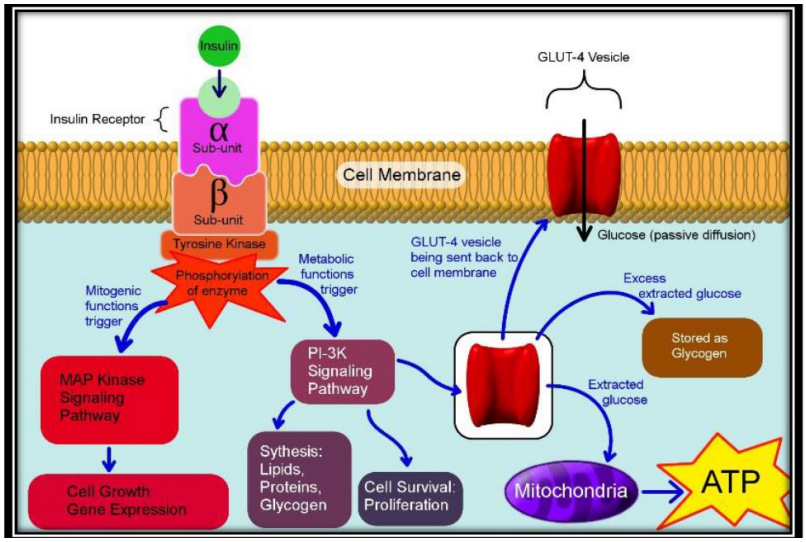

Figure 2.6 Insulin signaling pathway (Fu *et al.* 2012)

2.1.6 Treatment aapproaches methods that are both old and new

By 2021, diabetes will be the sixth leading cause of mortality in the United States. As a result of the $174 billion economic impact of diabetes-related complications in 2007, governments throughout the globe place a high premium on its treatment and control (Dall *et al.* 2007).

Insulin therapy, on the other hand, is only able to alleviate the symptoms for a short period of time in patients with this long-term, degenerative illness. Despite the newest pharmacotherapies, patients continue to suffer macro- and microvascular issues. Non-trauma lower-limb amputations are up to 60% more likely in people with diabetes than in those without the disease, and heart attacks, strokes, renal failure, and blindness are all associated to diabetes. In-depth and responsible study into alternative treatment options for various kinds of this illness is required. After gastrointestinal bypass surgery, it is possible to restore control of diabetes without insulin treatment (Caspersen *et al.* 2012).

- Insulin treatment

Diabetes therapy involves the use of insulin, a hormone that maintains healthy blood sugar levels in diabetics (glucose). Because of its closeness to human insulin, it may be manufactured from pig (porcine) or beef (no longer accessible in the United States). It can also be genetically modified. Exogenous insulin (typically delivered subcutaneously) is required for the survival of a diabetic patient with type I diabetes mellitus. insulin resistance, inadequate insulin production, or the combination of these are the hallmarks of type 2 diabetes. Insulin therapy may be necessary for certain Type 2 diabetes patients if other therapies are unable to control blood glucose levels adequately.

Insulin is used to treat diabetes in a number of ways. When it comes to how soon they start working, when they reach their "peak" activity (which is when blood insulin concentrations are at their highest), and how long their effects last, they are categorized (Buysschaert *et al.* 2000).

- Insulin that works quickly and lasts for many hours is known as rapid-acting insulin.
- The term "regular" or "short" insulin refers to insulin that takes 30 minutes to kick in and lasts 3 to 6 hours.
- Insulin with an intermediate half-life takes 2 to 4 hours to kick action, but it may last up to 18 hours.
- Insulin that works for up to 24 hours but takes 6 to 10 hours to enter the circulation is known as long-acting insulin.

For the treatment of diabetes, insulin may be injected subcutaneously (under the skin) or intravenously (into the bloodstream) (intravenously). All patients with type I diabetes and the vast majority of those with type II diabetes are treated with subcutaneous insulin injections. Insulin may be administered using a needle and syringe, a cartridge system, or a prefilled pen. Additionally, glucose-monitoring equipment, such as insulin pumps, may be found on the market (Gallen *et al.* 2004).

Insulin injections either intravenously or subcutaneously may be used to treat diabetes (intravenously). All patients with type I diabetes and the vast majority of those with type II diabetes are treated with subcutaneous insulin injections. Insulin injections may be performed using a needle and syringe, a cartridge system, or a prefilled pen device. Purchase of insulin pumps is also possible.

- Oral diabetes treatment

The progression of T2D may be slowed down in the early stages by maintaining a nutritious diet, regular exercise, and other aspects of a well-managed lifestyle. Most people with type 2 diabetes will eventually need medication, including oral hypoglycemic agents (OHA) and insulin. There are two types of OHA agents based on their roles. By improving insulin sensitivity, Metformin and Thiazolidinediones, for example, may help treat type 2 diabetes. By urging the pancreas to secrete more insulin, Sulfonylureas and Meglitinides, for example, are effective (Tsang 2012). DPP-4 and GLP-4 inhibitors have been created in recent years and used as T2D medicines, respectively, for the management of the disease.

In contrast, effective therapies for T2D include metformin, sulfonylureas, thiazolidinediones, pioglitazones, and glycosidase inhibitors, which may be used alone or in combination. Anti-diabetic drugs, on the other hand, have several adverse effects, including gastrointestinal toxicities and weight gain as well as swelling and hypoglycemia. Consequently, novel therapeutic targets and agents will be needed to treat T2D. Anti-diabetic medicine process is shown in (Figure 2.7). A variety of anti-diabetic pharmaceutical classes are shown in (Table 2.2) for the treatment of type 2 diabetes.

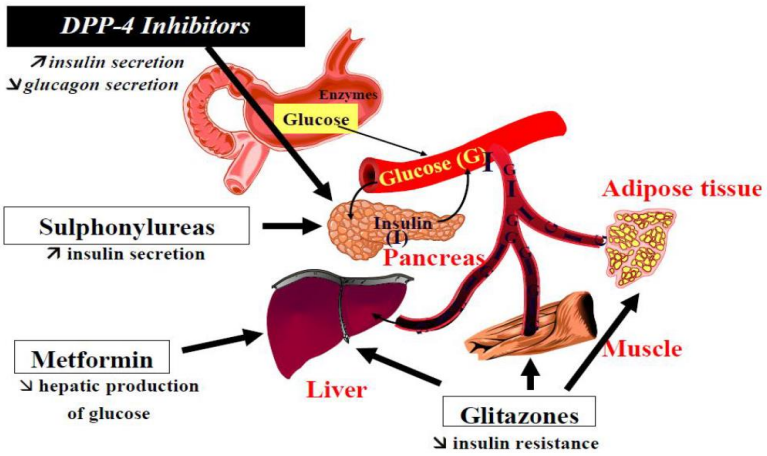

Figure 2.7 Mechanism of anti-diabetic drugs

Table 2.2 Depicted various anti-diabetic medication classes for the treatment of T2D (Ghate and V. Jain 2013)

NAME	COMPANY	DEVELOPMENT STAGE
Sitaglaptin (Januvia)	Mearck& Co	Approved in 2006
Vlidaglaptin (Galvus)	Novartis	Approved in 2008
Saxaglaptin (Onglyza)	Bristol-Mayers-Squibb	Approved in 2009
Linaglaptin (Tradjenta)	BI and Eli Lilly and Company	Approved in 2011
Aloglaptin (SRY-322)	Takeda	Approved in 2013
Dutoglaptin (PHX1149)	Phenomix	Dropped from the market
Omarigliptin (MK-3102)	Mearck	Approved in Japan 2015
PSN9301	OSI Pharmaceuticals	Phase 2
RI38	Roche	Phase 2
GRC8200	Glenmark pharmaceuticals	Phase 1
SSR162369	Sanofi-Aventis	Phase 1
TS-021	Taisho pharmaceuticals	Phase 1
ALS 2-0426	Alantos pharmaceuticals	Phase 1
SYR-619	Takeda	Phase 1

2.1.7 Metformin

Metformin, a guanidine derivative derived from the French Lilac Galega officinalis plant, has been used to treat diabetes since the 1950s as shown in (Figure 2.8). Jean Sterne dubbed this naturally produced medicine 'Glucophage,' which literally means 'glucose eater,' because it effectively lowers blood glucose levels (Bailey and Day 2004), Because of its low toxicity, it has become the most preferred first-line oral treatment for diabetes. Metformin has been shown to reduce all-cause mortality in diabetic individuals when compared to those who do not take the medicine (Roussel *et*

al. 2010) demonstrating its usefulness. Three forms of biguanides, metformin (MF), phenformin, and buformin, were used in the early therapy regimens. Despite the fact that the effective dose of phenformin was much lower than that of MF, both buformin and phenformin have been linked to lactic acidosis, and their usage has been discontinued in most countries. As a result, MF is the only biguanide used in clinical practice, and it is the current standard of care for T2DM (Kilmer 2010).

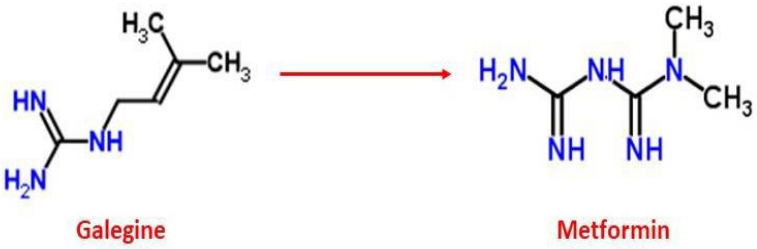

Figure 2.8 Metformin is a Galegine-derived drug (Prepared by author)

- Metformin pharmacokinetics

Metformin is considered to be a positively charged molecule due to its guanidine-like structure (Figure 2.8). In biological fluids, the commonly supplied form of MF, MF-hydrochloride, appears as a sort of free base (Scheen 1996). MF is slowly absorbed by the small intestine after intake, with a bioavailability of 50-60% .Several organic cation transporters (OCTs) enhance its uptake despite its hydrophilic character, which precludes diffusion through cellular membranes (Todd and Florez 2014), Because MF is not entirely absorbed by the intestine, is not metabolized by the liver, and is excreted through the urine system, it has a direct effect on the kidneys (Figure 2.9).

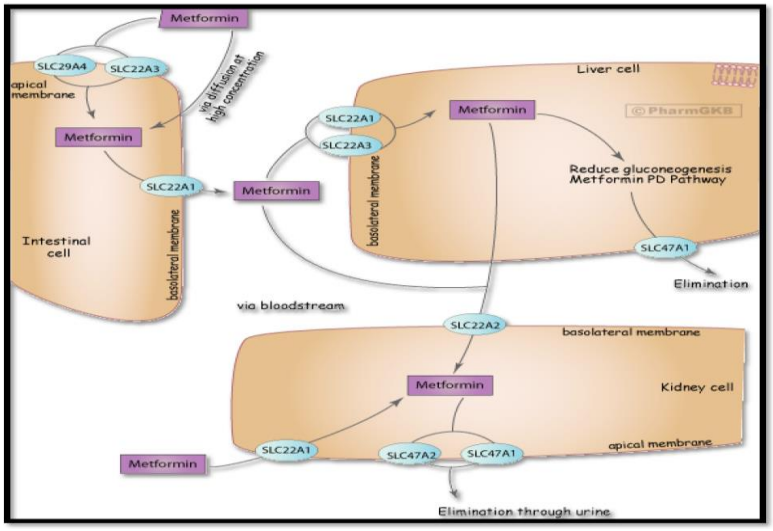

Figure 2.9 Metformin transport pharmacokinetics (Gong *et al.* 2012)

2.1.8 Dipeptidyl-peptidase (DPP4)

This 110-kDa glycoprotein, also known as CD26, is extensively expressed in the body. As soon as it was discovered, it was identified by Hopsu Havu and Glenner (Hopsu-Havu and Glenner 1966). An enzyme known as sheddin cleaves the transmembrane protein, DPP4, from its membrane and injects it into the circulation In recent years, the scientific and medical sectors have been more interested in DPP4 as a potential therapy for type 2 diabetes because of the availability of DPP4 inhibitors. Known as gliptins, they increase incretin levels and so extend insulin's post-prandial effect.

Extracellular matrix proteins (ECMPs) and the enzyme DPP4 are among the many peptides that DPP4 may bind to (Cordero *et al.* 2009, Mulvihill and Drucker 2014a). A serine protease, DPP4 may cleave a broad variety of substrates, further complicating its role in the body. Due to its involvement in signaling and activation of immune cells, DPP4 has been associated to a wide range of diseases (Huecas and Andreu 2004).

- Biology of DPP4

To understand how DPP4 functions, we'll look at the domain structure. As a member of the serine peptidase family S9B, DPP4 (EC3.4.14.5) joins other enzymes including FAP, the resident cytoplasmic proteins DPP8 and DPP9, as well as the non-enzymatic DPP6 and the enzyme-inactive DPP10. DPP4 is a transmembrane protein of the type II kind. All of these proteins have the same / hydrolase fold. In (Mulvihill et al. 2014b). In the transmembrane domain (TMD) (7–28), the flexible stalk segment is split into four sections: a heavily glycosylated area, a cysteine-rich area, and a catalytic area. In addition, there are four portions to the extracellular domain (40–766) (Figure 2.10).

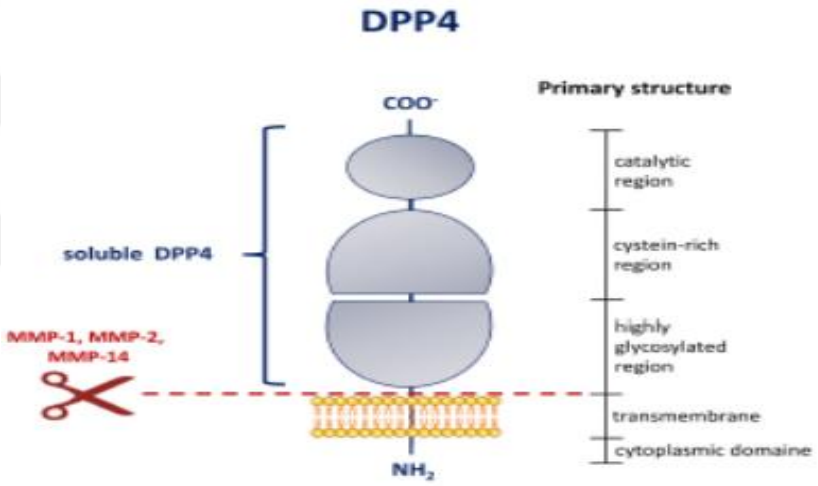

Figure 2.10 Domain structure of DPP4 (Lambeir *et al.* 2003)

In order to be found in the endoplasmic reticulum and begin its journey across the membrane, DPP4 contains a signal peptide that is required. The signal peptide, in contrast to other released proteins, is not broken off but rather functions as an anchor in the membrane. To produce a circulating version of DPP4 (sDPP4) that lacks its cytoplasmic domain and transmembrane area, adipocytes and smooth muscle cells are broken down by matrix metalloprotases (MMPs) (Huecas and Andreu 2004).

In oligopeptides, dipeptidyl peptidase -4/CD26 removes the N-terminal dipeptides preferentially by eliminating the hydrophobic N-terminal domain, transmembrane region, and catalytic triad located at the protein C-terminus (Engel *et al.* 2003).

In the cytoplasm, DPP-4 contains six amino acids (residues 1-6), 22 amino acids (residues 7-28), 295 amino acids (residue 29-323), and 215 amino acids (residues 552-776) are found in the transmembrane region of each monomer (Medrano *et al.* 1998). Catalytic domain (hydrolase) and eight-bladed propeller (N-terminal domain) are positioned on opposite ends of the molecule. Thr251 and Ala717 and Thr736 form the dimer interface of Ile237 and Thr251. The inhibitors' carbonyl carbons from the catalytic triad (Ito et al. 2000) form adducts with the carbaminic acid of Ser630 and His740 (Figure 2.11) depicts the -propeller domain, the /-hydrolase domain, and the ligand binding site.

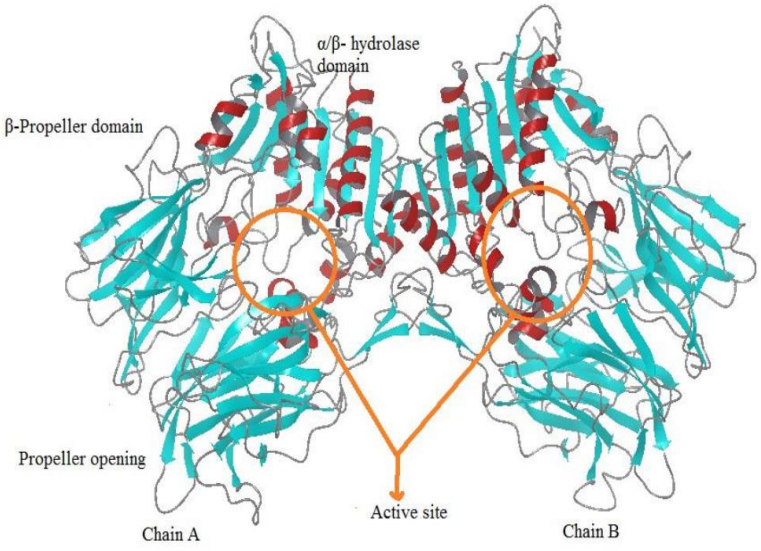

Figure 2.11 DPP-4 enzyme secondary structure and binding location (orange circle) (Ghate and Jain 2013)

At the point where the propeller and hydrolase domains meet, DPP-4's active site is located. As with DPP8 and DPP9, the EE-Helix and S1 pocket residues (Tyr548, Ser630, Tyr631, Val656, Trp659, Asp663, Tyr666, Asn710, Val711, and His741) are placed into the first sheet of the fourth propeller blade. Propeller blades 5 and 6 are

connected to the DPP-4 EE-Helix via an S2-loop (Agr358 and Phe357) (Figure 2.12) (Goettig *et al.* 2002).

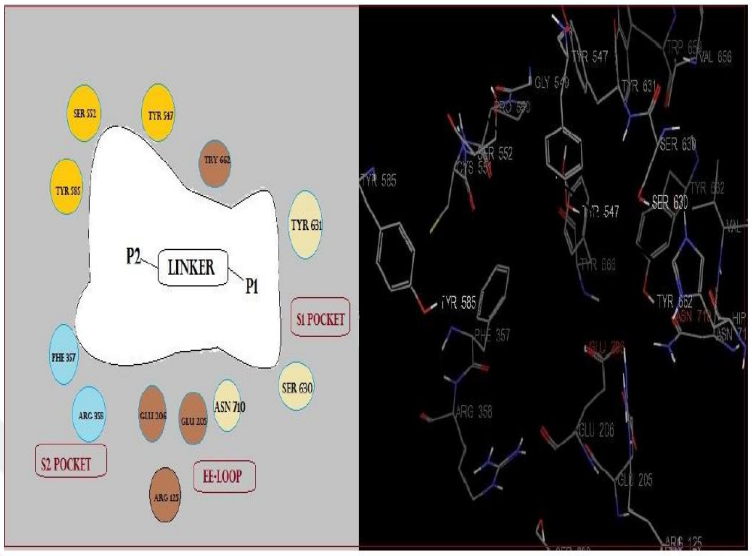

Figure 2.12 S1 pocket, S2 pocket, and EE-Loop are all situated in the DPP-4 active site at the interface of the /-hydrolase domain (Ghate and Jain 2013)

Because they form a covalent link with the catalytic residue Ser630, some commonly used DPP-4 inhibitors have 5-membered heterocyclic rings that mimic proline (Kang *et al.* 2007). Selective binding of the DPP-4 enzyme to substrates containing proline at the P1-position has been shown. However, nitriles may also be boronic acid or diphenylphosphonate, which produce bonding in certain situations. Since the free amino group of P2-amino acid interacts with cyanopyrrolidines with a nitrile functional group, scientists at Zeria Pharmaceuticals found in 1994 that the imidate formed by these cyanopyrrolidines with nitrile functional groups is unstable, Other dipeptidyl peptidase inhibitors, such as those lacking the electrophilic group, have been found, but their toxicity has been shown by their affinity for DPP-2, DPP-8, and DPP-9 (Janardhan 2011).

- DPP4's physiological importance

In addition to its role in glucose homeostasis, DPP4 has a wide range of other functions, including the control of the cardiovascular, pain, immunological, cancer development, nutrition, bone marrow mobilization, and cell adhesion mechanisms. Inflammatory modulation of TGF-1-dependent inflammatory responses by CD26 enhances the proliferation and generation of Th1-dependent cytokines that communicate antigenic stimulation (Orita *et al.* 2009).

Leucocyte trafficking, lymphocyte maturation, and lymphoid tissue development are all regulated by chemokines, which are substrates of DPP4 (Rollinger *et al.* 2009). (Figure 2.13) emphasized the importance of DPP4's physiological role. The effect of DPP4 inhibitors on T-cell proliferation, cytokine production.

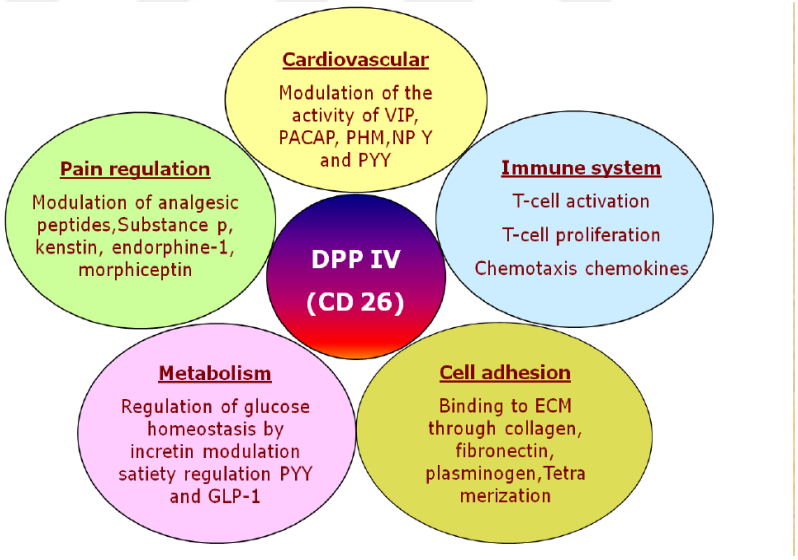

Figure 2.13 Physiological importance of DPP4 update (Rollinger *et al.* 2009)

- DPP4 inhibitors

Overweight and inactivity are risk factors for type 2 diabetes (T2D), which is more common in urban settings. According to the World Health Organization, there are over 170 million diabetes worldwide (WHO). Each year, diabetes medications bring in more than $12 billion in revenue. Between 2000 and 2030, the predicted populations of diabetes in India, China, and the United States are expected to be the greatest, second, and third largest, respectively (Ghate and Jain 2013). Selective DPP4 inhibitors have been considered potential medicines for the treatment of type 2 diabetes based on data from preclinical and clinical research. Type 2 diabetes drugs based on preclinical and clinical research have been identified as selective DPP4 inhibitors.

Long-term effectiveness and tolerance of current T2D therapy are limited, as are undesired side effects include weight gain, hypoglycemia and stomach discomfort. As a consequence, novel T2D treatment strategies and medications are required to address these issues. As a result, the pharmaceutical industry has lately shown interest in the invention of DPP4 inhibitors to treat Type 2 Diabetes (T2D). Reversible inhibitors pyrrolidines, non-peptide heterocyclic inhibitor xanthines, and aminomethyl pyrimidines have been filed by many pharmaceutical firms. These chemical families comprise amino acid amide, carbocycle, and alkylamine derivatives of aminomethyl pyrimidines. Today's drug discovery is hampered by the difficulty of creating potent, selective DPP4 inhibitors that do not inhibit DPP8, DPP9, QPP, or FAP. DPP4 gene related enzymes, on the other hand, can be improved by introducing a specific chemotype with the correct substitution groups to their active site residues.

- DPP-4 inhibitor drug development protocol

Using hit-to-lead strategies to uncover novel DPP-4 inhibitors might be a more potent tool for finding new scaffolds. The creation of novel DPP-4 inhibitors may benefit from the use of 3D pharmacophore models. Inhibitors of DPP-4 are represented by pharmacophore models that characterize their chemical characteristics. These models may also be used to filter fragment design in a virtual database. Lead optimization has

been used to look for tiny primary pieces that might be inserted to DPP-4's S1 and S2 places (Lu *et al.* 2008). The 3D architectures of ligand-protein complexes are utilized to generate novel, more effective ligands with higher binding affinity. Structure-activity relationships are updated after synthesis and testing, and a new design cycle begins. A drug's bioavailability and metabolic stability may be neglected in the quest to find ligands with nanomolar affinities, which may take several design cycles (Keseru and Makara 2006).

- DPP-4 inhibitor classification

Despite the fact that several DPP-4 inhibitors have been discovered, there is still a great need for novel ones in the treatment of diabetes. Developing DPP-4 inhibitors that just work against DPP-4 is very challenging because to the potential adverse effects and increased mortality that would result if these inhibitors were combined with DPP-2, DPP-8, or DPP-9. Diabetes patients also want long-acting inhibitors that are very successful in the management of the disease. Substances being tested in people to see whether they can be used to treat illness fall into two broad categories. DPP-4 inhibitors with peptididomimetic and nonpeptidomimetic structural characteristics have been discovered. The peptidomimetic class includes (a) glycine-based (-series) and (b) alanine-based (-series) inhibitors (-series) (Figure 2.15).

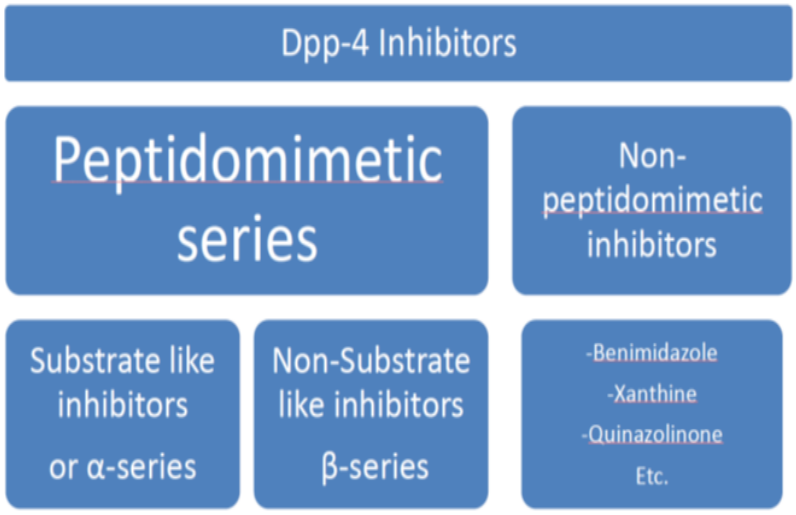

Figure 2.14 Classification of DPP-4 inhibitors (Ghate and Jain 2013) Revisons

For example, the X-ray crystal structure of DPP-4 and computer modeling studies show two key interactions between the enzyme and -series inhibitors. Carbonyl oxygen is linked to Arg125 by a hydrogen bond, whereas the free amino group is linked to Glu205 and/or Glu206 via a salt bridge.

This series had a salt bridge but the hydrogen link to carbonyl oxygen was not well defined (Anon n.d.).

Studying how DPP4 inhibitors affect a variety of metabolic processes requires a detailed evaluation of data associating changes in the molecular forms and levels of a suspected DPP4 substrate to actions arising from treatment with an in vivo DPP4 inhibitor.

- Clinical advancements

For more than 24 hours, oral DPP-4 inhibitors block more than 90% of plasma DPP activity in humans when taken as small molecules. NVP-DPP728 (Ahrén *et al.* 2002), a short-acting Novartis inhibitor, followed these encouraging preclinical results with a clinical proof of concept**.**

- Sitagliptin

Sitagliptin (Januvia®; MK-0431) was the first of a new class of oral antihyperglycemic medicines manufactured from -amino acids. Drug sales were expected to exceed $1 billion in 2010 after sitagliptin was licensed for use in the treatment of type 2 diabetes by the US Food and Drug Administration in 2006. Trifluoromethane (2R) trifluoromethane] sitagliptin -4,3,a] -[4-oxo-4-(trifluoromethyl) -5,6 dihydro [1,2,4] triazolo pyrazin-7(8H)-yl] Generated and sold by Merck -1-(2,4,5 Trifluorophenyl) butan-2-amine is a potent, selective, and orally accessible DPP4 inhibitor. The amino acid derivative of sitagliptin had no effect on other enzymes connected to DPP4, such as

DPP8, DPP9, FAP-, and QPP. It reduces DPP-4 activity for a lengthy period of time and retains its efficacy after a single daily dosage. Blood levels of GIP and GLP1 were shown to be lower after meals in both acute and chronic tests. During clinical trials, patients with type 2 diabetes were given the DPP-4 inhibitor sitagliptin, an oral medication with high selectivity, or the diabetes medication metformin. T2DM patients throughout the spectrum benefit from sitagliptin's fasting and postprandial blood glucose management.

Sitagliptin medication improved indications of cell function as well (Xu et al. 2008) High blood glucose levels were generally tolerated (Scott et al. 2007) without causing any notable adverse effects, with a low risk of hypoglycemia or gastrointestinal difficulties and a neutral effect on weight (Todd and Bloom 2007) A runny or stuffy nose, sore throat, upper respiratory infection, stomach pain, diarrhea, and a headache are all possible adverse effects of the drug sitagliptin. It is possible that sitagliptin has a benefit over vildagliptin in the event that off-target effects from inhibition of other DPP subtypes are linked to negative outcomes since sitagliptin has greater DPP-4 selectivity. Patients with T2DM and moderate to severe renal impairment, including those on dialysis, found that sitagliptin was well tolerated and offered good glycemic control.

- Linagliptin

There are eight different forms of Linagliptin (TradjentaR BI-1356, Linagliptin (TradjentaR BI-1356)) (3R) -3-aminopiperidin-1-yl] -7-(but-2-yn-1-yl) the carboxylic acid 3-[(4-methoxyquinazoline-2-yl)] the first methyl group from 1-[(4-methylquinazolin-2-yl)] One of [(4-methylquinazolin-2-yl)]. Orally effective and selective DPP4 inhibitor -3,7-dihydro-1H-purine-2,6-dione) was developed by Boehringer Ingelheim and Eli Lilly. On May 2, 2011, the FDA authorized the first xanthine derivative of Linagliptin as an anti-hyperglycemic medicine for the treatment of type 2 diabetes. GLP-1 and GIP incretin levels are increased by linagliptin, whereas blood sugar and insulin levels are decreased by inhibiting DPP4 specifically (BANIK, KAISAR, and HOSSAIN 2013).

It was linagliptin binding to its DPP-4 target that accounted for the concentration-dependent plasma protein binding at therapeutic plasma levels. Long-term exposure to the drug linagliptin is unlikely to result in tissue accumulation because the binding capacity of DPP-4 to the drug is saturated. Taking metformin (ten milligrams per day) with metformin had no effect on metformin exposure (850 mg three times a day). Linagliptin has demonstrated no clinically meaningful pharmacokinetic interactions with other commonly used oral glucose-lowering medications, such as metformin, pioglitazone, and glibenclamide (warfarin, digoxin). Except for these issues, Linagliptin was well tolerated and did not cause weight gain or other drug-drug interactions when being used in conjunction with metformin or pioglitazone. Linagliptin has a high effectiveness and safety profile, which indicates that it is a new DPP-4 inhibitor (Ahmad *et al.* 2018, Ghate and Jain 2013).

- Dutogliptin

An anti-diabetic drug called Dutogliptin has been shown to boost GLP-1 levels in the blood and the stomach. In the pancreas, insulin production rises and glycogen storage decreases when GLP-1 levels are elevated. In a 12-week, double-blinded, randomized research with 422 participants, type 2 diabetes was effectively treated with dutogliptin, metformin, and glitazone. HbA1c levels dropped statistically significantly (Pattzi *et al.* 2010).

In the treatment of type 2 diabetes, Merck and Co.'s Omarigliptin (MK-3102), an oral DPP-4 inhibitor, is administered once a week (Krishna *et al.* 2016)

Incretin levels (GLP-1 and GIP) rise when DPP-4 activity is reduced, reducing glucagon release and lowering blood glucose levels. Omarigliptin (25mg and 12.5mg tablets) were approved by the Japan Food and Drug Administration (PMDA) on September 28th, 2015. (Burness 2015) It was in Japan when omarigliptin was first approved for usage. Merck intends to stop marketing its products in the United States and Europe.

Sitagliptin (Januvia®; MK-0431) was included in MK-0431, the first of a new class of oral antihyperglycemic medicines derived from -amino acids. Type 2 diabetes was diagnosed and treated with Sitagliptin, which was licensed for use by the FDA in 2006. a medicine called sitagliptin, for example (2R) Excessive exposure to sunlight may lead to the formation of cancerous tumors. -5,6-dihydro [1,2,4] triazolo -4-oxo-4-[3-oromethyl) -5,6-dihydro [1,2,4] Pyrazin-7(8H)-yl] Merck developed and marketed a DPP4 inhibitor called -1-(2,4,5-trifluorophenyl) butan-2-amine. Sitagliptin, an amino acid derivative of DPP4, was reported to have favorable pharmacokinetics by another DPP4-related enzyme, DPP8. Improved fluorine substitution on the phenyl ring resulted to an improvement in DPP4 affinity of 2,5 and 2,4,5-tri-F from 3,4-di-F to 2,5. For the triazolo pyrazine ring, we added a trifluoromethyl moiety to boost effectiveness and bioavailability in laboratory rats. When it comes to reducing blood glucose levels, clinical investigations in phase IIb have demonstrated that sitagliptin is both well-tolerated and safe to use. Merck is actively working on MK-431A, a combination of Sitagliptin and Metformin, to treat Type 2 Diabetes (T2D) (Duffy *et al.* 2007).

- Glucose homeostasıs and – 4 enzymes

GLP-1, an intestinal hormone, was shown to be a DPP-4 substrate, and this discovery provided evidence that the enzyme is involved in maintaining glucose homeostasis. It was determined in 1986 that GLP1, a newly discovered peptide (Bell *et al.* 1983, Rskov *et al.* 1986), regulates blood sugar levels and has a substantial influence on the pancreas. It has been shown via studies done on healthy humans and animals that the incretin hormone GLP-1 has strong insulinotropic and glucagonostatic effects in vitro utilizing perfused pancreas from pig and rat pancreas. A great deal of attention has been paid to the insulinotropic and glucose-lowering properties of GLP-1 in people with type 2 diabetes (T2DM) (Nathan *et al.* 1992). Michael Nauck showed that intravenous GLP-1 infusion might regulate hyperglycemic fasting glucose levels in people with Type 2 Diabetes Mellitus (T2DM) (Nauck *et al.* 1993) When blood glucose levels rise, insulin synthesis increases and glucagon production decreases, which means that GLP-1's anti-diabetic effects diminish as euglycemia approaches. However, despite glucose levels remaining well within the hyperglycemic range and circulating immunoreactive GLP-1

levels being elevated for several hours after a single subcutaneous injection of insulin, the insulinotropic effects were brief and insulin levels returned to baseline within 90 minutes (Nauck *et al.* 1996).

The secret was first brought to light by previously unknown diabetics and non-diabetics alike. Tests in vitro showed that GLP1 is a substrate for DPP-4 in kinetic studies, which led researchers to uncover that DPP-4-cleaved GLP-1 was the predominant circulating component of GLP-1-like immunoreactivity in healthy persons, as well as T2DM patients.

Exogenous GLP treatment had comparable outcomes in rats, validating our initial findings. Blocking DPP-4's degradative activity increased endogenous GLP1 levels and enhanced its anti-hyperglycemic actions, they discovered, since GLP-1 was in fact DPP-4's substrate. Because of its similarity to the angiotensin-converting enzyme inhibitors used to treat hypertension, DPP-4 inhibition was proposed as a possible treatment for type 2 diabetes Consequently (Carr 2016).

- Inhibiting DPP-4 as a T2DM therapy

GLP-1 metabolism must begin and be dominated by DPP-4 cleavage in order for this technique to be effective. Because alternate clearance mechanisms would simply take over once the DPP-4 route had been shut down, there would be no increase in the amounts of intact peptide if this wasn't the case. Since no human-safe form of DPP-4 inhibitor was available at the time, preclinical trials served as a proof of concept. Methods of GLP1 metabolism that don't need DPP-4 cleavage aren't applicable. The DPP-4 pathway would therefore be blocked, and peptide levels would not rise as a result. Preclinical investigations were used to test the notion that DPP-4 inhibitors were not acceptable for human usage, as they had been previously reported (Huang *et al.* 2022). The discovery of DPP-IV enzyme inhibitors has given T2DM patients a new treatment alternative (Silveira *et al.* 2013) Blood sugar levels in type 2 diabetes (T2DM) patients have been lowered with the use of gut hormones such GLP-1, GIP and gastric inhibitory polypeptide. Findings like these suggest that this condition could benefit from

a therapeutic approach including these hormones. - The importance of these hormones in maintaining healthy cells, building new ones, and producing insulin cannot be emphasized. Sugar homeostasis and insulin levels in the blood are both enhanced. In the year of our Lord 1929, King Kong (Lammi and colleagues 2016) DPP-IV in blood plasma, on the other hand, deactivates GLP-1 and GIP, causing -cells to generate insulin. This results in a significant increase in incretin hormone half-life as a result of DPP-4 inhibitors increasing the amount of time they spend in blood circulation. Preventing the cleavage of the gut incretins GLP-1 and GIP is a popular T2DM therapy approach.

Figure 2.16 shows the DPP-IV inhibitors' method of action. After meals, the GI tract secretes a hormone called GLP-1 incretin, which is broken by an enzyme called DPP-IV. If there is a DPP-IV inhibitor in the plasma, which inhibits the DPP-IV enzyme, protecting GLP-1 cleavage, blood glucose levels may be managed by boosting pancreatic insulin production. DPP-IV inhibitors like vildagliptin and sitagliptin, according to the FDA, are totally reversible, orally active, and competitive. Diabetes mellitus type 2 patients may now use DPP-IV inhibitors for the first time, according to an approval by the FDA (T2DM) (Bharti *et al.* 2012, Jadav *et al.* 2012)

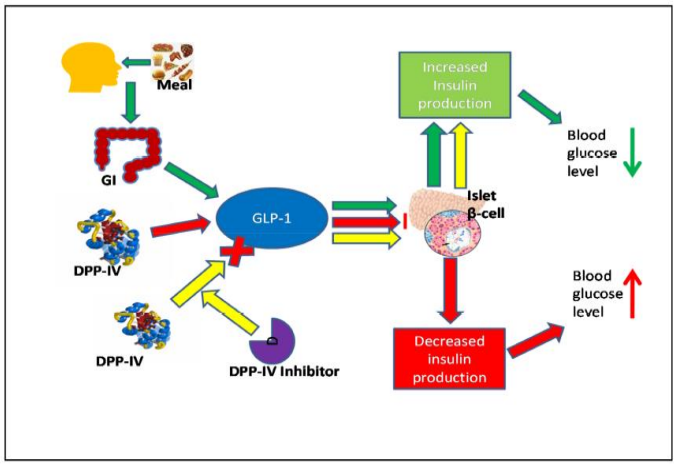

Figure 2.16 What this figure illustrates about DPP-mechanism IV's and possible role in maintaining a homeostatic blood glucose level by guarding against incretin hormone degradation by the DPP-IV enzyme is (Singh *et al.* 2021)

GLP-1 antagonists and DPP-4 inhibitors are two incretin-based medications that have been suggested as T2DM treatments. Weight reduction, hypoglycemia, and minimal side effects are all associated with DPP-IV inhibition. It is possible that these traits will help us achieve our aim by enabling better treatment.

2.1.9 Cytokines

Inflammation and immunity are both regulated by cytokines, which are non-structural proteins with molecular weights of less than 30 kDa (200 amino acids). Immune cells, such as leukocytes, create cytokines at the site of damage in order to control inflammation and hematopoiesis (Deverman and Patterson 2009).

Macrophages, B lymphocytes, and T lymphocytes produce cytokines, as do endothelial cells and fibroblasts, among other stromal cells (Elhannan *et al.* 2015).

When one cytokine stimulates its target cells to produce more cytokines, it is called a "cascade" system, since one activating molecule triggers the production of many other cytokines. They interact with a wide range of receptors in the brain and are involved in a wide range of mental processes (Elhannan *et al.* 2015). For this reason, cytokines control the synthesis and activity of other anti-inflammatory and pro-inflammatory cytokines, as well as the activity and differentiation of immune cells and their ability to proliferate and survive.

As endogenous inflammatory and immunomodulatory mediators, cytokines affect a broad spectrum of cells, both positively and negatively, with both positive and negative consequences (Sultani *et al.* 2012). Inflammatory and anti-inflammatory cytokines are subcategories of cytokines, which are distinguished by their role in inflammatory conditions. There is an inflammatory response at the infected site due to the activation of multiple inflammatory cell types including fibroblasts, endothelial cells and tissue macrophages. This cytokine family is referred to as "proinflammatory cytokines" because of its ability to increase inflammation in response to tissue damage and

infection (Ong *et al.* 2010) Immune cells produce pro-inflammatory cytokines, which act as a kind of signaling molecule that encourages inflammation. TNF, IL-1, IL-12, IL-6, and IL-1 are all important components of the innate immune response, and they all play a role (Zhang *et al.* 2016).

As opposed to this, alterations in the immune system are directly linked to weight gain and type 2 diabetes (T2D) (Ieronymaki *et al.* 2019)

Research on macrophages, for example, has revealed the continual dynamic cross-talk between metabolism and immune systems.

Immune metabolism is the phrase used to describe how they interact. Inflammatory cytokines have a critical part in the pathophysiology of T2D, which has emerged as an essential pathophysiological component. Chronic, low-grade inflammation caused by cells is described by inflammatory cytokines that characterize the inflammatory state. These cytokines have been dubbed'metabolic inflammation'.

Metabolic and inflammatory responses are triggered when you consume an excessive amount of active nutrients (Gregor and Hotamisligil 2011).

Insulin sensitivity is adversely affected since pro-inflammatory cytokines such as TNF- and IL-1 interfere with insulin signals as well as fat metabolism (Sethi and Hotamisligil 1999).

Studies show that treating T2D patients by targeting pro-inflammatory cytokines improves insulin sensitivity and glucose metabolism in T2D patients (Donath 2014).

Proinflammatory cytokines are regulated by anti-inflammatory cytokines, which are immunoregulatory molecules (Andargie and Diro Ejara 2016).

Immunomodulatory agents in physiologic situations include anti-inflammatory cytokines and specific cytokine inhibitors, which minimize the potentially harmful consequences of prolonged or severe inflammatory responses. If these anti-inflammatory mediators fail to moderate proinflammatory activity in immune-mediated illnesses or overcompensate and decrease the immune response, the host may be at risk of systemic infection.

2.1.10 Interleukin-1ß potential role in the onset of type 2 diabetes

An antimicrobial reaction to pathogen-carrying molecular patterns An key part of the body's defense against infection and damage is IL-1ß, a potent pro-inflammatory cytokine (Bagust and Beale 2003). Of the 11 IL-1 family members, this is the one that has been investigated and described the most thoroughly. Many cell types produce and release pro-IL-1, however the bulk of study has concentrated on monocytes and macrophages, which are innate immune system cells characterized as "pathogen associated molecular patterns" (PAMPs). Gene expression pathways are affected by PAMPs because they operate on macrophages via pattern recognition receptors (PRR) (Takeuchi and Akira 2010). Prime phase stimulation of pro-IL-1 expression is an inefficient trigger for secretion. The primed cell must now come into touch with another PAMP or DAMP in order to initiate the processing and secretion of an activated IL-1 molecule (danger-associated molecular pattern, endogenous chemicals released from dead cells). Mandrup observed that interleukin-1 beta had a substantial influence on beta cell activity and/or mass in his research on the effect of the cytokine on people with type 2 diabetes. Beta cell death, the role of glucose and FFA in beta cell development, and the role of lipid reserves in systemic inflammation were all discovered after more than two decades of preclinical research. Endogenous levels of IL-1Ra and the impact of IL-1ßblockade on macrophage infiltration on beta-cell function (or cluster). Targeting this cytokine solely in people with type 2 diabetes might have a major effect on treating the illness and maybe reduce cardiovascular events if current studies continue to demonstrate that suppressing IL-1ß restores beta-cell activity (Mandrup-Poulsen *et al.* 1987, Mandrup-Poulsen *et al.* 2010).

3. MATERIALS AND METHODS

3.1 Study Design

In this study, (181) samples were taken. After obtaining the required official approvals, during the period from the beginning of September 2020 until the end of February 2021. Using the web software OSSE (osse.bii.astar.edu.sg/calculation1.php), the sample size and study power were estimated.

3.1.1 Patients group

There were 181 patients participating in this work and they were divided into four main groups: (49) patients who were newly diagnosed with type 2 diabetes, (42) the group of patients taking metformin treatment and 41 the group of patients taking DDP4 treatment, and the fourth group (49) is the group of healthy individuals. They were chosen from the Diabetes Center of the Teaching Hospital (AL-Sadder) in the province of Al Najaf Al-Ashraf. Specialist physicians have identified and recognized them as measures of inclusion. The participants' ages ranged from 30 to 80 years old, with a mean SD 52.5±10.65 of years.

3.1.2 Criteria for inclusion

The following are some of the inclusion criteria:

Any patient with diabetic symptoms (nocturia, polyuria, and polyphagia) who has been diagnosed with T2DM by a physician has:

- Fasting blood glucose levels of less than 126 mg/dl.
- Glucose levels in the postprandial period are less than 200 mg/dl
- A cut point of 6.5 percent HbA1c was proposed for diagnosing diabetes.

3.1.3 Criteria for exclusion

The following are examples of exclusionary criteria:

- Between the ages of 30 and 40.
- They are taking insulin or have T1DM.
- Heart illness, heart failure, congenital heart disease, or cardiomyopathy.
- Having an autoimmune disease (infections, cancers, Rheumatic arthritis, severe renal or liver diseases, and pregnancy).

3.2 Control Group

In the control group, there were 49 individuals. A mean of 52.4±8.15 years separated the 33 to 70-year-old individuals. Family, friends, and medical professionals were all in attendance. This research did not include anybody who had diabetes, hypertension, heart disease, cardiovascular disease, or kidney disease, or who was even suspected of having one of these conditions as a participant..

3.3 Samples in Study Groups

3.3.1 Blood samples

Five milliliters of venous blood were drawn from each patient in both groups using a 5milliliter disposable syringe in an EDTA tube in the two study groups (DM patients with newly diagnosed patients and DM patients taking antidiabetic drugs and healthy groups) using. Samples of diabetic and healthy patients were preserved in diabetes laboratories. All blood collection tubes were gently shaken to mix, incubated for 5 min, and then stored in a deep freezer.

3.3.2 Measurements of phenotype

The questionnaire for this study, which was based on laboratory tests performed in a specialized diabetic laboratory, included information on the demographics of diabetes patients, such as height and weight, type of therapy, date of illness onset, and vital signs (systolic and diastolic blood pressure). In the Al Sadder hospital's diabetes laboratory, the following tests were used: BMI was calculated by dividing a person's weight in kilograms by their square height in meters (m2). Obesity and excess weight were classified as either an illness or a condition by WHO criterion 155. Obesity is defined as a BMI of 30 kg/m2 or higher, whereas overweight is defined as a BMI of 24.9 kg/m2 or less. Serum glucose (SG), serum CRP, total cholesterol (TC), LDL-C, triglycerides, VLD-C, HDL-C, HbA1C, urea, and creatinine are all biochemical parameters that are examined in this study.

3.4 Equipments

The equipments and apparatus utilized in this study are stated in Table 3.1.

Table 3.1 Manufacturers, equipment, and country

N0.	EQUIPMENT	MANUFACTURER	COUNTRIES
1	ELISA (Microplate Washer)	Paramedical PKL	Italy
2	ELISA (Microplate Reader)	Paramedical PKL	Italy
3	Deep freeze	Hitachi	Japan
4	Centrifuge	Labtech	Germany
5	Mindray(bc10)	CBC analyzer	China
6	Incubator(37c)	Memmert	Japan
7	Spectrophotometer	Ecolab	Japan
8	Water bath	Memmert	Japan
9	Microplate reader	Humareder HS	Germany
10	Mechanical pipette multi-channel and disposable tips	Ecolab	Japan
11	semi-automatic analyzer	Bio system 350	India
12	Mechanical pipette-single channel and disposable tips	Ecolab	Japan

3.5 Materials

3.5.1 Kits with chemical's materials

As shown in Table 3.2, a variety of companies provided the chemical materials and kits used in the current study.

Table 3.2 Companies that manufacture chemicals and manufactured goods

NO	CHEMICAL AND KITS	COMPANY
1	Blood glucose Kit	Biomaghreb, Tunes
2	Glycosylated hemoglobin (HbA1c) kit	Stanbio / U.S.A
3	CD26/DPP4 human ELISA Kit	U.S.A
4	Interleukin 1B ELISA Kit	U.S.A
5	Serum urea kit	Human/Germany
6	Serum creatinine kit	Human/Germany
7	Triglycerides Kit	Spinreact, Spain
8	Cholesterol Kit	Spinreact, Spain
9	High-density lipoprotein cholesterol Kit	Spinreact, Spain
10	Low-density lipoprotein cholesterol Kit	Spinreact, Spain
11	Very low-density lipoprotein Kit	Spinreact, Spain
12	c-reactive protein Kit	Latex / Germany

3.6 Methods

3.6.1 Estimation of serum glucose level in sera

Principle: The colorimetric indicator used in this study is quinoneimine, which is made from 4-amino antipyrine and phenol by the catalytic activity of H2O2 inside peroxidase. (AMBADE *et al.* 1998). The reagents were prepared and used according to the instructions in the kit's leaflet (appendix 1)

3.6.2 Determination of serum total cholesterol in blood

Following oxidation and enzymatic hydrolysis, the content of cholesterol was determined. With the presence of peroxidase and phenol, quinonemine was produced as

an indicator from H2O2 and 4-amino antipyrin (KESKİN *et al.* 2021) Reagents were prepared and used according to the instructions in the kit's leaflet (appendix 2).

3.6.3 Determination of serum total triglyceride in blood

Following enzymatic hydrolysis with lipase, the concentration of TG was determined. H2O2, 4-chlorophenol, and 4-amino phenazone were used to make aquinonimine as an indicator under the influence of peroxidase catalysis. The reagents were prepared and used according to the instructions in the kit's brochure (appendix3)

3.6.4 Determining the amount of HDL-C in the blood

With the presence of Mg ions, VLDL, LDL, and chylomicron fractions have been quantitively precipitated by adding phospho-tungstic acid. Following the centrifugation process, the concentration of cholesterol in the HDL fraction that remains in the supernatant was assessed using the method described in 3.6.2

The reagents were prepared and used according to the instructions in the kit's leflet.

3.6.5 Determining the concentration of VLDL-C in the blood

By dividing the TG value obtained in section 3.6.3 by 5, the concentration of VLDL was calculated Equation 3.1

$$VLDL - C = Triglyceride\ concentration/5 \quad (3.1)$$

3.6.6 Determination of serum concentration of LDL-C

The concentration of LDL-C was evaluated from the following Equation 3.2

$$LDL\ cholesterol\ = Total\ cholesterol - (VLDL\ +\ HDL - cholesterol) \quad (3.2)$$

3.6.7 Serum urea measurement

Procedure:

- One vial of urease R1a was added to one bottle of phosphate buffer R1b to prepare the working reagent.
- Three plain tubes were prepared included blank, standard, and sample.
- Add 1ml of reagent to the first tube to prepare the blank.
- Ten µl from the standard and one ml from working reagent were
- added to the second tube to prepare the standard.
- Then ten µl of sample and one ml of reagent were added to the third tube to prepare the sample.

Then, the shaken and incubate tubes for five minutes at (20.25) °C. Then, measure standard and sample vs. blank through two hours.

3.6.8 Measurement of glycosylated hemoglobin (HbA1c)

- To make the working reagent, one vial of urease R1a was added to one bottle of phosphate buffer R1b.
- Three plain tubes, including a blank, a standard, and a sample, were constructed.
- To prepare the blank, add 1ml of reagent to the first tube.
- To make the standard, ten mL of the standard and one mL of the working reagent were added to the second tube.
- To prepare the sample, ten liters of sample and one ml of reagent were added to the third tube. The tubes should then be shaken and incubated for five minutes at 20.25 °C. Then, over the course of two hours, compare the standard and sample to the blank. During sixty minutes, the absorbance of the three tubes was measured against water at 415 nm.

- 5.0 mL distilled water was pipetted into S, U, and C labelled tubes.
- Hemolysate (20µl) was pipetted into the indicated tube, mixed thoroughly, and transferred to the cuvette for absorbance measurements.
- The absorbance of S, U, and C vs. water was measured at 415 nm for sixty minutes.

3.6.9 Determination's concentration of serum c-reactive protein

Principle: Antigen-antibody complexes are formed in the buffer and transferred to the nitrocellulose matrix, where they are captured by the other immobilized antibodies on the test strip, using a sandwich imumuno detection technique. A stronger fluorescence signal is generated from detector antibodies when there are more antigens in the sample, which is processed by the device for ichroma TM tests to evaluate CRP concentration. Instructions for making and using the kit's components and procedures were followed exactly.

3.6.10 Determination of DDP4-CD26 enzyme

DDP4-CD26 enzyme was measured for all participating patients, as well as other pathway probes such as

- Blood glucose.
- C-reactive protein
- Lipid profile.
- HBA1C,
- IL-1B

Principle: CD26 ELISA Kit employs a sandwich enzyme-linked immunosorbent test (ELISA). Precoated onto 96-well plates is a mouse monoclonal antibody specific for CD26. Standard samples (NSO, D34-P766) are added to the wells, followed by the addition of biotinylated detection polyclonal antibodies from goats specific for CD26. Rinsing with PBS or TBS solution is then carried out on the wells. The unbound

conjugates were removed using PBS or TBS buffer after injection of the Avidin-Biotin-Peroxidase Complex. The TMB substrate was used for the HRP reaction. The HRP enzymatic process may be readily shown using this method. After being catalyzed by HRP, the blue TMB became yellow when exposed to an acidic stop solution. Plate yellow density is correlated with the number of CD26 samples in the human body.

Preparation and storage of reagents

A. It is recommended to prepare the CD26 standard solution no more than two hours before to the experiment's start time in circumstances where there are two tubes of the CD26 standard (20 ng per tube).

CD26 is the most common treatment for human CD26 Mix 1 mL of sample diluent buffer with 1 mL of one tube at room temperature for 10 minutes.

Human CD26 standard solution is utilized at a concentration of 312 pg/ml. You'll need six Eppendorf tubes, each labeled with the concentrations of 10,000 pg/ml, 5, 000 ml, 2, 500 pg/ml, 1, 250 pg/ml, and 312 pg/ml, as well as 625 nl and 312, respectively. Dilution of the sample diluent buffer with the equivalent amount of water results in 0.3 ml in each tube. In the second tube, 0.3ml of the first tube's 20,000pg/ml CD26 standard solution should be added. Two tubes of 0.3ml each will suffice. Transfer 0.3ml from the second tube to the third tube, and so on.

B. Preparation of the working solution for the Avidin-Biotin-Peroxidase Complex (ABC) is summarized below. It's recommended that you prepare the solution no later than an hour before you begin the experiment.

The total volume for each well should be 0.1ml multiplied by the number of wells (the number of wells). Always have an additional 0.1-0.2 mL on hand.

Dilution buffer and ABC should be properly mixed, and the Avidin, Biotin, and Peroxidase Complex (ABC) should be diluted to 1:100. For example, one liter of ABC diluent buffer may be mixed with 99 liters of ABC diluent.

Assay Procedure: Before using, the TMB color development agent should be warmed to 37°C for 30 minutes. Before dilution, samples and reagents must be well mixed and equally dispersed. Each experiment should have its own set of CD26 detection curves. The sample dilution fold may be estimated by measuring the amount of CD26 present in samples.

Aliquot Fill the precoated 96-well plates with 0.1 milliliters of each of the human CD26 standard solutions at concentrations of 20,000 picomolar, 10,000 picomolar, 5000 picomolar, 2500 picomolar, 1250 picomolar. The control well should be filled with 0.1 ml of the sample diluent buffer (Zero well). 0.1 ml of each sample (serum, plasma, saliva, or urine) should be put to each empty well. Find out how to dilute samples in the "Sample Dilution" section above. According to the suggestion, each human CD26 standard solution and sample should be tested twice.

After closing the plate with the lid, incubate at 37°C for 90 minutes.

Discard the food on the plate and wipe the plate dry with paper towels or another absorbent material after removing the lid. The wells should never be completely dry at any time.

At 37°C, incubate each well for 60 minutes with the biotinylated anti-human CD26 antibody working solution.

After rinsing the plate three times with 0.01M TBS or 0.01M PBS, the washing buffer should be allowed to stay in the wells for one minute each time. With a paper towel or other absorbent material, remove the washing buffer from the plate and allow it to air dry.

The prepared ABC working solution should be poured into each well and incubated for 30 minutes at 37°C.

TBS or PBS may be used to wash the plate five times, leaving the washing buffer to sit in the wells for around 1-2 minutes each time. With a paper towel or other absorbent material, remove the washing buffer from the plate and allow it to air dry.

Incubate for 20-25 minutes at 37°C in the dark with 90 l of TMB color development agent in each well.

Each well should be filled with 0.1ml of the TMB stop solution produced earlier. In a matter of seconds, the color shifts from blue to yellow.

Analyze the optical density (OD) absorbance (450 nm) in a microplate reader within 30 minutes after applying the stop solution.

In order to calculate the "relative O.D.450," use the following values: (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve is a plot of the O.D.450 ratio (Y) vs the concentration (C) of each standard solution (Kameoka *et al.* 1993, DARMOUL *et al.* 1990).

The kit component in appendix 8.

3.7 Determination of Serum IL-1ß

Principles of testing: An enzyme linked immunosorbent test (ELISA) double antibody sandwich approach was used to quantify human Interleukin 1beta (IL-1) (ELISA). Adding both the Standard and the Sample to wells pre-coated with the goal antibody creates an immunological complex. Remove any unattached enzyme by using the HRP-Conjugated reagent. Substrate A and B are the last two ingredients. When exposed to

acid, the solution will first become blue, then yellow. A lack of depth or brightness in the color or light.

Procedure for performing an assay

- Get all of the reagents ready before commencing the test procedure. We recommend running all standards and samples through the Microelisa Stripplate twice.
- Standardize: Using the standard wells, do sample well testing. Add 50 liters of water to a standard well.
- Add 10 l of sample to the testing sample well, followed by 40 l of sample diluent; the blank well has no influence.
- Using a seal plate membrane, cover each well with 100 l of HRP-conjugated reagent and gently shake for 60 minutes at 37 °C to mix.
- How to make the laundry detergent: Distilled or deionized water should be used to dilute the washing concentration (20X) for future usage.
- Remove the film, drain the liquid, dry the area, fill each well with washing solution, leave aside for a minute, then drain the liquid, repeat five times. Pat dry.
- Pour 50 ml of chromogen solution A into each well, followed by 50 ml of chromogen solution B. This is how you produce color. Gently whisk the ingredients together to blend. Color development requires 15 minutes of incubation at 37°C with no light.
- Add 50 l of Stop Solution to each well to bring the reaction to a halt.
- Assay: The absorbance (OD) of each well should be measured under a 450nm wavelength 15 minutes after the stop solution has been added by using a control well.
- Regression equation for standard curve based on concentrations and their corresponding OD values may be calculated Based on the OD values of the samples, determine the concentration of the applicable sample.

3.8 Statistical Analysis

The Kruskal-Wallis test for Windows version 20.0 of the Statistical Package for the Social Sciences (SPSS) was used for the statistical study. The mean, standard deviation (mean ± SD), maximum, minimum, and range of the data were used to display the findings. Mann-Whitney Comparing variables between groups was done using U test analysis. This study's correlation analysis, using Pearson's correlation coefficients, revealed a substantial association between the variables that could be measured and those that could be predicted. The p-value, on the other hand, was used when the variation between groups was less than 0.05.

4. RESULTS AND DISCUSSION

4.1 Analytical Results of Anthropometric and Biochemical Features

There were 132 cases of diabetic patients in total in this study. The first group consisted of 42 patients who were taking metformin drugs, while the second group consisted of 49 patients who had newly been diagnosed with type 2 diabetes, and the third group consisted of 41 patients who were taking DDP4 inhibitors drugs. The mean average age was (53.5±11.65) years in patients who were taking metformin drugs, with males and females. The second group of patients who had newly been diagnosed with type 2 diabetes had an average age of (50.2±10.41) years, with males and females, and the third group of patients taking DDP4 inhibitors drug had an average age (53.5±8.30). A healthy control group of 49 healthy people was included in the study, with a mean age of (52.4±8.15) years and males and females. There were highly significant differences in age and BMI across the groups, according to the Chi-square test (age: P = 0.002, BMI=0.02), while gender had no significant differences between groups Table 4.1 As shown in Figure 4.1, Figure 4.2 and Figure 4.3.

Table 4.1 Statistical distribution (frequency and percentage) of study groups by their age, gender and BMI

Items	Sub-groups	Metformin Group (N= 42)		New Diabetic Group (N= 49)		DDP4 Group (N= 41)		Control Group (N= 49)		Chi Square (P value)
		Freq	%.	Freq	%.	Freq	%.	Freq	%.	
Age / Years	**36-47**	9	21.4	15	30.6	8	19.5	12	24.5	26.0 (0.002) HS
	48-59	19	45.2	23	46.9	11	26.8	13	26.5	
	60-71	8	19.0	7	14.3	22	53.7	20	40.8	
	72-83	6	14.3	4	8.2	0	0.0	4	8.2	
Mean±SD		**53.5±11.65**		**50.2±10.41**		**53.5±8.30**		**52.4±8.15**		
Gender	**Male**	28	66.7	26	53.1	20	48.8	27	55.1	2.99 (0.39) NS
	Female	14	33.3	23	46.9	21	51.2	22	44.9	
BMI	**Underweight**	2	4.8	4	8.2	0	0.0	0	0.0	19.15 (0.02) S
	Normal	3	7.1	4	8.2	2	4.9	5	10.2	
	Overweight	20	47.6	10	20.4	23	56.1	22	44.9	
	Obese	17	40.5	31	63.3	16	39.0	22	44.9	
Mean±SD		**29.9±8.61**		**30.8±7.67**		**29.1±4.67**		**34.9±12.75**		

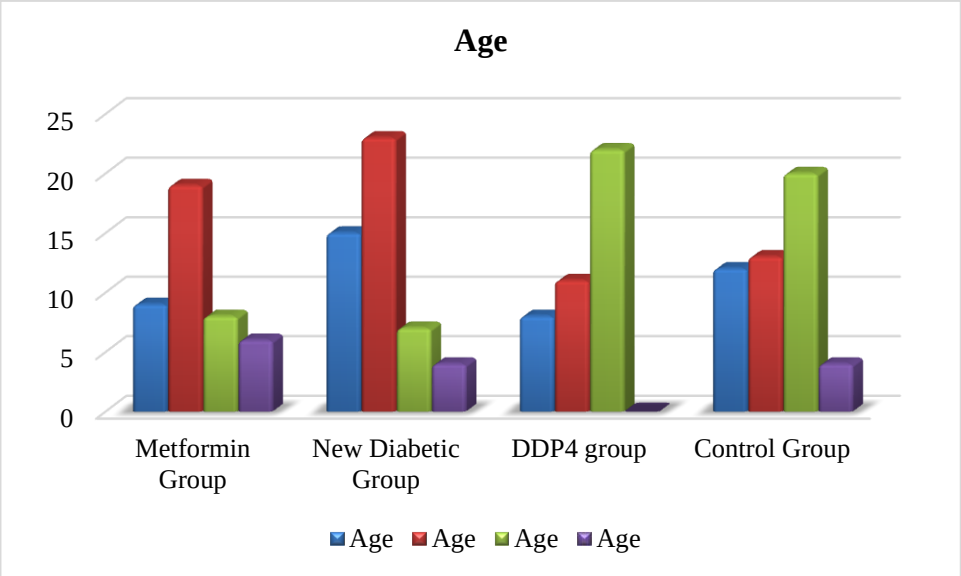

Figure 4.1 Bar chart for statistical distribution (frequency and percentage) of study groups by their age

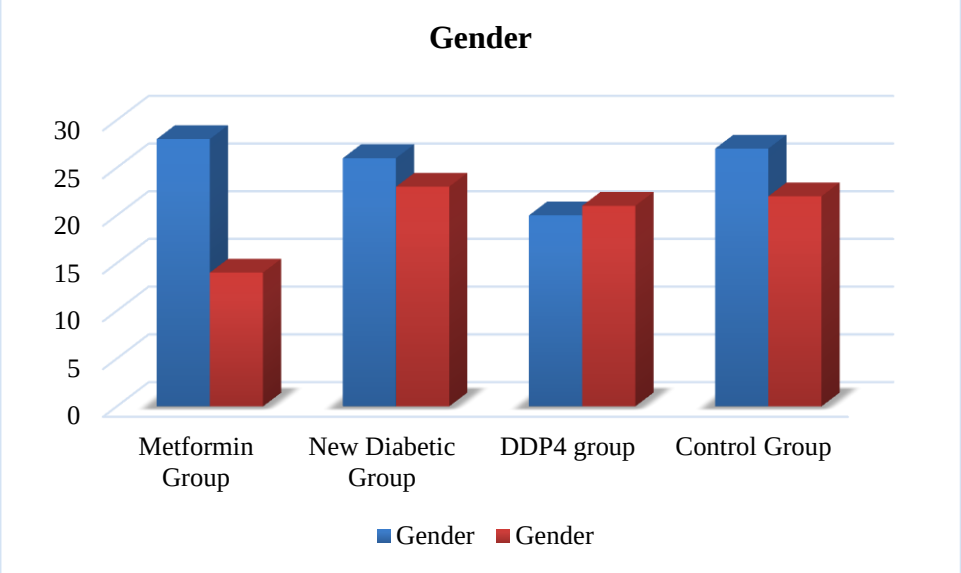

Figure 4.2 Bar chart for statistical distribution (frequency and percentage) of study groups by their gender

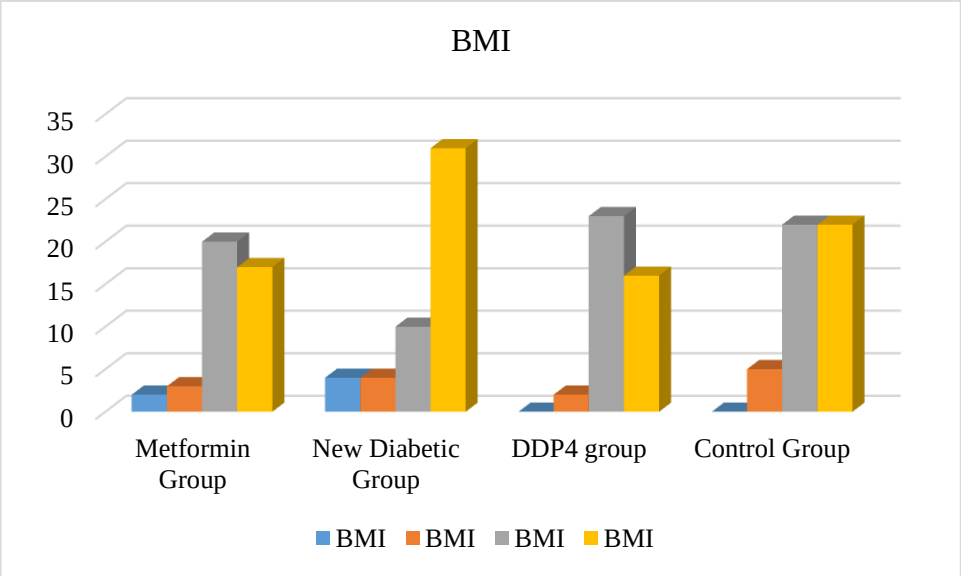

Figure 4.3 Bar chart for statistical distribution (frequency and percentage) of study groups by their BMI

4.2 The Biochemical for Parameters

The p-value for Bartlett's Test for inequality of Population Variances was less than 0.05, indicating that the variances are not homogeneous and that the ANOVA may not be acceptable. As a result, the Kruskal-Wallis test was performed to calculate the P-value.

4.3 Blood Glucose

With regard to blood glucose, there were significant differences among the groups, 106.46, p <.000, The means and standard deviations are presented in table 4.2 and (figure 4.4). Patients taking DDp4 inhibitors drugs showed significantly lower blood glucose levels (197.62± 29.84) than patients who had recently been diagnosed with type 2 diabetes (225.86 ± 54.83), p < .000, and patients taking metformin drugs (189.66 ± 17.71). The mean blood glucose level in healthy groups (87.22± 10.60) was significantly lower than that in patients newly diagnosed with type 2 diabetes (225.72 ±101.35), p<.000. There have been no additional significant effects found Table 4.2.

4.4 Blood HBA1C

HBA1C levels differed significantly between the groups, = 39.57, p< 001, Table 4.2 shows the averages and standard deviations. In comparisons between each pair of readings, figure 4.4 demonstrated that the mean HBA1C for newly diagnosed patients (7.91±1.56) was considerably higher than the control (5.46±5.51), p<.001. The mean HBA1C for patients newly diagnosed with diabetes (7.91±1.56) was substantially higher than for patients taking metformin drugs (7.42 ±1.41), p <.01, and patients taking DDP4 inhibitors drugs. The mean of HBA1C for Control (5.46 ± 5.51) was considerably lower than the other groups, for the main effect of the group.

Table 4.2 Measurement and difference in the levels of Blood Glucose and HBA1C among study groups

Items		Metformin Group (N= 42)	New Diabetic Group (N= 49)	DDP4 Group (N= 41)	Control Group (N= 49)	Kruskal Wallis Test	P value
Blood Glucose (mg/dl)	Mean	189.66 ^{A}	225.86 ^{B}	197.62	130.80 ^{C}	112.72	0.000 HS
	SD	17.71	54.83	29.84	16.20		
HBA1C	Mean	7.42 ^{AC}	7.91 ^{AB}	6.85 ^{AC}	5.46 ^{C}	39.57	0.01 S
	SD	1.41	1.56	5.08	5.51		

SD: Standard Deviation ABC: Different letters refer to significant difference at P<0.05 by using Mann-Whitney U test for each two independent groups

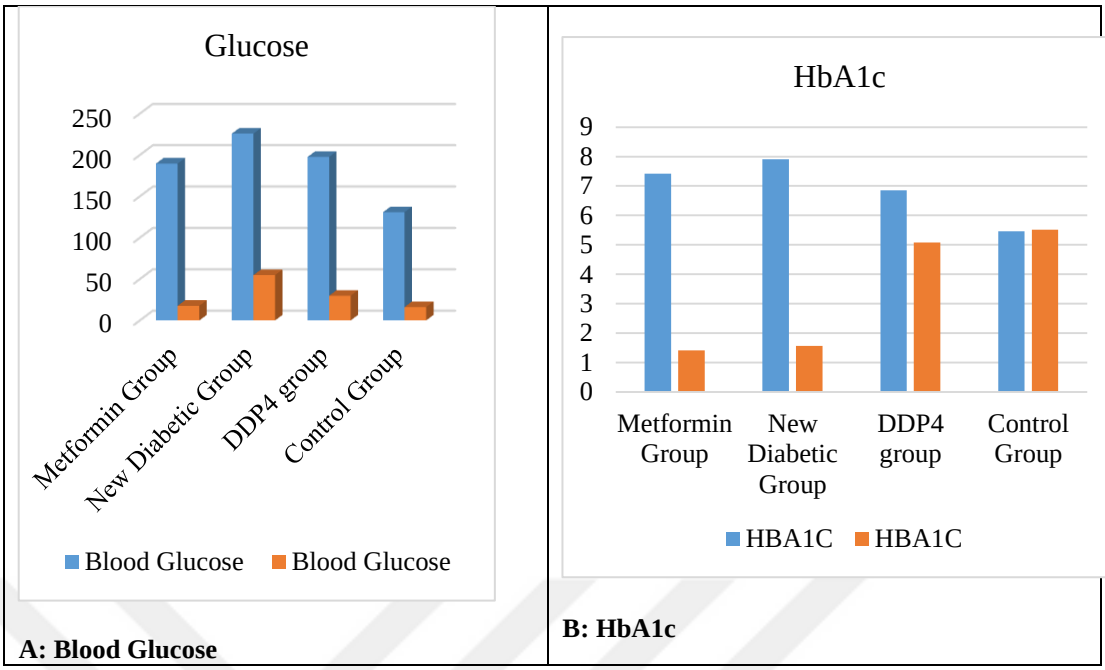

Figure 4.4 Bar Charts of the measurement and difference in the levels of Blood Glucose and HBA1C among study groups

4.5 Lipid Profile

Lipid profile was examined in all groups of the study, the distribution of observation was analyzed as follows

4.5.1 Triglyceride

Triglyceride the results of the Table 4.3 were significant for serum triglyceride, indicating there were significant differences among the group. Using Mann-Whitney U test revealed the mean of Serum triglyceride for patients taking DDP4 inhibitors drugs (194.54 ± 40.58) mg/dl was significantly higher than for control (131.78 ±18.14) mg/dl. Patients taking metformin drugs groups 175.62 ±27.73 mg/dl did not show a significant effect as compared to controls nor patients who had recently been diagnosed with type 2 diabetes groups. recently been diagnosed with type 2 diabetes groups. As shown ın Fıgure 4.5.

Table 4.3 Measurement and difference in the levels of lipid profile markers among study groups

Items		Metformin Group (N= 42)	New Diabetic Group (N= 49)	DDP4 Group (N= 41)	Control Group (N= 49)	Kruskal Wallis Test	P value
TG (mg/dl)	Mean	151.29 ^{A}	194.54 ^{B}	175.62	131.78 ^{AD}	13.26	0.004
	SD	51.33	40.58	27.73	19.14		
CH (mg/dl)	Mean	177.90 $^{B\,A}$	186.00 ^{B}	164.46 ^{B}	120.78 ^{C}	72.3	0.000
	SD	37.94	17.78	39.32	25.96		
VLDL (mg/dl)	Mean	30.75^{A}	45.52^{B}	33.47^{ABC}	24.00^{C}	26.94	0.000
	SD	7.54	35.97	23.33	5.20		
LDL (mg/dl)	Mean	116.59^{CB}	118.37	109.84	101.57^{A}	29.89	0.000
	SD	24.59	8.50	17.53	20.49		
HDL (mg/dl)	Mean	41.19 ^{A}	36.20 ^{B}	39. 04 ^{A}	57.74 ^{A}	32.16	0.000
	SD	24.71	10.81	22.54	22.54		

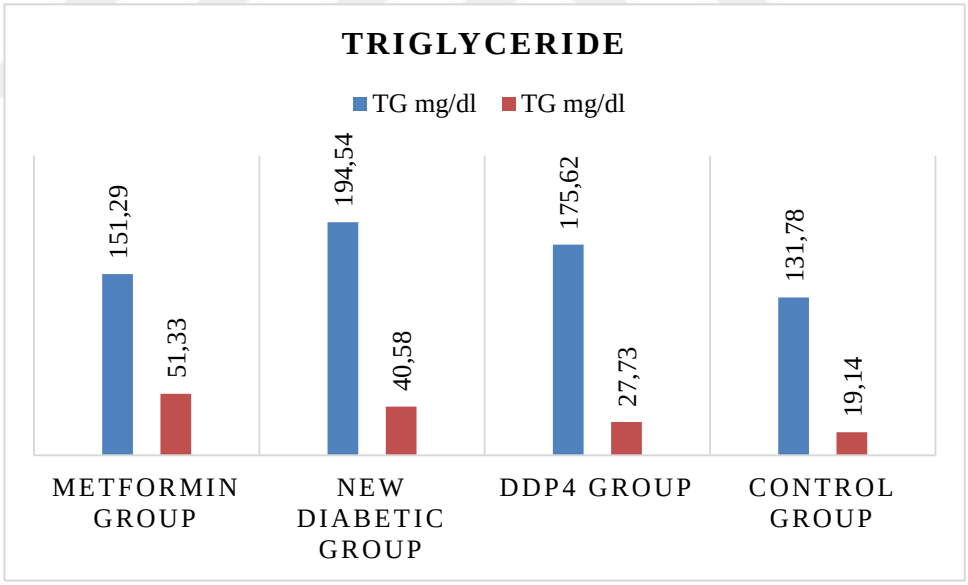

Figure 4.5 Bar Charts of the measurement and difference in the levels of Triglyceride among study groups

4.5.2 Cholesterol

The Kruskal Wallis test was used to calculate descriptive statistics for serum cholesterol. It's a non-parametric alternative to one-way ANOVA that doesn't require normally distributed data (Conover and Iman, 1981). The Kruskal-Wallis test revealed significant differences in cholesterol between the groups, with a p value of > .000. Table 4.3 shows the results.

By using Mann-Whitney U test were calculated between each pair of measurements to examine the differences among the groups. the mean of cholesterol for newly diabetics group (186.00 ±17.87), was significantly higher than for Control (120.78 ± 25.96) p <0.001. The mean of cholesterol for Control was significantly smaller than for patients taking metformin drugs (177.90 ±37.94), and patients taking DDP4 inhibitors drugs (164.46±39.32) as shown in Figure 4.6.

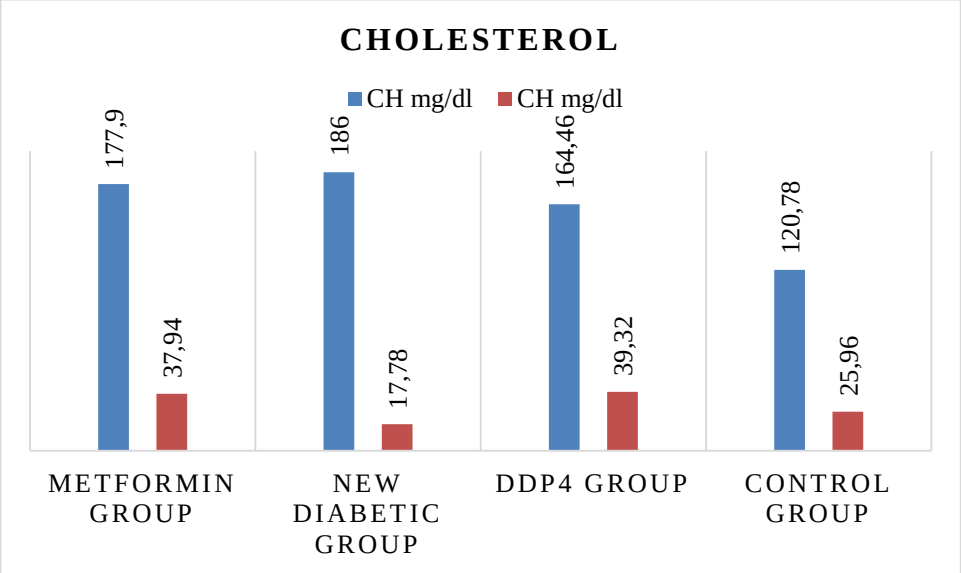

Figure 4.6 Bar Charts of the measurement and difference in the levels of Cholesterol among study group

4.5.3 Serum HDL

To see if there were any significant changes in serum HDL levels across groups, the Kruskal-Walli's classification test was used. A value of p < 0.05, = 32.16, < 0.001 was used heavily in the Kruskal-Walli's test. S. HDL exhibited an extension of 41.19±24.71mg/dL in participants receiving metformin. S. HDL was 39.04 ± 22.54 mg/dL in individuals on DDP4 inhibitors and 36.20 ±10.81 mg/dL in patients newly diagnosed with diabetes. S. HDL levels in the three groups were considerably lower than the controls (49.68 8.39). There were no other significant differences found. Show in Figure 4.7.

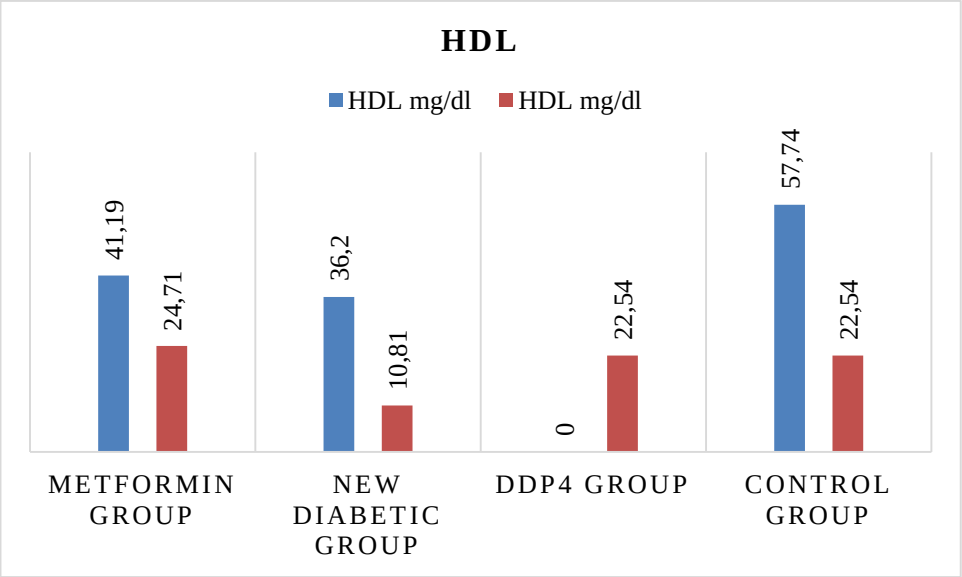

Figure 4.7 Bar Charts of the measurement and difference in the levels of HDL among study groups

4.5.4 Serum LDL

For Control, S. LDL was118.37 ± 8.50 mg/dl. For patients taking metformin drugs, serum LDL had an average of 101.57 ± 20.49. For patients taking DDP4 inhibitors drugs had an average of 116.59 ± 24.59 and group patients newly diagnosis diabetes 109.84±17.53. The Kruskal-Wallis results were significant, p < 05, indicating there were significant differences in LDL mg/ dl among the groups Table 4.3. The means and

standard deviations are presented in Table 4.3. Manna Whitney U test comparisons showed the mean of LDL mg/dl for patients taking metformin were significantly lower than for Control p < .001. Control means on the other hand was significantly higher than for patients newly diagnosed with diabetes p < .001and patients taking DDP4 inhibitors drugs. patients taking metformin mean LDL was also significantly different from that for patients taking DDP4 inhibitors drugs p < .001and newly diagnosed with diabetes. As shown in Figure 4.8.

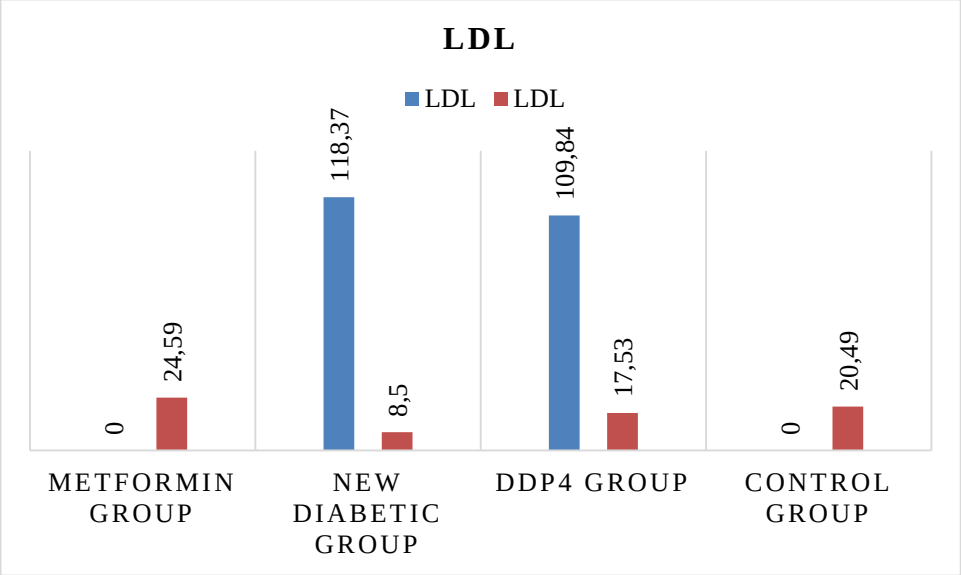

Figure 4.8 Bar Charts of the measurement and difference in the levels of LDL-cholesterol among study groups

4.5.5 Serum VLDL

VLDL had an average of 45.52 ± 35.97 for group patients newly diagnosed with diabetes. 24.00 ± 5.20 for Control. S. VLDL had patients taking DDP4 inhibitors drugs average of 33.47± 23.33 and group patients taking metformin drugs 30.75±7.54. In 4.3 table The Kruskal-Walli's test revealed that the mean rank of VLDL mg/dl was statistically different across groups, with an alpha value of 0.05, = 26.94, p < 001, showing that the mean rank of VLDL mg/dl was significantly different among groups. In Figure 4.9 multiple comparisons revealed significant differences between the three groups and control on an alpha value of 0.05.

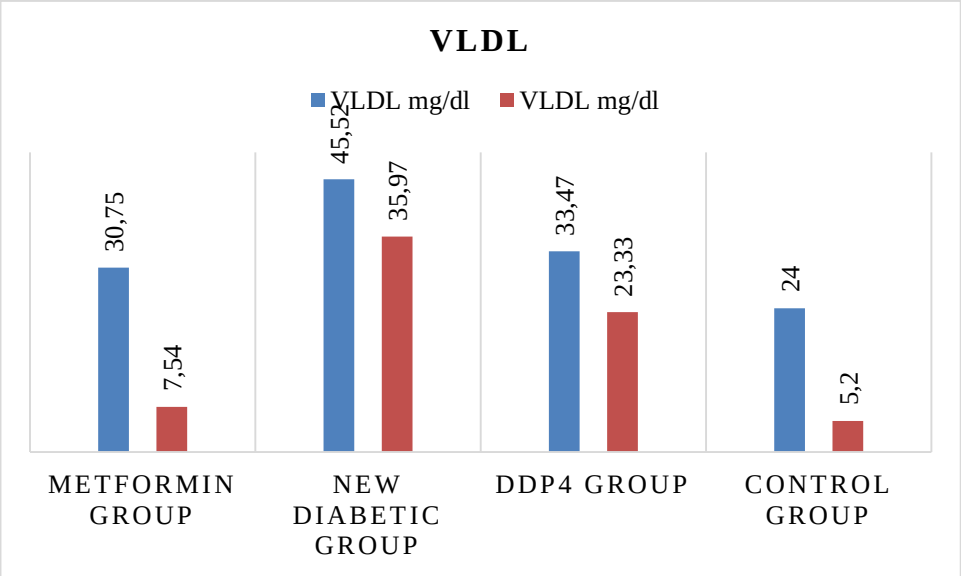

Figure 4.9 Bar Charts of the measurement and difference in the levels of VLDL-Cholesterol among study groups

4.6 Biochemical Parameters of Kidney Functions Markers Among Study Groups

4.6.1 Serum urea

The difference in urea mg/dl between the groups was significant, 40.82, p<.001. Table 4.4 and Figure 4.10 shows the means and standard deviations. Mann-Whitney to investigate the differences between the variables, U test comparisons were calculated between each pair of measurements. The mean value of urea in the DDP4 inhibitors group (41.00±6.30) was considerably greater than in the Control group (32.00±7.09), p<001. The mean urea for the Control group (32.00± 7.09) was significantly lower than the urea for the metformin drug group (34.12 ±5.29), p<.001, and the urea for the newly diagnosed diabetes group (38.67 ±3.36). There were no additional major consequences discovered.

4.6.2 Serum creatinine

S. Creatinine levels differed significantly between the groups, 26.87, p<001. Table 4.4 shows the averages and standard deviations. Figure 4.10 Mann-Whitney U-test the mean Creatinine for a group of patients taking metformin medicine (1.03± 0.32) was substantially greater than the Control (0.80 0.09), p < 001, according to U comparisons between each pair of readings. The mean of S. creatinine in a group of patients taking metformin drug (1.03 ± 0.32) was significantly greater than in a patient group taking DDP4 inhibitors drug (0.87 0.21), p < 001, and in a group of patients newly diagnosed with diabetes.

Table 4.4 Measurement and difference in the levels of kidney functions markers among

Items		Metformin Group (N= 42)	New Diabetic Group (N= 49)	DDP4 Group (N= 41)	Control Group (N= 49)	Kruskal Wallis Test	P value
Urea (mg/dl)	Mean	34.12 [A]	38.67 [B]	41.00 [B]	32.00 [A]	40.82	0.000
	SD	5.29	3.36	6.30	7.09		
Creatinine (mg/dl)	Mean	1.03 [A]	0.83 [B]	0.87 [B]	0.80 [B]	26.87	0.000
	SD	0.32	0.19	0.21	0.09		

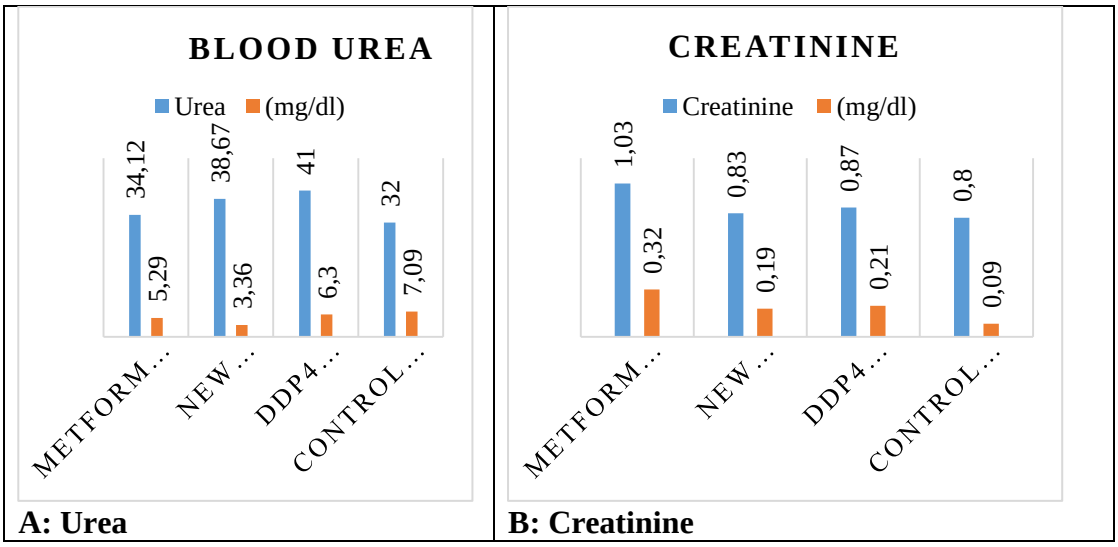

Figure 4.10 Bar Charts of the measurement and difference in the levels of serum urea and creatinine among study groups

4.7 C-reactive Protein

There were substantial disparities between the groups in terms of CRP. Table 4.5 shows the means and standard deviations Figure 4.11. To further investigate the differences between variables, paired t-tests were calculated between each set of measurements. For each independent group comparison, the Mann-Whitney U test was used, and the mean CRP of the DDP4 inhibitor group (5.62) was substantially lower than the metformin group (8.96), p<001, and the newly diagnosed diabetes group (9.18). The control group's mean CRP (4.80) was significantly lower than the other two groups. According to the findings of the current study. In comparison to those using metformin drugs and those newly diagnosed with diabetic Mellitus, DDP4 inhibitors had a lower CRP level in other groups.

Table 4.5 Measurement and difference in the levels CRP

Items		Metformin Group (N= 42)	New Diabetic Group (N= 49)	DDP4 Group (N= 41)	Control Group (N= 49)	Kruskal Wallis Test	P value
CRP (mg/dl)	Mean	4.8 [A]	9.18 [B]	8.96 [B]	5.62 [A]	36.12	0.000
	SD	1.22	1.26	1.30	1.09		

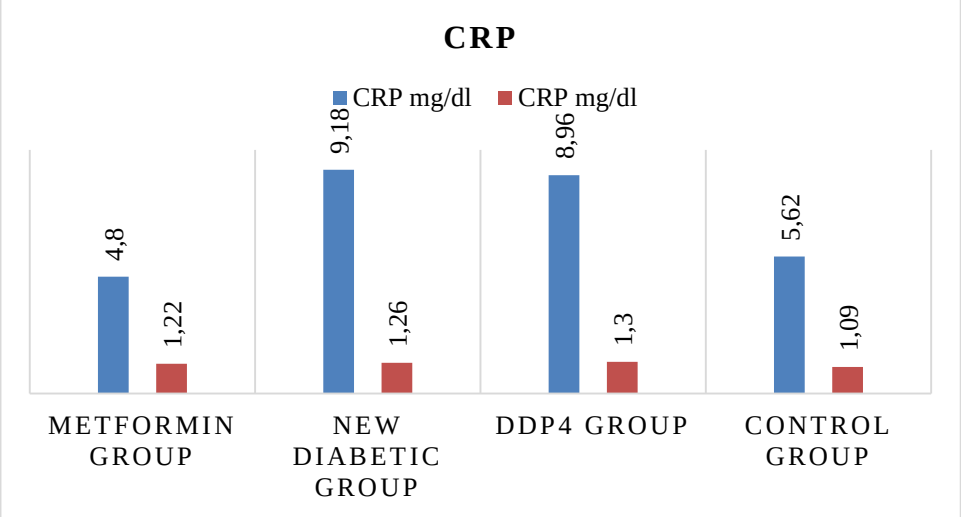

Figure 4.11 Bar Charts of the measurement and difference in the levels of serum C-reactive protein among study groups

4.8 DDP4 Enzyme and IL-ß Levels in the Study Groups

4.8.1 DDP4 enzyme

Patients newly diagnosed with diabetes Miletus had mean levels of DDP4 (58.89 ±7.42). DDP4 levels were (55.08 ±8.02) on average in the metformin group. DDP4 levels in the control group were (50.98± 5.44). The mean levels of DDP4 in the group of patients taking a DDP4 inhibitor medication were (51.00 ± 5.80), which was lower than the other groups. To see if there were any significant variations in DDP4 levels between groups, the Kruskal Wallis classification test was used. The test results were significant (33.06, p <0.001), showing that the mean rank of DDP4 levels in the two groups was considerably different. The Kruskal-Wallis rank-sum test results are presented in Table 4.5. The Kruskal-Wallis rank-sum test results are shown in Table 4.5. The Bar charts of the ranked values of DDP4 levels by groups are shown in Figure 4.12. Overall results of the pairwise testing showed significant differences between the groups based on an alpha value of 0.05. the pairwise testing showed significant differences between the groups based on an alpha value of 0.05.

Table 4.5 Measurement and difference in the levels of DDP4 enzyme and IL-B among study groups

Items		Metformin Group (N= 42)	New Diabetic Group (N= 49)	DDP4 Group (N= 41)	Control Group (N= 49)	Kruskal Wallis Test	P value
DDP4	Mean	55.08 [A]	58.89 [B]	51.00 [A]	50.98 [A]	33.06	0.000
	SD	8.02	7.42	5.80	5.44		
IL-ß Pg/µl	Mean	180.90 [A]	215.57[B]	157.73 [A]	148.40	8.288	0.000
	SD	27.51	29.62	46.59	44.50		

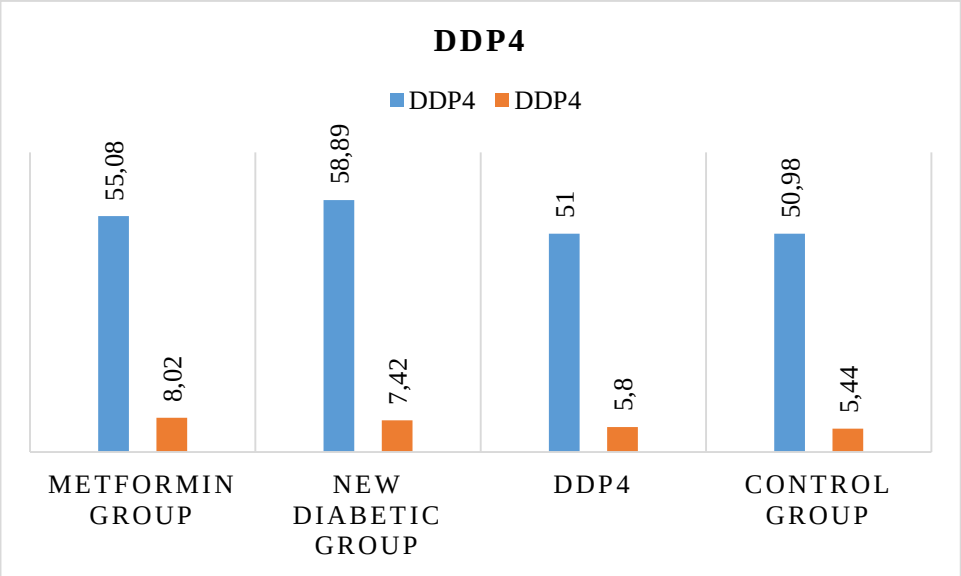

Figure 4.12 Bar Charts of the measurement and difference in the levels of DDP4 among study group

4.9 IL-1ß

The results of interleukin-1-beta showed that individuals newly diagnosed with type 2 diabetes had considerably greater interleukin concentrations than the other groups, with a mean concentration of (215.57 ±29.62). The mean IL-1ß findings for patients taking a metformin drug were (180.90 ± 27.5), while the mean IL-1ß findings for individuals taking DDP4 inhibitors drug were (157.73± 46.59). The mean IL-1ß concentration readings for the control group were (148.40 ±44.50). Table 4.5 provides a summary of the statistics.

To see if there were any significant differences in IL-1ß levels between the research groups, the Kruskal-Wallis rank-sum test was used. The test yielded a significant value of 82.88, p>0.001, suggesting that the mean rank of IL-1ß differed considerably between the groups. The Kruskal-Wallis rank sum test results are shown in Table 4.5. The bar charts of the ranking values of IL-1ß by groups are shown in Figure 4.13. The results of the multiple comparisons revealed that the following variable pairings had significant differences.

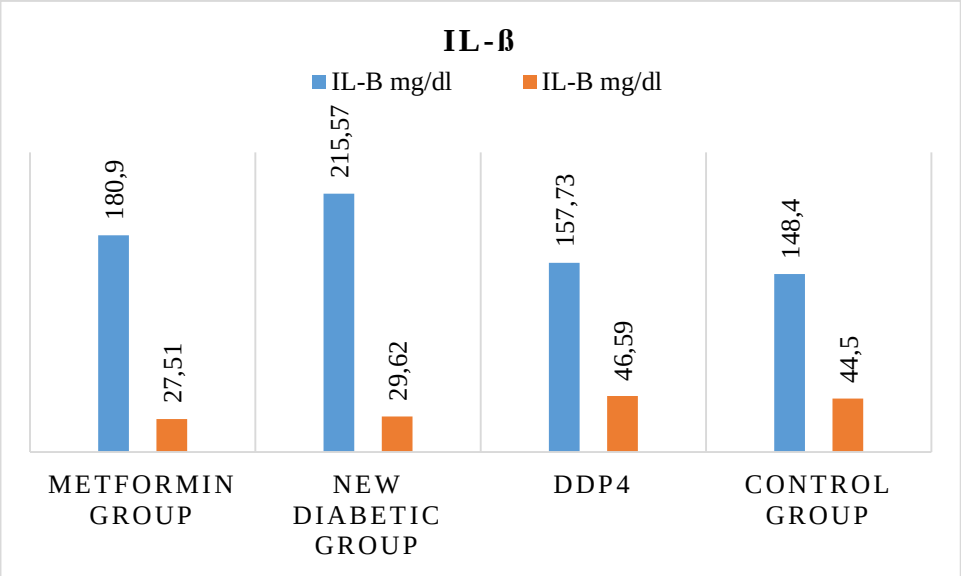

Figure 4.13 Bar Charts of the measurement and difference in the levels of IL-ß among study groups

4.10 Correlation Coefficient (r) Between Biomarkers and Each of DDP4 and BMI

Table 4.6 shows the correlation between biomarkers and each of the DDP4 and IL-1ß levels in diabetic type 2 individuals. In all groups, the results show a positive correlation between blood sugar and IL-1ß, and LDL-C mg/dl and DDP4 enzymes. Other parameters had no link to the measured variables

Table 4.6 Spearman Correlation Coefficient (r) between biomarkers and each of DDP4 and IL-1ß

MARKERS	P	DDP4	IL-B
Blood Sugar	r	-0.211	0.374
	P value	0.184	0.031*
TG	r	0.200	-0.078
	P value	0.210	0.628
CH	r	0.212	-0.048
	P value	0.183	0.766
VLDL	r	-0.031	-0.101
	P value	0.847	0.528
LDL	r	0.383	0.072
	P value	0.05	0.655
HDL	r	0.163	-0.127
	P value	0.310	0.429
HBA1C	r	-0.261	0.045
	P value	0.099	0.779
CRP	r	0.096	0.119
	P value	0.549	0.460
Urea	r	-0.222	0.038
	P value	0.164	0.811
Creatinine	r	-0.155	-0.056
	P value	0.332	0.729
DDP4	r	1.000	-0.017
	P value	--	0.914
IL-ß	r	-0.017	1.000
	P value	0.914	---

The study population as a whole had gender differences. The comparison of male and female patients with type 2 diabetes is shown in Table 4.7

The Mann-Whitney U Test finding of the present study revealed that there was a significant difference in blood glucose, cholesterol, triglycerides, LDL-C, VLDL, and Creatinine between male and female patients in Table 4.7. As shown in Figure 4.14 and Figure 4.15. In the comparison of DDP4, IL-1B, HBA1C, CRP, and urea between male and female patients by mean±SD, the results demonstrate that there is no significant difference between the two categories. This means these parameters in patients are not related to gender. As shown in Figure 4.16, Figure 4.17 and Figure 4.18.

Table 4.7 Measurement and difference in the levels of studied biomarkers among gender groups

MARKERS		MEAN	SD	SE	MANN-WHITNEY U TEST	P VALUE
DDP4	Male	135.78	14.35	2.76	191.00	0.62
	Female	124.68	16.55	3.53		
IL-ß	Male	181.26	14.74	2.84	202.50	0.84
	Female	160.14	17.63	3.76		
Blood Glucose	Male	128.93	24.67	4.75	93.00	0.00*
	Female	110.77	24.43	5.21		
TG	Male	25.48	4.92	0.95	117.00	0.02*
	Female	22.18	5.06	1.08		
CH	Male	117.59	9.30	1.79	113.50	0.01*
	Female	119.32	7.49	1.60		
VLDL	Male	41.30	11.55	2.22	182.00	0.04*
	Female	36.27	10.17	2.17		
LDL	Male	5.47	2.74	0.53	129.00	0.03*
	Female	5.45	7.76	1.65		
HDL	Male	5.34	0.51	0.10	185.00	0.51
	Female	5.95	0.94	0.20		
HBA1C	Male	33.00	6.79	1.31	140.00	0.06
	Female	30.77	7.40	1.58		
CRP	Male	0.78	0.04	0.01	209.00	0.98
	Female	0.82	0.12	0.03		
Urea	Male	50.41	5.59	1.08	155.50	0.14
	Female	51.67	5.30	1.13		
Creatinine	Male	153.26	49.87	9.60	97.50	0.00*
	Female	163.21	42.72	9.11		

SD: Standard Deviation; SE: Standard Error; TG: Triglycerides; CH: Cholesterol; VLDL: Very Low-Density Lipoproteins; LDL: Low Density Lipoproteins

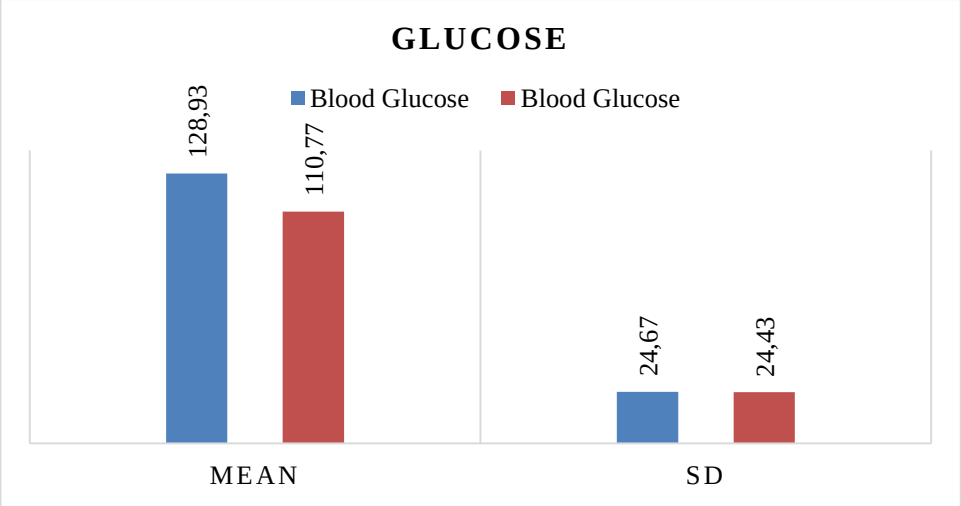

Figure 4.14 Bar Chart of the measurement and difference in the levels of blood glucose regarding gender

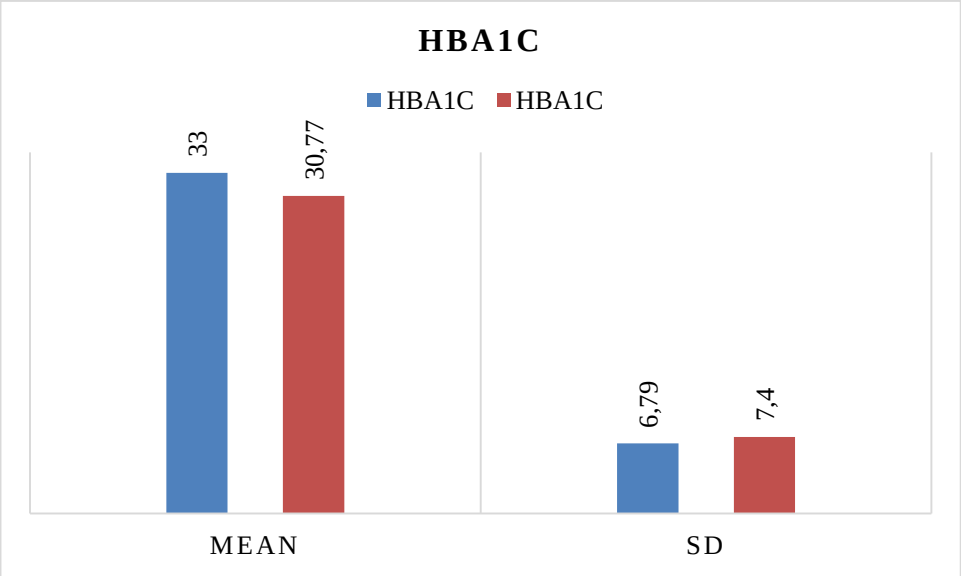

Figure 4.15 Bar Chart of the measurement and difference in the levels of HBA1C regarding gender

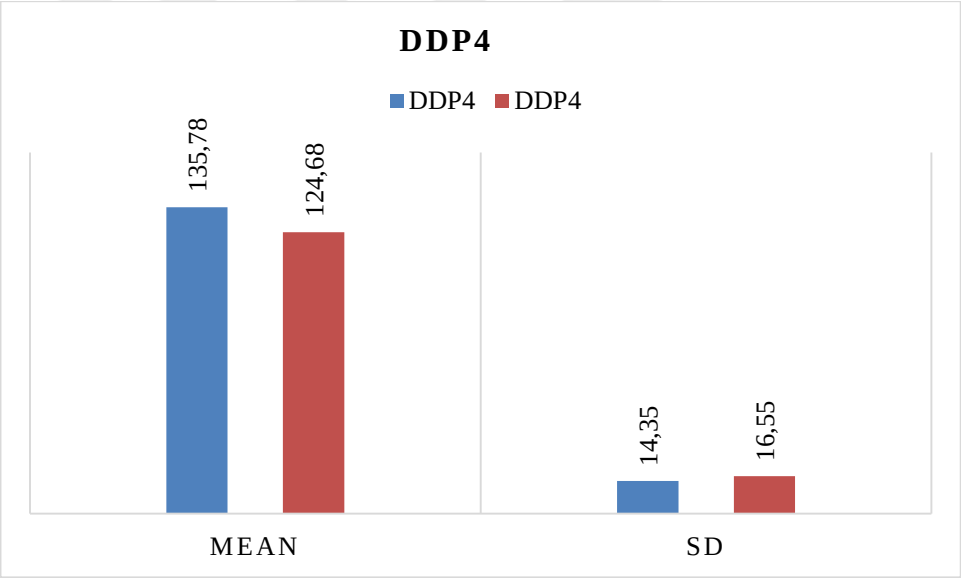

Figure 4.16 Bar Chart of the measurement and difference in the levels of DDP4 regarding gender

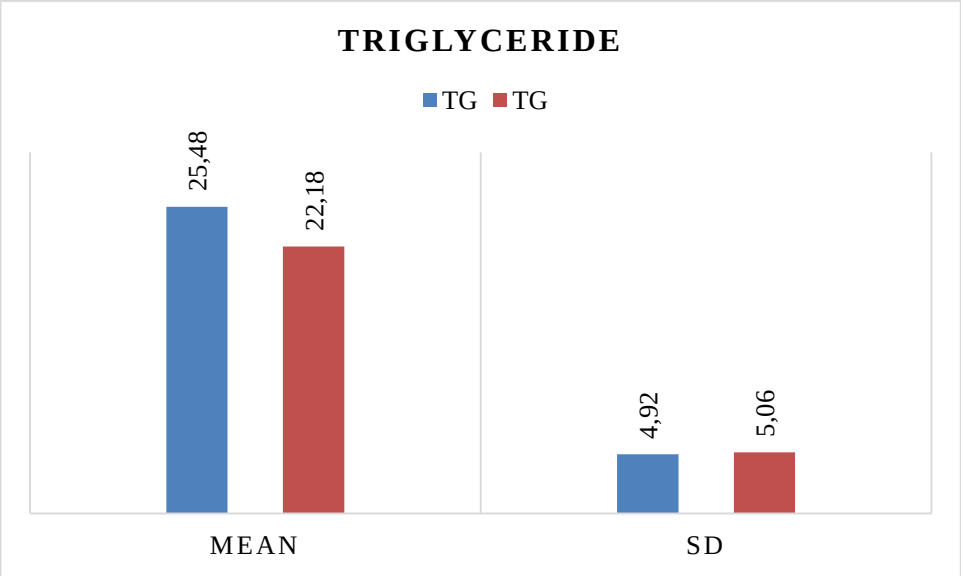

Figure 4.17 Bar Chart of the measurement and difference in the levels of Triglyceride regarding gender

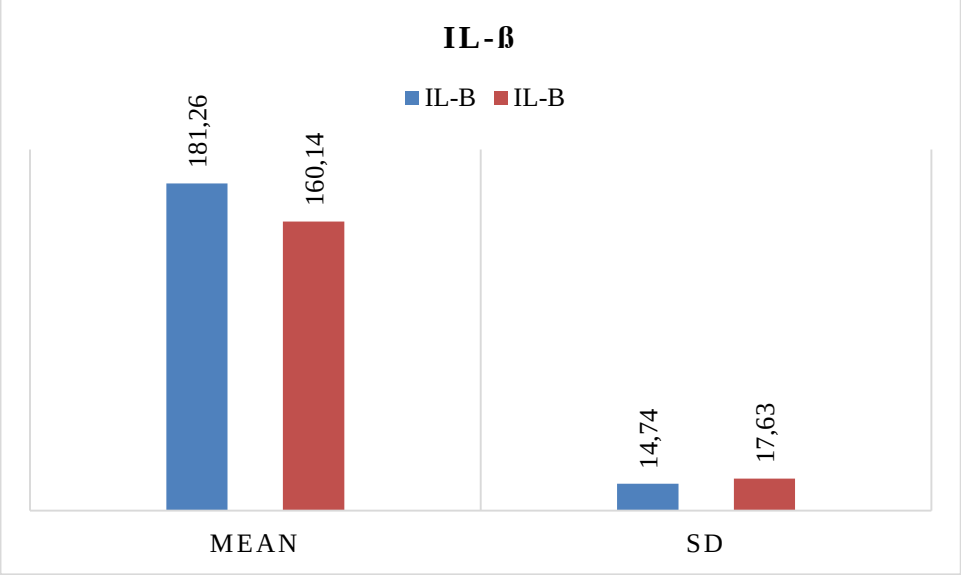

Figure 4.18 Bar Chart of the measurement and difference in the levels of IL-ß regarding gender

4.11 Correlation of Age and BMI With Other Variables

Table 4.8 shows sera and the correlation between age and BMI blood sugar, lipid profile, HBA1C, CRP, IL-1ß, DDP4, Urea, and Creatinine in diabetes mellitus type 2 patients. According to the data, there was a positive correlation between age and BMI.

The findings show that the lipid profile Triglyceride TG mg/dl, cholesterol mg/dl, HDL, and VLDL have a positive correlation with BMI, while HDL has a negative correlation with BMI. Age and other variables had no effect on the assessed variables.

Table 4.8 Pearson Correlation Coefficient (r) between biomarkers and each of Age and BMI

MARKERS	r	AGE	BMI
BMI	r	0.414	
	P value	0.031*	
Blood Sugar	r	-0.032	-0.042
	P value	0.784	0.679
TG	r	-0.019	0.394
	P value	0.897	0.034*
CH	r	-0.344	0.345
	P value	0.123	0.007*
VLDL	r	-0.201	0.346
	P value	0.333	0.021*
LDL	r	-0.069	0.453
	P value	0.234	0.001*
HDL	r	-0.04	-0.532
	P value	0.245	0.002*
HBA1C	r	-0.083	0.081
	P value	0.567	0.623
CRP	r	-0.231	0.182
	P value	0.522	0.643
Urea	r	0.099	0.288
	P value	0.463	0.129
Creatinine	r	0.108	0.055
	P value	0.478	0.345
DDP4	r	0.058	0.024
	P value	0.567	0.765
IL-ß	r	0.049	0.099
	P value	0.599	0.489

4.12 Discussion

Many medical conditions may be traced back to diabetes. In the long run, it may lead to serious health issues including heart disease, vascular disease, renal disease, and even blindness if untreated (Singh *et al.* 2014). Because it takes significant time and resources, is expensive, and has a detrimental influence on a person's quality of life, researchers have focused their attention on the causes and effects of type 2 diabetes in people.

In order to combat diabetes, anti-diabetic drugs work by reducing blood glucose levels or the overall amount of sugar in the body. Treatment options for gestational, type 1 and type 2 diabetes all rely on these medications. A wide array of anti-diabetic medicines are available. It is thought that the anti-diabetic drugs act by raising the body's insulin levels, improving its sensitivity to insulin (or lowering insulin resistance), or decreasing gastrointestinal glucose absorption.

Patients with type 2 diabetes and metabolic syndrome were given metformin, an insulin sensitizer, and a DDP4 inhibitor in this study to assess the impact on serum biomarker levels. Novel drugs such as DPP-4 inhibitors may help us better understand how inflammation IL-1b. plays a part in the development of type 2 diabetes, as well as increase our arsenal against it.

4.12.1 Results of the study group's demographic and clinical features

Those who were newly diagnosed with type 2 diabetes compared to those who were previously diabetic but using DDP4 inhibitors exhibited a difference in mean glucose levels of P 0.05 or less. The third trial contrasted diabetics who were taking metformin with those who were not. It was found that the three groups had slight differences (P 0.05) that were not significant. Findings that conflict with one another are found here. For example, (Campos and Unger 2021), According to the study's results, there were large variations across the study's participant age groups. It was discovered that the mean ages of people with diabetes and those in the control group were significantly different in the present investigation, which was in line with the previous results of (Kameda *et al.* 2020). Males and females who took part in the research showed no discernible disparities in their results based on gender.

Patients' groups receiving metformin, DDP4 inhibitors, and newly diagnosed diabetes mellitus type 2 all had BMIs of 4.9–12.75, 30.8–7.67, 29.1–4.67, and 29.9–8.61 Kg/m2 accordingly in Table 3-1 of the outcomes of the study. Between the healthy control group and the other groups, the mean SD of BMI differed significantly (Rafiq and Jeppesen 2018). Patients using DDP4 with metformin show a substantial drop in BMI

compared to the control group. Anti-diabetic medicines, particularly DDP4 inhibitors, have a considerable impact on weight reduction, according to clinical studies (Mitri and Hamdy 2009) found similar findings.

Diabetes and obesity are well-known risk factors for mortality from any cause as well as death from cardiovascular disease, according to the findings of this research. Type 2 diabetes mellitus patients are recommended to maintain a healthy weight as a therapeutic guideline since obesity has been connected to the disease's progression and prognosis (Jong *et al.* 2019). Weight gain associated with antidiabetic medicine is also considered an adverse effect. It's still unclear whether or not BMI is associated with death in patients with type 2 diabetes. Poorer results aren't shown in all groups when the ages of 2, 3, or obesity are present. As a result of the obesity paradox, obesity is linked with a lower death rate than normal weight in certain persons with heart failure, stable coronary artery disease, and acute coronary syndromes (ACS)

4.12.2 Blood glucose

The results of the current study showed that for blood glucose, there was a significant difference between the groups. 112.72, $p < .001$, means and standard deviations are shown in Table 3.3 and Figure 3.4. The mean serum glucose for the DDP4 inhibitor drugs (189.66 ± 17.71) was significantly lower than that of the other groups, $p < .001$. The mean blood glucose for the control (130.80 ± 16.20) was significantly lower than that of the metformin drug group (225.72 ± 101.35), $p < .001$ and the newly diagnosed type 2 diabetes group. In a study conducted by (Deshmukh *et al.* 2013). Control group blood glucose levels were lower than those of either of the other two groups, and researchers identified a strong correlation between blood glucose levels in the three groups and those of the control groups. The results of (Esfandiari et al et al, 2019), (Rafael Simó and Cristina Hernández) are in accord with these findings. Because glucose regulation is compromised, insulin is needed to keep blood sugar levels within normal ranges. Constantly elevated blood glucose levels cause long-term and irreversible damage to the human body. High blood sugar has been linked to an increased risk of cardiovascular disease and nerve damage. End-organ damage occurs in

anything from coronary artery disease to skin infections and strokes to microvascular consequences including diabetic nephropathy, peripheral neuropathy, and diabetic retinopathy. All of the above symptoms of diabetes (as well as weight loss, numbness or tingling in the extremities, inability to concentrate, impaired vision, increased urination) should be taken seriously (Rehani *et al.* 2019).

4.12.3 HBA1C

According to a comprehensive study, antidiabetic medicines had a smaller impact on HbA1c among newly diagnosed diabetics compared to those who are not using any anti-diabetic medications .This study showed significant differences in HbA1c mg/dL between groups, = 39.57, $p < 001$, the mean HBA1C for newly diagnosed patients (7.91 ± 1.56) was significantly higher than that of the control group (5.46 ± 5.51), $p < 001$ and also was Significantly higher than in patients taking metformin (7.42 ± 1.41), $p < .01$, and then in the group of patients taking DDP4 inhibitor drugs. The mean HBA1C for control (5.46 ± 5.51) was significantly lower than the other groups, for the main effect of the group. This study had previously agreed by (Kaiafa *et al.* 202, Sherifali *et al.* 2010). In line with Krishna et al. 2015, who reported that anti-diabetic therapy such as metformin and glibenclamide substantially lowered HbA1c percent levels compared to pre-treatment values (Krishna *et al.* 2015), this data confirms that the majority of anti-diabetes drugs cut HbA1c levels. Later studies indicated a strong correlation between HbA1c levels and glycemic control over a period of two to three months, leading to the use of the test in clinical practice in the 1980s. HbA1c has become a cornerstone in the care of diabetes thanks to the Diabetic Control and Problems Trial and the UK Diabetes Prospective Study, both of which link HbA1c readings to the development of diabetes problems. The Kim et al. research indicated that Asians and non-Asian (non-Japanese) groups had a larger drop in HbA1c after using anti-diabetic medication compared to Caucasians (Kim *et al.* 2013).

4.12.4 The impact of DDP4 inhibitors drug and metformin on lipid profile

These results in this study found comparing the extra glycemic effects of various antihyperglycemic agents on the lipid profile in type 2 diabetes in agreement with (Monami *et al.* 2012) there performed a meta-analysis examining the extra glycemic effects of numerous antihyperglycemic drugs on the lipid profile in type 2 diabetes. According to a recent study, the mean Serum Cholesterol for the newly diabetics group (186.00 ±17.87), was significantly higher than for Control (120.78 ± 25.96) p <0.001. The mean cholesterol for Control was significantly smaller than for patients taking metformin drugs (177.90 ±37.94), and for patients taking DDP4 inhibitors drugs (164.46±39.32). In this study analysis, DPP-4 inhibitors lowered total cholesterol and triglycerides. DPP-4inhibitors decreased total cholesterol somewhat more than other antihyperglycemic medications. There is some debate about the therapeutic significance of this tiny variation, however the effect of DPP-4 inhibitors is listed here as a "off-target" effect. While the control group had normal triglyceride levels, this group's levels were considerably higher (p> 0.05) (newly diagnosed patients). Serum triglycerides in diabetes individuals have increased, which might be attributable to an increase in hepatic triglyceride production. In comparison to the control group and metformin treatment, the groups treated with DDP4 inhibitors and those treated with metformin therapy differed significantly. DDP4 and lipid profile in diabetes mellitus patients were shown to be compatible with these results (Monami *et al.* 2012). Glucagon-like peptide 1 (GLP-1) and neuropeptide Y have been shown to be inactivated by DPP4 in lipid metabolism. Fat and oil metabolism is influenced directly by DPP4 (Fadini *et al.* 2012). An increase in the activity of the peroxisome proliferator-activated receptor (PPAR) and a reduction in the production of lipogenesis may be seen when the DPP4 gene is suppressed. While previous studies have shown a decrease in DPP4 levels in diabetic individuals, this new study shows the opposite effect. Steatosis of the liver is avoided. According to the research, diabetes duration, patient age, and blood glucose management may all play a role in these discrepancies. When the body is exposed to high levels of glucose for an extended period of time, researchers found that DPP4 production was also boosted (Itou *et al.* 2013). The HDL cholesterol (HDL-C) levels of those with type 2 diabetes are lower and the HDL function of those with type 2 diabetes

is reduced. Low HDL-C may be caused by insulin resistance, an increase in low-density lipoprotein production, and an increased activity of cholesteryl ester transfer protein and hepatic lipase, although the particular cause of low HDL-C in type 2 diabetes is not yet clear (Faddini *et al.* 2014). With type 2 diabetes, it's typical for patients to have abnormally low levels of high-density lipoprotein cholesterol (HDL-C) (high density lipoprotein). There is no evidence that low HDL-C levels induce type 2 diabetes. A rise in the activity of liver lipase and the cholesterol ester protein transporter may be caused by insulin resistance and high levels of HDL (low density lipoprotein) in the blood. This research was given the go-ahead by (Rizzo *et al.* 2009). The findings of serum low density lipoprotein for newly diagnosed diabetic patients and those receiving DDP4 inhibitors medicines were significantly different from those of the control group (p> 0.05). Type 2 diabetics had elevated LDL levels, according to a study published in (Kumar *et al.* 2011). Removal of VLDL particles leads to an increase in the production of IDL and LDL, both of which are intermediate density lipoproteins (Cagatay *et al.* 2009).

VLDL-C levels were shown to be significantly higher in diabetes patients as compared to controls (Gordon and Song 2009). In type 2 diabetics, increased hepatic VLDL-C synthesis is likely due of insulin resistance's effect on VLDL lipoprotein size and subclass particle concentrations.

4.12.5 The influence of age and BMI in type 2 diabetes patients with other parameters

Table 4.8 displays the serum concentrations of several biomarkers in diabetics with type 2 diabetes, as well as the relationship between age and BMI, blood sugar, lipid profile, HBA1C, CRP, IL-1ß, DDP4, urea, and creatinine. Age and BMI had a favorable relationship, according to the study. There is a positive link between BMI and TG mg/dl, cholesterin mg/dl, HDL and VLDL, but a negative correlation between HDL and BMI, according to the research results.

Today, being overweight or obese is described as having an excessive accumulation of adipose tissue, which is harmful to both one's physical and mental health. At this point in our society, obesity is the most frequent chronic physical illness. As the number of overweight and obese individuals continues to climb, it has become a major public health issue. When it comes to (Naser *et al.* 2006). Obesity is considered a public health concern in both developed and underdeveloped countries.. According to the World Health Organization, diabetes mellitus type 2 is a main impairment cause.

A rise in insulin resistance is linked to the development of both type 2 diabetes and obesity. Insulin resistance does not cause hyperglycemia in the overwhelming majority of obese individuals. When the pancreas' islet of Langerhans cells produce enough insulin to counteract drops in insulin levels, normal glucose tolerance is reached. Type 2 diabetes' natural history shows that endothelial dysfunction and obesity/insulin resistance are inseparable. Inclusion is made for those with reduced glucose tolerance and/or low fasting glucose. It is possible that cells may adapt to a lower glucose tolerance, although this is not guaranteed. A rise in insulin resistance and lipids, as well as a reduction in insulin sensitivity, are all possible causes of type 2 diabetes. Obese people's adipose tissue generates non-esterified fatty acids (NEFAs), which shows a connection between insulin resistance and cellular malfunction (Sowers and Frohlich 2004).

In terms of age-related BMI heterogeneity, no one knows what mechanism is at work. According to Lumeng and Saltiel, young-onset obesity is associated with an increased risk of metabolic disorders due to circulating levels of free fatty acids and adipokines, which impair insulin sensitivity and may result in an increase in reactive oxygen species and insulin secretion (Lumeng and Saltiel 2011). Young-onset obesity may be connected to maternal undernutrition or overnutrition as a child, which has been linked to an increased risk of type 2 diabetes in the long run (Abdullah *et al.* 2010).

Pettitt DJ, et al. approved the correlation between age and BMI in this investigation. Obesity, insulin resistance, hyperglycemia, and other cardiovascular risk factors may be caused by a sedentary lifestyle in those who are already fat. (Pettitt and colleagues

2008) As a consequence, the diabetes preventive advantages that come with being a young adult are lost on obese young people. Sarcopenia, as established by Dollman J et al., is thought to be the cause of the weight loss that occurs as individuals age, since it causes muscle mass to decline while fat mass rises somewhat. In 2005, (Dollman J, Norton K), (Another aging-related characteristic is fat redistribution. Metabolic problems are more likely to occur in people who are viscerally obese because of the shift of subcutaneous fat into visceral fat. As a result, these age-related increases in BMI are more likely to worsen rather than alleviate the relationship between obesity and diabetes. According to our data, BMI is associated with an increased risk of developing diabetes, regardless of age. As this study demonstrates, BMI has a far higher impact on the development of diabetes in children and adolescents than previously thought. This new study may help explain why so many young people are acquiring diabetes as a consequence of their obesity and weight gain. Obesity and weight increase, especially in young individuals, are critical risk factors for developing diabetes. They agreed to participate in this study because Chen et al. found that being overweight was associated with a higher risk of developing type 2 diabetes in China (Chen *et al.* 2018).

4.12.6 Prevalent type 2 diabetes and anti-inflammatory cytokines IL-1ß

Advanced stages of type 2 diabetes need insulin treatment because cell activity deteriorates. Because of the combination of hereditary and environmental variables, together with secondary events, such as hyperglycemia-induced cell function impairment and apoptosis, islet degeneration in late type 2 diabetes is considered to occur. Another possibility is that apoptotic cells might trigger an immune response when they are exposed to cytokines like IL-1ß or are in high abundance (Bellone *et al.* 1997, Henry 2000). It was shown that higher glucose concentrations stimulated IL-1ß, which was discovered by Lacy and Finke in 1991 as part of an autoimmune process. Islet macrophages are critical to the development of autoimmune diabetes. IL-1ß is generated and released when local macrophages are stimulated. Arnush and colleagues 1998. IL-1ß generation in rat islet cells was recently shown to be boosted by viral replicative intermediate double-stranded RNA, indicating that viral infection may be a factor in the development of autoimmune diabetic cell damage. According to the 2001

research by Heitmeier and colleagues, mouse cells stimulated in this way generate the IL-1ß converting enzyme needed for IL-1ß activation, and similar findings apply to human cells that may make IL-1ß in response to glucose without the assistance of a virus or an immune response. Found in tissue sections from diabetes individuals are cells that produce interleukin-1ß. Insulin-producing cells are damaged and destroyed by hyperglycemia, and this process resembles an immune-mediated process. Type 2 diabetes may have its genesis in the proinflammatory cytokine IL-1ß, which may contribute to cell glucotoxicity.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

- The biochemical analyses done on large groups spanning the whole T2D population as part of this research are an essential addition to the significant clinical studies to regulate and enhance the treatment of individuals with type 2 diabetes.
- Diabetic patients' cytokines, in particular IL-1ß, are highlighted by this research.
- Supporting the current recommendation for DDP inhibitors as a first-line agent for T2D. These results indicate that more patients can benefit from DDP4.
- Gender has no effect on patient type 2 diabetes.
- The inflammatory such as IL-1ß and c-reactive protein biomarkers are good predictors for accelerating the DM2disease .
- Obesity induced the type 2 diabetes, and may contribution development of this disease.

5.2 Recommendations

- More valuable research and studies should be provided about the long-term efficacy, side effects, and safety of this new class of drug agents.
- Next studies should be planned to include more than 50,000 patients with T2DM and follow them for 4-5 years.
- We conclude that our understanding of anti-inflammatory proteins and their role in the normal development of insulin resistance, cell dysfunction, and type 2 diabetes is currently limited.
- The same can be said about the ability of cytokines, especially interleukin-1-beta, to improve metabolic regulation by modulating immune systems.
- we have a long list of prospective proteins that need to be examined and will be investigated in the future.

REFERENCES

Abdullah, A., Peeters, A., de Courten, M. and Stoelwinder, J. 2010. The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies. Diabetes research and clinical practice., 89(3): 309-319.

Abusaib, M., Ahmed, M., Nwayyir, H. A., Alidrisi, H. A., Al-Abbood, M., Al-Bayati, A. and Mansour, A. 2020. Iraqi experts consensus on the management of type 2 diabetes/prediabetes in adults. Clinical Medicine Insights: Endocrinology and Diabetes., 13: 1179551420942232.

Ahmed, A., Jawad, S. R., Majeed, and Hussein, M. O. 2020. "Biochemistry and Molecular Cell Biology of Diabetic Complications." Systematic Reviews in Pharmacy., 11(11): 50–60.

Ahrén, B. O., Simonsson, E., Larsson, H., Landin-Olsson, M., Torgeirsson, H., Jansson, P. A. and Holmes, D. 2002. Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. Diabetes care., 25(5): 869-875.

Ambade, V. N., Sharma, Y. V. and Somani, B. L. 1998. Methods for estimation of blood glucose: a comparative evaluation. Medical Journal Armed Forces India., 54(2): 131-133.

Andargie, T. E. and Ejara, E. D. 2015. Pro-and anti-inflammatory cytokines in visceral leishmaniasis. Journal of Cell Science and Therapy., 6(2): 1.

Andrade-Cetto, A. and Heinrich, M. 2005. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. Journal of ethnopharmacology., 99(3): 325-348.

Angerås, O., Albertsson, P., Karason, K., Råmunddal, T., Matejka, G., James, S. and Omerovic, E. 2013. Evidence for obesity paradox in patients with acute coronary syndromes: a report from the Swedish Coronary Angiography and Angioplasty Registry. European heart journal., 34(5): 345-353.

Arafat, E. S., Taha, I. M., Kattan, S. W., Babteen, N. and Fawzy, I. 2020. Associations between Vitamin D and Type 2 Diabetes Mellitus: The Role of Vitamin D Receptor and Binding Protein. Journal of Diabetes Mellitus., 10(04): 222.

Arnush, M., Scarim, A. L., Heitmeier, M. R., Kelly, C. B. and Corbett, J. A. 1998. Potential role of resident islet macrophage activation in the initiation of autoimmune diabetes. The Journal of Immunology., 160(6): 2684-2691.

Atlas, I. D. F. 1955. International Diabetes Federation. Sciences., 2: 66.

Augeri, D. J., Robl, J. A., Betebenner, D. A., Magnin, D. R., Khanna, A., Robertson, J. G. and Hamann, L. G. 2005. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Journal of medicinal chemistry., 48(15): 5025-5037.

Bagust, A. and S. Beale. 2003. "Deteriorating Beta-Cell Function in Type 2 Diabetes: A Long-Term Model." QJM - Monthly Journal of the Association of Physicians., 96(4): 281–88.

Bailey, C. J. and Day, C. 2004. Metformin: its botanical background. Practical Diabetes International., 21(3): 115-117.

Banik, S., Kaisar, M. M. and Hossain, M. S. 2013. Development and validation of a simple and rapid uv spectrophotometer method for assay of linagliptin in bulk and marketed dosage form. Indian. J. Nov. Drug. Delivery., 5: 221-224.

Bell, G. I., Santerre, R. F. and Mullenbach, G. T. 1983. Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature., 302(5910): 716-718.

Bellone, M., Iezzi, G., Rovere, P., Galati, G., Ronchetti, A., Protti, M. P. and Manfredi, A. A. 1997. Processing of engulfed apoptotic bodies yields T cell epitopes. The Journal of Immunology., 159(11): 5391-5399.

Berger, J. and D. Moller. 2002. "The Mechanisms of Action of Ppars." Annual Review of Medicine., 53: 35–40.

Berghe, G. 2004. How does blood glucose control with insulin save lives in intensive care. The Journal of clinical investigation., 114(9): 1187-1195.

Bharti, S. K., Krishnan, S., Kumar, A., Rajak, K. K., Murari, K., Bharti, B. K. and Gupta, A. K. 2012. Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of Castanospermum australe: Investigation by experimental validation and molecular docking. Phytomedicine., 20(1): 24-31.

Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A. and Legssyer, A. 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). International Journal of Diabetes and Metabolism., 14(1): 1.

Brison, D. W. 2017. "Definition, Diagnosis, and Classification." Ameliorating Mental Disability: Questioning Retardation., 1: 19.

Brubaker, P. L. and D. J. Drucker. 2004. "Minireview: Glucagon-like Peptides Regulate Cell Proliferation and Apoptosis in the Pancreas, Gut, and Central Nervous System." Endocrinology., 145(6): 53–59.

Burness, C. B. 2015. Omarigliptin: first global approval. Drugs., 75(16): 1947-1952.

Buysschaert, M., Dramais, A. S., Wallemacq, P. E. and Hermans, M. P. 2000. Hyperhomocysteinemia in type 2 diabetes: relationship to macroangiopathy, nephropathy, and insulin resistance. Diabetes care., 23(12): 1816-1822.

Cagatay, P., Susleyici-Duman, B., Alasya, H. and Ipbuker, A. 2009. Effects of oral antidiabetic drugs over lipid parameters in Turkish type 2 diabetes patients. Acta Medica Academica., 38(2): 77-85.

Campos, C. and Unger, J. 2021. Primary care management of type 2 diabetes: a comparison of the efficacy and safety of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Postgraduate medicine., 133(8): 843-853.

Care, D. 2019. of Medical Care in Diabetesd2019. Diabetes Care., 42: S7.

Carr, R. D. 2016. "Drug Development from the Bench to the Pharmacy: With Special Reference to Dipeptidyl Peptidase-4 Inhibitor Development." Diabetic Medicine., 33(6): 18–22.

Caspersen, C. J., Thomas, G. D., Boseman, L. A., Beckles, G. L. and Albright, A. L. 2012. Aging, diabetes, and the public health system in the United States. American journal of public health., 102(8): 1482-1497.

Cefalu, W. T., Berg, E. G., Saraco, M., Petersen, M. P., Uelmen, S. and Robinson, S. 2019. Glycemic Targets: Standards of Medical Care in Diabetes-2019. Diabetes Care., 42: S61-S70.

Chen, Y., Zhang, X. P., Yuan, J., Cai, B., Wang, X. L., Wu, X. L. and Li, X. Y. 2018. Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. BMJ open., 8(9): e021768.

Clifford, R. M., Davis, W. A., Batty, K. T. and Davis, T. M. 2005. Effect of a pharmaceutical care program on vascular risk factors in type 2 diabetes: the Fremantle Diabetes Study. Diabetes care., 28(4): 771-776.

Conget, I. 2002. Diagnóstico, clasificación y patogenia de la diabetes mellitus. Revista española de cardiología., 55(5): 528-535.

Cordero, O. J., Salgado, F. J. and Nogueira, M. 2009. On the origin of serum CD26 and its altered concentration in cancer patients. Cancer immunology, immunotherapy., 58(11): 1723-1747.

Dahlström, E. and Sandholm, N. 2017. Progress in defining the genetic basis of diabetic complications. Current diabetes reports., 17(9): 1-13.

Dall, T. M., Zhang, Y., Chen, Y. J., Wagner, R. C. A., Hogan, P. F., Fagan, N. K. and Tornberg, D. N. 2007. Cost Associated with Being Overweight and with Obesity, High Alcohol Consumption, and Tobacco Use within the Military Health System's TRICARE Prime—Enrolled Population. American Journal of Health Promotion., 22(2): 120-139.

Darmoul, D., Lacasa, M., Chantret, I., Swallow, D. M. and Trugnan, G. 1990. Isolation of a cDNA probe for the human intestinal dipeptidylpeptidase IV and assignment of the gene locus DPP4 to chromosome 2. Annals of human genetics., 54(3): 191-197.

DePaula, A. L., Macedo, A. L. V., Rassi, N., Machado, C. A., Schraibman, V., Silva, L. Q. and Halpern, A. 2008. Laparoscopic treatment of type 2 diabetes mellitus for patients with a body mass index less than 35. Surgical endoscopy., 22(3): 706-716.

Deshmukh, Vinod, Vijay Motghare, Sudhir Padwal, Swapnil Jaykare, Jyoti Patil, and Harshal Pise. 2013. “Newer Drugs in the Management of Diabetes Mellitus.” International Journal of Basic and Clinical Pharmacology 2(1):4. doi: 10.5455/2319-2003.ijbcp20130102.

Deverman, B. E. and Patterson, P. H. 2009. Cytokines and CNS development. Neuron., 64(1): 61-78.

Diabetes, Description O. F. 2013. “Diagnosis and Classification of Diabetes Mellitus.” Diabetes Care., 36(1): 67–74.

Dollman, J., Norton, K. and Norton, L. 2005. Evidence for secular trends in children's physical activity behaviour. British journal of sports medicine., 39(12): 892-897.

Donath, M. Y. 2014. Targeting inflammation in the treatment of type 2 diabetes: time to start. Nature reviews Drug discovery., 13(6): 465-476.

Dreyer, M. 2019. Typ-1-Diabetes. Der Diabetologe., 15(5): 400-407.

Duffy, J. L., Kirk, B. A., Wang, L., Eiermann, G. J., He, H., Leiting, B. and Weber, A. E. 2007. 4-aminophenylalanine and 4-aminocyclohexylalanine derivatives as potent, selective, and orally bioavailable inhibitors of dipeptidyl peptidase IV. Bioorganic and medicinal chemistry letters., 17(10): 2879-2885.

Elhannan, S., Taha, S., Ben Khalaf, N., Bakheit, H., Fathallah, M. D. and Bakhiet, M. 2015. Induction of dissociated cytokine profiles by ISRAA with selective critical involvement of ERK1/2 in its signaling functions. International journal of molecular medicine., 36(6): 1583-1592..

Engel, M., Hoffmann, T., Wagner, L., Wermann, M., Heiser, U., Kiefersauer, R. and Brandstetter, H. 2003. The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. Proceedings of the National Academy of Sciences., 100(9): 5063-5068.

Fadini, G. P., Albiero, M., Menegazzo, L., De Kreutzenberg, S. V. and Avogaro, A. 2012. The increased dipeptidyl peptidase-4 activity is not counteracted by optimized glucose control in type 2 diabetes, but is lower in metformin-treated patients. Diabetes, Obesity and Metabolism., 14(6): 518-522.

Fadini, G. P., Iori, E., Marescotti, M. C., de Kreutzenberg, S. V. and Avogaro, A. 2014. Insulin-induced glucose control improves HDL cholesterol levels but not reverse cholesterol transport in type 2 diabetic patients. Atherosclerosis., 235(2): 415-417.

Fu, Z., R Gilbert, E. and Liu, D. 2013. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Current diabetes reviews., 9(1): 25-53.

Gallen, I. W. 2004. Helping the athlete with type 1 diabetes. The British Journal of Diabetes and Vascular Disease., 4(2): 87-92.

Ghate, M. and V Jain, S. 2013. Structure based lead optimization approach in discovery of selective DPP4 inhibitors. Mini Reviews in Medicinal Chemistry., 13(6): 888-914.

Ghosh, S., Liu, H., Yazdankhah, M., Stepicheva, N., Shang, P., Vaidya, T. and Sinha, D. 2021. βA1-crystallin regulates glucose metabolism and mitochondrial function in mouse retinal astrocytes by modulating PTP1B activity. Communications biology., 4(1): 1-19.

Goettig, P., Groll, M., Kim, J. S., Huber, R. and Brandstetter, H. 2002. Structures of the tricorn-interacting aminopeptidase F1 with different ligands explain its catalytic mechanism. The EMBO journal., 21(20): 5343-5352.

Gordon, G. and Song. S. 2009. J Ournal of Bi Omedical S Cience and E Ngineering. Journal of Biomedical Science., 2(3): 24–30.

Gregor, M. F. and Hotamisligil, G. S. 2011. Inflammatory mechanisms in obesity. Annual review of immunology., 29(1): 415-445.

Hahl, J., Hämäläinen, H., Sintonen, H., Simell, T., Arinen, S. and Simell, O. 2002. Health-related quality of life in type 1 diabetes without or with symptoms of long-term complications. Quality of Life Research., 11(5): 427-436.

Haque, N., Salma, U., Nurunnabi, T. R., Uddin, M. J., Jahangir, M. F., Islam, S. M. and Kamruzzaman, M. 2011. Management of type 2 diabetes mellitus by lifestyle, diet and medicinal plants. Pakistan journal of biological sciences: PJBS., 14(1): 13-24.

Haus, J. M., Solomon, T. P., Marchetti, C. M., Edmison, J. M., Gonzalez, F. and Kirwan, J. P. 2010. Free fatty acid-induced hepatic insulin resistance is attenuated following lifestyle intervention in obese individuals with impaired glucose tolerance. The Journal of Clinical Endocrinology and Metabolism., 95(1): 323-327.

Havale, S. H. and Pal, M. 2009. Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. Bioorganic and medicinal chemistry., 17(5): 1783-1802.

Heitmeier, M. R., Arnush, M., Scarim, A. L. and Corbett, J. A. 2001. Pancreatic β-cell damage mediated by β-cell production of interleukin-1: a novel mechanism for virus-induced diabetes. Journal of Biological Chemistry., 276(14): 11151-11158.

Henry, W. L. 2000. Perspectives in Diabetes. Journal of the National Medical Association., 54(1): 76–78.

Holst, J. J., Ørskov, C., Vagn Nielsen, O. and Schwartz, T. W. 1987. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS letters., 211(2): 169-174.

Hopsu-Havu, V. K. and Glenner, G. G. 1966. A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-β-naphthylamide. Histochemie., 7(3): 197-201.

Horwich, T. B., Fonarow, G. C., Hamilton, M. A., MacLellan, W. R., Woo, M. A. and Tillisch, J. H. 2001. The relationship between obesity and mortality in patients with heart failure. Journal of the American College of Cardiology., 38(3): 789-795.

Huang, J., Liu, X., Wei, Y., Li, X., Gao, S., Dong, L. and Zhong, J. 2022. Emerging Role of Dipeptidyl Peptidase-4 in Autoimmune Disease. Frontiers in Immunology., 1: 3.

Huecas, S. and Andreu, J. M. 2004. Polymerization of nucleotide-free, GDP-and GTP-bound cell division protein FtsZ: GDP makes the difference. FEBS letters., 569(1-3): 43-48.

Ieronymaki, E., Daskalaki, M. G., Lyroni, K. and Tsatsanis, C. 2019. Insulin signaling and insulin resistance facilitate trained immunity in macrophages through metabolic and epigenetic changes. Frontiers in Immunology., 10: 1330.

Ito, K., Inoue, T., Kabashima, T., Kanada, N., Huang, H. S., Ma, X. and Yoshimoto, T. 2000. Substrate recognition mechanism of prolyl aminopeptidase from Serratia marcescens. The Journal of Biochemistry., 128(4): 673-678.

Itou, M., Kawaguchi, T., Taniguchi, E. and Sata, M. 2013. Dipeptidyl peptidase-4: a key player in chronic liver disease. World journal of gastroenterology: WJG., 19(15): 2298.

Jadav, P., Bahekar, R., Shah, S. R., Patel, D., Joharapurkar, A., Kshirsagar, S. and Sairam, K. V. 2012. Long-acting peptidomimetics based DPP-IV inhibitors. Bioorganic and medicinal chemistry letters., 22(10): 3516-3521.

Janardhan, S. and Padmanabha Reddy, Y. 2011. Homology modeling and molecular docking studies of human DPP8 and DPP9. International Journal of Pharma Research and Development., 2(12): 131-146.

Jong, C. B., Li, H. Y., Pan, S. L., Hsieh, M. Y., Su, F. Y., Chen, K. C. and Wu, C. C. 2019. Relationship between body mass index, antidiabetic agents, and midterm

mortality in patients with both type 2 diabetes mellitus and acute coronary syndrome. Journal of the American Heart Association., 8(7): e011215.

Kaiafa, G., Veneti, S., Polychronopoulos, G., Pilalas, D., Daios, S., Kanellos, I. and Savopoulos, C. 2021. Is HbA1c an ideal biomarker of well-controlled diabetes?. Postgraduate Medical Journal., 97(1148): 380-383.

Kameda, T., Kumamaru, H., Nishimura, S., Kohsaka, S. and Miyata, H. 2020. Use of oral antidiabetic drugs in Japanese working-age patients with type 2 diabetes mellitus: dosing pattern for metformin initiators. Current Medical Research and Opinion., 36(5): 749-756.

Kameoka, J., Tanaka, T., Nojima, Y., Schlossman, S. F. and Morimoto, C. 1993. Direct association of adenosine deaminase with a T cell activation antigen, CD26. Science., 261(5120): 466-469.

Kang, N. S., Ahn, J. H., Kim, S. S., Chae, C. H. and Yoo, S. E. 2007. Docking-based 3D-QSAR study for selectivity of DPP4, DPP8, and DPP9 inhibitors. Bioorganic and medicinal chemistry letters., 17(13): 3716-3721.

Keserű, G. M. and Makara, G. M. 2006. Hit discovery and hit-to-lead approaches. Drug discovery today., 11(15-16): 741-748.

KESKİN, A., Recai, A. C. İ., DURAN, U. and SUGEÇTİ, S. 2021. Physiological and Anti-obesity Effects of Melatonin and Niacin Supplements in Rat Models. Caucasian Journal of Science., 8(1): 27-37.

Kilmer, P. D. 2010. Review Article: Doug Underwood Journalism and the Novel: Truth and Fiction, 1700—2000 New York: Cambridge University Press, 2008. 269 pp. ISBN 978 0 89952 9 Jan Whitt Settling the Borderland: Other Voices in Literary Journalism Lanham, MD: University Press of America, 2008. 178 pp. ISBN 978 07618 4093 0 Sonja Merljak Zdovc Literary Journalism in the United States of America and Slovenia Lanham, MD: University Press of America, 2008. 146 pp. ISBN 978 0 7618 4156 2. Journalism., 11(3): 369-373.

Kim, Y. G., Hahn, S., Oh, T. J., Kwak, S. H., Park, K. S. and Cho, Y. M. 2013. Differences in the glucose-lowering efficacy of dipeptidyl peptidase-4 inhibitors between Asians and non-Asians: a systematic review and meta-analysis. Diabetologia., 56(4): 696-708.

Kong, T. H. and Hong. E. 1929. Medical Diary. The Lancet., 213(5521): 34–35.

Koya, D. and King, G. L. 1998. Protein kinase C activation and the development of diabetic complications. Diabetes., 47(6): 859-866.

Krishna, R., Addy, C., Tatosian, D., Glasgow, X. S., Gendrano III, I. N., Robberechts, M. and Stoch, S. A. 2016. Pharmacokinetics and pharmacodynamics of omarigliptin, a once-weekly dipeptidyl peptidase-4 (DPP-4) inhibitor, after single and multiple doses in healthy subjects. The Journal of Clinical Pharmacology., 56(12): 1528-1537.

Kumar, A. and Singh, G. 2011. Relationship among HbA1c and lipid profile in Punajbi type 2 diabetic population. Journal of Exercise Science and Physiotherapy., 7(2): 99-102.

Lacy, P. E. 1994. The intraislet macrophage and type I diabetes. The Mount Sinai journal of medicine, New York., 61(2): 170-174.

Lacy, P. E. and Finke, E. H. 1991. Activation of intraislet lymphoid cells causes destruction of islet cells. The American journal of pathology., 138(5): 1183.

Lakhtakia, R. 2013. The history of diabetes mellitus. Sultan Qaboos University Medical Journal., 13(3): 368.

Lambeir, A. M., Durinx, C., Scharpé, S. and De Meester, I. 2003. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. Critical reviews in clinical laboratory sciences., 40(3): 209-294.

Lammi, C., Zanoni, C., Arnoldi, A. and Vistoli, G. 2016. Peptides derived from soy and lupin protein as dipeptidyl-peptidase IV inhibitors: in vitro biochemical screening and in silico molecular modeling study. Journal of agricultural and food chemistry., 64(51): 9601-9606.

Lebowitz, D., Gariani, K., Kressmann, B., Von Dach, E., Huttner, B., Bartolone, P. and Uçkay, I. 2017. Are antibiotic-resistant pathogens more common in subsequent episodes of diabetic foot infection?. International Journal of Infectious Diseases., 59: 61-64.

Lee, A. A., Graham, D. A., Dela Cruz, S., Ratcliffe, A. and Karlon, W. J. 2002. Fluid shear stress-induced alignment of cultured vascular smooth muscle cells. J. Biomech. Eng., 124(1): 37-43.

Lu, I. L., Tsai, K. C., Chiang, Y. K., Jiaang, W. T., Wu, S. H., Mahindroo, N. and Wu, S. Y. 2008. A three-dimensional pharmacophore model for dipeptidyl peptidase IV inhibitors. European journal of medicinal chemistry., 43(8): 1603-1611.

Lumeng, C. N. and Saltiel, A. R. 2011. Inflammatory links between obesity and metabolic disease. The Journal of clinical investigation., 121(6): 2111-2117.

Malviya, N., Jain, S. and Malviya, S. A. P. N. A. 2010. Antidiabetic potential of medicinal plants. Acta pol pharm., 67(2): 113-118.

Mandrup-Poulsen, T., Bendtzen, K., Dinarello, C. A. and Nerup, J. 1987. Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic beta-cell cytotoxicity. The Journal of Immunology., 139(12): 4077-4082.

Mandrup-Poulsen, T., Pickersgill, L. and Donath, M. Y. 2010. Blockade of interleukin 1 in type 1 diabetes mellitus. Nature Reviews Endocrinology., 6(3): 158-166.

Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C. and del Cañizo-Gómez, F. J. 2016. Update on the treatment of type 2 diabetes mellitus. World journal of diabetes., 7(17): 354.

Medrano, F. J., Alonso, J., Garcia, J. L., Romero, A., Bode, W. and Gomis-Ruth, F. X. 1998. Structure of proline iminopeptidase from Xanthomonas campestris pv. citri: a prototype for the prolyl oligopeptidase family. The EMBO journal., 17(1): 1-9.

Merz, C. N. B., Buse, J. B., Tuncer, D. and Twillman, G. B. 2002. Physician attitudes and practices and patient awareness of the cardiovascular complications of diabetes. Journal of the American College of Cardiology., 40(10): 1877-1881.

Michael, E. S., Covic, L. and Kuliopulos, A. 2019. Trace amine–associated receptor 1 (TAAR1) promotes anti-diabetic signaling in insulin-secreting cells. Journal of Biological Chemistry., 294(12): 4401-4411.

Mitri, J. and Hamdy, O. 2009. Diabetes medications and body weight. Expert opinion on drug safety., 8(5): 573-584.

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S. and Devasagayam, T. P. A. 2007. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition., 40(3): 163-173.

Mojsov, S., Weir, G. C. and Habener, J. F. 1987. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. The Journal of clinical investigation., 79(2): 616-619.

Monami, M., Lamanna, C., Desideri, C. M. and Mannucci, E. 2012. DPP-4 inhibitors and lipids: systematic review and meta-analysis. Advances in therapy, 29(1), 14-25.

Mulvihill, E. E. and Drucker, D. J. 2014. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocrine reviews., 35(6): 992-1019.

Mulvihill, E. E. and Drucker, D. J. 2014. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocrine reviews., 35(6): 992-1019.

Naser, K. A., Gruber, A. and Thomson, G. A. 2006. The emerging pandemic of obesity and diabetes: are we doing enough to prevent a disaster. International journal of clinical practice., 60(9): 1093-1097.

Nathan, D. M. and DCCT/Edic Research Group. 2014. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes care., 37(1): 9-16.

Nathan, D. M., Schreiber, E., Fogel, H., Mojsov, S. and Habener, J. F. 1992. Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects. Diabetes Care., 15(2): 270-276.

Nauck, M. A., Kleine, N., Ørskov, C., Holst, J. J., Willms, B. and Creutzfeldt, W. 1993. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia., 36(8): 741-744.

Nauck, M. A., Wollschläger, D., Werner, J., Holst, J. J., Ørskov, C., Creutzfeldt, W. and Willms, B. 1996. Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7–36 amide]) in patients with NIDDM. Diabetologia., 39(12): 1546-1553.

Ong, N. T. T., Bowcock, A. and Hodges, N. J. 2010. Manipulations to the timing and type of instructions to examine motor skill performance under pressure. Frontiers in Psychology., 1: 196.

Orita, M., Warizaya, M., Amano, Y., Ohno, K. and Niimi, T. 2009. Advances in fragment-based drug discovery platforms. Expert Opinion on Drug Discovery., 4(11): 1125-1144.

ØRSKOV, C., Holst, J. J., Knuhtsen, S., Baldissera, F. G., Poulsen, S. S. and Nielsen, O. V. 1986. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. Endocrinology., 119(4): 1467-1475.

Pasinetti, G. M., Wang, J., Porter, S. and Ho, L. 2011. Caloric intake, dietary lifestyles, macronutrient composition, and Alzheimer'disease dementia. International journal of Alzheimer's disease., 2: 11.

Pathirana, M. M., Lassi, Z. S., Ali, A., Arstall, M. A., Roberts, C. T. and Andraweera, P. H. 2021. Association between metabolic syndrome and gestational diabetes mellitus in women and their children: a systematic review and meta-analysis. Endocrine., 71(2): 310-320.

Pattzi, H. M. R., Pitale, S., Alpizar, M., Bennett, C., O'Farrell, A. M., Li, J. and PHX1149-PROT202 Study Group. 2010. Dutogliptin, a selective DPP4 inhibitor, improves glycaemic control in patients with type 2 diabetes: a 12-week, double-blind, randomized, placebo-controlled, multicentre trial. Diabetes, Obesity and Metabolism., 12(4): 348-355.

Pettitt, D. J., Lawrence, J. M., Beyer, J., Hillier, T. A., Liese, A. D., Mayer-Davis, B. and Dabelea, D. 2008. Association between maternal diabetes in utero and age at offspring's diagnosis of type 2 diabetes. Diabetes care., 31(11): 2126-2130.

Prabhakar, P. K. and Doble, M. 2008. A target based therapeutic approach towards diabetes mellitus using medicinal plants. Current Diabetes Reviews., 4(4): 291-308.

Prabhakar, P. K. and Doble, M. 2011. Mechanism of action of natural products used in the treatment of diabetes mellitus. Chinese journal of integrative medicine., 17(8): 563-574.

Rafiq, S. and Jeppesen, P. B. 2018. Body mass index, vitamin D, and type 2 diabetes: a systematic review and meta-analysis. Nutrients., 10(9): 1182.

Rehani, P. R., Iftikhar, H., Nakajima, M., Tanaka, T., Jabbar, Z. and Rehani, R. N. 2019. Safety and mode of action of diabetes medications in comparison with 5-aminolevulinic acid (5-ALA). Journal of Diabetes Research., 9: 1.

Rizzo, M., Rizvi, A. A., Spinas, G. A., Rini, G. B. and Berneis, K. 2009. Glucose lowering and anti-atherogenic effects of incretin-based therapies: GLP-1

analogues and DPP-4-inhibitors. Expert opinion on investigational drugs., 18(10): 1495-1503.

Rollinger, J. M., Schuster, D., Danzl, B., Schwaiger, S., Markt, P., Schmidtke, M. and Stuppner, H. 2009. In silico target fishing for rationalized ligand discovery exemplified on constituents of Ruta graveolens. Planta medica., 75(03): 195-204.

Rosenblat, J. D., Cha, D. S., Mansur, R. B. and McIntyre, R. S. 2014. Inflamed moods: a review of the interactions between inflammation and mood disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry., 53: 23-34.

Roussel, R., Travert, F., Pasquet, B., Wilson, P. W., Smith, S. C., Goto, S. and Reduction of Atherothrombosis for Continued Health (REACH) Registry Investigators. 2010. Metformin use and mortality among patients with diabetes and atherothrombosis. Archives of internal medicine., 170(21): 1892-1899.

Saisho, Y. 2014. Glycemic variability and oxidative stress: a link between diabetes and cardiovascular disease?. International journal of molecular sciences., 15(10): 18381-18406.

Scheen, A. J. 1996. Clinical pharmacokinetics of metformin. Clinical pharmacokinetics., 30(5): 359-371.

Scott, R., Wu, M., Sanchez, M. and Stein, P. 2007. Efficacy and tolerability of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy over 12 weeks in patients with type 2 diabetes. International journal of clinical practice., 61(1): 171-180.

Serreze, D. V., Ottendorfer, E. W., Ellis, T. M., Gauntt, C. J. and Atkinson, M. A. 2000. Acceleration of type 1 diabetes by a coxsackievirus infection requires a preexisting critical mass of autoreactive T-cells in pancreatic islets. Diabetes., 49(5): 708-711.

Sethi, J. K. and Hotamisligil, G. S. 1999. The role of TNFα in adipocyte metabolism. In Seminars in cell and developmental biology., 10(1): 19-29.

Sherifali, D., Nerenberg, K., Pullenayegum, E., Cheng, J. E. and Gerstein, H. C. 2010. The effect of oral antidiabetic agents on A1C levels: a systematic review and meta-analysis. Diabetes care., 33(8): 1859-1864.

Silva Xavier, G. 2018. The cells of the islets of Langerhans. Journal of clinical medicine., 7(3): 54.

Silveira, S. T., Martínez-Maqueda, D., Recio, I. and Hernández-Ledesma, B. 2013. Dipeptidyl peptidase-IV inhibitory peptides generated by tryptic hydrolysis of a whey protein concentrate rich in β-lactoglobulin. Food chemistry., 141(2): 1072-1077.

Sima, C. and Glogauer, M. 2013. Diabetes mellitus and periodontal diseases. Current diabetes reports., 13(3): 445-452.

Sinclair, A., Saeedi, P., Kaundal, A., Karuranga, S., Malanda, B. and Williams, R. 2020. Diabetes and global ageing among 65–99-year-old adults: Findings from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice., 162: 108078.

Singh, A. K., Yadav, D., Sharma, N. and Jin, J. O. 2021. Dipeptidyl Peptidase (DPP)-IV inhibitors with antioxidant potential isolated from natural sources: A novel approach for the management of Diabetes. Pharmaceuticals., 14(6): 586.

Singh, R., Rao, H. K. and Singh, T. G. 2020. Advanced glycated end products (ages) in diabetes and its complications: an insight. Plant Arch., 20(1): 3838-3841.

Skyler, J. S., Bakris, G. L., Bonifacio, E., Darsow, T., Eckel, R. H., Groop, L. and Ratner, R. E. 2017. Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes., 66(2): 241-255.

Sowers, J. R. and Frohlich, E. D. 2004. Insulin and Insulin Resistance: Medical Clinics of North America., 88(1): 63–82.

Sultani, M., Stringer, A. M., Bowen, J. M. and Gibson, R. J. 2012. Anti-inflammatory cytokines: important immunoregulatory factors contributing to chemotherapy-induced gastrointestinal mucositis. Chemotherapy research and practice., 8: 2.

Takeuchi, O. and Akira, S. 2010. Pattern recognition receptors and inflammation. Cell., 140(6): 805-820.

Temelkova-Kurktschiev, T. S., Koehler, C., Henkel, E., Leonhardt, W., Fuecker, K. and Hanefeld, M. 2000. Postchallenge Plasma Glucose and Fasting Glucose or HbA 1c Level. Diabetes Care., 23(12): 1–5.

Todd, J. F. and Bloom, S. R. 2007. Incretins and other peptides in the treatment of diabetes. Diabetic medicine., 24(3): 223-232.

Todd, J. N. and Florez, J. C. 2014. An update on the pharmacogenomics of metformin: progress, problems and potential. Pharmacogenomics., 15(4): 529-539.

Tsang, M. W. 2012. The management of type 2 diabetic patients with hypoglycaemic agents. International Scholarly Research Notices., 12: 8.

WHO. 2017. Comprehensive Assessment of Pakistan's Health Information System 2017.

Xu, L., Man, C. D., Charbonnel, B., Meninger, G., Davies, M. J., Williams-Herman, D. and Stein, P. P. 2008. Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on beta-cell function in patients with type 2 diabetes: a model-based approach. Diabetes, Obesity and Metabolism., 10(12): 1212-1220.

Y Ahmad, T., KH Tawfeeq, F. and A AL-Ameen, S. 2018. Biochemical Study of Dipeptidylpeptidase-4 from Normal Human Serum. Rafidain Journal of Science., 27(1): 123-132.

Ye, F. Z., Liu, W. Y., Zheng, K. I., Pan, X. Y., Ma, H. L., Wang, X. D. and Zheng, M. H. 2020. Homeostatic model assessment of insulin resistance closely related to lobular inflammation in nonalcoholic fatty liver disease. European Journal of Gastroenterology and Hepatology., 32(1): 80-86.

Yin, J., Zhang, H. and Ye, J. 2008. Traditional Chinese medicine in treatment of metabolic syndrome. Endocrine, Metabolic and Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine and Metabolic Disorders)., 8(2): 99-111.

Zhang, L., Liu, K., Li, H., Li, D., Chen, Z., Zhang, L. L. and Guo, L. L. 2016. Relationship between body mass index and depressive symptoms: the "fat and jolly" hypothesis for the middle-aged and elderly in China. BMC public health., 16(1): 1-15.

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3-Review Study of Interleukin-6 and its Association with Coronavirus.SR Majeed, AJ Ahmed, WAA Hassan.Journal of Kufa for Chemical Science Vol 2 (6)2020

4-The study of Cytokines and Chemokines in patient's infectious diseases and the impact of age and sex. Bushra Ismail Ibrahim, Athmar Jawad Ahmed.International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 04 2020

5-The effect of selenium, magnesium and zinc level in seminal plasma infertile men. Ahmar Jawad ahmed11 ,(698) 692 . Al-Kufa University Journal for Biology2014

6-In vitro sperm effect of incubation period and vitamin c on in vitro sperm activation in oligospermic infertile patients by using Fert cult flushing medium. Dhirgam Alshimerty, Athmar Jawad ahmed.Kufa for nursing sciences 3 (10), 102013.